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Influence of pH of culture medium on fungal contaminants and growth response of Barhi cultivar date palm explants (*Phoenix dactylifera* L.) *in vitro*

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Influence of pH of culture medium on fungal contaminants and growth response of Barhi cultivar date palm explants (*Phoenix dactylifera* L.) *in vitro*

Abstract: Date palm is commonly propagated through tissue culture techniques; however, this method often encounters complications, particularly fungal contamination. The effects of varying pH levels on fungal development and explant response in vitro are the focus of the research. Morphological and molecular assessments led to the identification of three fungal species: *Alternaria alternata* (Fr.) Keissl., *Chaetomium globosum* Kunze and *Nigrospora osmanthi* Mei Wang & L. Cai were the most prevalent, with *A. alternata* being the most frequently observed. Among the tested pH levels, pH 5 had the most pronounced impact on the radial expansion of the fungi. Additionally, pH variations affected the fungal dry biomass, with *N. osmanthi* showing the highest sensitivity to changes in pH. In terms of shoot development within the tissue culture environment, overall shoot growth of date palm was not significantly influenced by pH levels. However, shoot length exhibited notable differences at pH 5, 5.5, and 6, measuring 4.2 cm, 4.6 cm, and 5.1 cm, respectively. Furthermore, the amino acid concentration in leaf tissues varied with pH, peaking at 50.6 mg per 100 g of tissue at pH 5. Carbohydrate levels in the leaves were also pH-dependent, with the highest value of 29.26 mg g^{-1} at pH 6 and the lowest (0.34 mg g^{-1}) at pH 5.

Key words: date palm tree, fungi, tissue culture, callus, shoots, contamination.

Vpliv pH gojišča na glivne kontaminante in odziv na rast eksplantatov datljevca 'Barhi' (*Phoenix dactylifera* L.) *in vitro*

Izvleček: Datljevo palmo običajno razmnožujejo s tehnikami tkivnih kultur; vendar se pri teh metodah pogosto pojavijo zapleti, zlasti okužba z glivami. V središču raziskave so učinki različnih pH na razvoj gliv in odziv eksplantatov *in vitro*. Morfološke in molekularne ocene so vodile do določitve treh vrst gliv, vrste *Alternaria alternata* (Fr.) Keissl., *Chaetomium globosum* Kunze in *Nigrospora osmanthi* Mei Wang & L. Cai so bile najbolj razširjene, pri čemer je bila vrsta *A. alternata* najpogostejša. Med testiranimi pH vrednostmi je imel pH 5 najizrazitejši vpliv na radialno širjenje gliv. Poleg tega so spremembe pH vplivale na suho biomaso gliv, pri čemer je vrsta *N. osmanthi* pokazala največjo občutljivost na spremembe pH. Kar zadeva razvoj poganjkov v okolju tkivne kulture, ravni pH niso bistveno vplivale na celotno rast poganjkov datljeve palme. Dolžina poganjkov je pokazala opazne razlike pri pH 5, 5,5 in 6, in sicer 4,2 cm, 4,6 cm oziroma 5,1 cm. Poleg tega se je koncentracija aminokislin v listnem tkivu spreminjala s pH in dosegla vrh pri 50,6 mg na 100 g tkiva pri pH 5. Ravni ogljikovih hidratov v listih so bile prav tako odvisne od pH, z največjo vrednostjo 29,26 mg g^{-1} pri pH 6 in najmanjšo (0,34 mg g^{-1}) pri pH 5.

Ključne besede: datljeva palma, glive, tkivna kultura, kalus, poganjki, kontaminacija.

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1 INTRODUCTION

In vitro plant tissue culture often encounters several technical obstacles, including tissue browning, vitrification, and, particularly, microbial contamination. Contamination caused by microorganisms remains a persistent and difficult challenge, While several problems can be minimized through careful management. Such contamination may originate from plant tissues themselves, the tools used, personnel handling, or the culture medium, often resulting in significant harm to cultured explants (Cassells, 1991; Reed & Tanprasert, 1995).

Certain antibiotics are introduced into the media in tissue culture to combat the growth of microbes, such as rifampicin, streptomycin, gentamicin, and chloramphenicol (Omar et al., 1992). Additionally, immersing explants in a 20 % sodium hypochlorite solution containing Tween-20 has shown some effectiveness in reducing bacterial and fungal contamination (Budiarni et al., 2024). Nonetheless, these microorganisms spread across the culture medium, adversely affecting plant material health, and frequently reappear during cultivation.

Nutrient-rich growth medium is a contributing factor to this issue, providing a perfect environment for the proliferation of microbes. Furthermore, the pH of the medium significantly influences nutrient uptake and the growth behavior of both plants and microbes (Aleandri et al., 2007; Chen et al., 2014;). Different organisms respond differently to the surrounding pH, with some thriving in acidic conditions while others favor alkaline environments. As such, adjustments to the culture medium's pH are typically achieved using either HCl or NaOH to cater to the growth requirements of specific organisms (Shi et al., 2017; Hamad et al., 2023).

Previous studies have outlined optimal pH ranges for various plant species, such as pH 5.6-6.0 for effective shoot multiplication in tetraploid black locust, and below pH 6.4 for Erqiao black locust (Huang & Liu, 2003). In apple tissue culture, the optimal pH range has been noted between 5.5 and 7.5 (Shi et al., 2017). Similarly, fungal species exhibit considerable variation in their preferred pH levels, although many can grow across a broad pH range (Landraud et al., 2013; Bousset et al., 2018). The surrounding pH can influence fungal virulence, gene expression, mycotoxin production, and the activity of enzymes that break down plant cell walls (Merhej, 2011).

Accordingly, pH shifts can have significant effects either beneficial or detrimental on both plant explants and microbial activity (Leifert et al., 1992; Bousset et al., 2018).

The date palm across the Middle East and North Africa, one of the oldest cultivated species in Iraq, holds major agricultural and nutritional significance. Its fruit is highly valued for its nutritional properties, making the crop vital in dry and arid climates (Yahia & Kader, 2011; Krueger et al., 2023).

Date palm is widely propagated using tissue culture, but this method has recurring problems, mainly due to fungal contamination. These fungal organisms can spread rapidly, colonize the growth medium, and degrade explant health, ultimately leading to the failure of the tissue culture process. Given the scarcity of research focused on fungal contamination in date palm tissue culture, the primary goal of this investigation was to explore how different pH levels affect fungal development and explant response under in vitro conditions.

2 MATERIALS AND METHODS

2.1 PREPARATION OF MURASHIGE AND SKOOGE (MS) CULTURE MEDIUM

The MS medium (Murashige and Skoog; Caisson Laboratory, USA) was prepared according to the formulation shown in Table 1. To begin, 4.44 g of MS powder was dissolved in 700 ml of distilled water inside a 1-liter flask. The following components were added to the solution in mg l^{-1}: 30000 mg sucrose, 0.170 g sodium hydrogen orthophosphate, 0.040 g adenine sulfate, 0.0005 g, 0.250 g activated charcoal, and 6g agar. The mixture was stirred thoroughly with a magnetic stirrer on a hot plate until fully dissolved. Plant growth hormones, including cytokinins and auxins, were incorporated as required by the experiment, for the callus stage 30 mg l^{-1} α-naphthalene acetic acid (NAA) and 3 mg l^{-1} 2-isopentenyl adenine (2iP) were added. While, For the shoots stage, 1 mg l^{-1} benzyl adenine (BA), 1 mg l^{-1} kinetin, and 0.5 mg l^{-1} NAA were added. The medium pH was adjusted to the target value using a digital pH meter. Once the medium was ready, 50 ml portions were dispensed into jars, sealed with plastic lids, and sterilized in an autoclave at 121 °C and 1.05 kg cm^{-2} pressure for 20 minutes. After autoclaving, the jars were allowed to cool before use for culture.

2.2 FUNGAL ISOLATION PROCEDURE

Fungal isolates were obtained from contaminated callus and shoot tissues of date palm cv. Barhi, sourced from the Date Palm Research Center's tissue culture lab. These explants were transferred to sterile Petri dishes containing potato dextrose agar (PDA) and incubated in complete darkness at 25 °C. This procedure was conducted under a laminar airflow cabinet using sterilized

Table 1: Components of basal medium Murashige and Skooge (MS) with vitamins.

Ingredients	Quantity mg l^{-1}
Ammonium nitrate	1650
Potassium nitrate	1900
Magnesium sulphate	370
Calcium chloride	440
Potassium phosphate	170
Sodium phosphate	-
Boric acid	6.20
Cobalt chloride	0.025
Magnesium sulphate	22.3
Copper sulphate	0.025
Sodium molybdate $2H_2O$	0.25
Potassium iodide	0.83
Zinc sulphate $7H_2O$	8.6
Ferrous sulphate $7H_2O$	27.84
EDTA disodium salt $2H_2O$	36.7
Thiamine HCl	0.5
Nicotinic acid	0.5
Pyridoxine HCl	0.5
Glycine	2
Myoinositol	100

tools to minimize contamination. After 4–5 days, visible mycelial segments were subcultured onto fresh PDA plates and incubated under the same conditions. Fungal growth was tracked regularly. The frequency of contamination was calculated using the formula: (NF / NT) × 100, where NF refers to the number of infected explant parts, and NT denotes the total number of explant samples tested.

2.3 FUNGI COLONIES MORPHOLOGY

Fungi were identified based on the macroscopic and microscopic characteristics observed after culturing on solid PDA medium. Colony traits such as pigmentation and radial growth were documented. Conidial features including shape, size, and structural arrangement were used to aid classification. Taxonomic identification was performed using resources and keys provided by Webster (1952), Chapman and Fergus (1975), Simmons (2007), and Wang et al. (2016). Observations of fungal mycelia and spores were made using a compound microscope at 40 × magnification, and representative images were captured.

2.4 MOLECULAR ANALYSIS

Genomic DNA was extracted from the fungal samples using the Geneaid Genomic DNA Mini Kit for Plants according to the manufacturer's protocol. PCR amplification of the internal transcribed spacer regions (ITS1 and ITS2) was carried out using AccuPower PCR PreMix (Bioneer) with the following primers:

- ITS1: 5'-TCCGTAGGTGAACCTGCGG-3'
- ITS4: 5'-TCCTCCGCTTATTGATATGC-3'

Thermocycling conditions included an initial denaturation at 94 °C for 1 minute, followed by 35 cycles of denaturation at 95 °C for 1 minute, annealing at 55 °C for 45 seconds, and extension at 72 °C for 1 minute, with a final extension at 72 °C for 10 minutes.

PCR products were sequenced by Macrogen Inc. (Korea). Sequences were aligned and edited in Mesquite v3.40 using Clustal v2.1. The best-fit model (GTR + F + I + G4) was selected using the Akaike Information Criterion in jModelTest v2.1.6. Phylogenetic relationships were inferred via maximum likelihood using IQ-Tree v1.6.10. Pairwise p-distances were computed using PAUP, accessed through the CIPRES Science Gateway. The sequence accession numbers were: *Alternaria alternata* (PP330021), *Nigrospora osmanthi* (PP330022), and *Chaetomium globosum* (OP090361).

2.5 EFFECT OF VARYING PH LEVELS ON FUNGAL RADIAL GROWTH

The influence of different pH levels on mycelial growth was evaluated using MS culture medium. The pH was modified to three settings: 5, 5.5, and 6. Each Petri dish received a 0.5 cm mycelial disc from the respective fungal isolate. Every pH condition was replicated three times. The plates were incubated at 25 °C, and radial fungal growth was recorded at intervals of 2, 4, 6, 8, and 10 days. Fungal colony characteristics, such as texture and pigmentation, were observed and documented at the end of the experiment.

2.6 MEASURMENT OF MYCELIUM DRY BIOMASS UNDER DIFFERENT PH CONDITIONS

To determine biomass yield, liquid MS medium (excluding agar) was prepared and adjusted to the designated pH. For each treatment, three 250 ml flasks containing 50 ml of medium were inoculated with 0.5 cm fungal discs. These flasks were incubated at 25 °C for 14 days. After incubation, the fungal biomass was harvested by filtration using filter paper Whatman No. 1, dried at 60 °C in an oven until a constant mass was obtained. and

weighed to assess the effect of pH on mycelial mass accumulation.

2.7 INFLUENCE OF PH ON SHOOT PROLIFERATION OF DATE PALM 'BARHI' IN VITRO

MS culture media were prepared and adjusted to pH 5, 5.5, and 6 using 0.1 M HCl or NaOH. In each jar, four shoots were cultured, and three jars were used per pH treatment. All cultures were kept in a growth chamber maintained at 27 ± 1 °C in complete darkness. Subculturing occurred every four weeks onto fresh culture media in new jars. After two months, the number of shoots, average shoot length, and multiplication rate were assessed to evaluate the effect of pH levels.

A 0.2 g of leaves were homogenized in 5 ml (95 % ethanol) and centrifuged at 6000 rpm for 15 minutes for biochemical analysis. The clear extract was reacted with 5 ml of ninhydrin reagent and heated at 70 °C for 20 minutes. Absorbance was measured at 570 nm using a spectrophotometer, as described by Moore & Stein (1954). To calculate amino acid concentrations from absorbance values, a standard curve was prepared using amino acid leucine in different concentrations. Leaf carbohydrate content was also measured. A 0.5 g leaf sample was boiled in 70 ml of distilled water at 90 °C for one hour. The extract was filtered using Whatman No. 1 filter paper, and 1 ml of the filtrate was reacted with 1 ml of phenol and sulfuric acid. Absorbance was read at 490 nm using a Shimadzu UV-1700 spectrophotometer, following the method of DuBois et al. (1965). To calculate carbohydrate concentrations from the absorbance values, a standard curve was prepared using known concentrations of glucose.

2.8 STATISTICAL ANALYSIS

All statistical analyses were performed using SAS software. A three-way ANOVA was conducted for radial growth comparisons among different pH treatments, with pH level, fungal species and incubation time, while a two-way ANOVA was employed to analyze the dry weight of fungal mycelium, with pH level and fungal species. One-way ANOVA was used to assess the effect of pH on shoot multiplication parameters in date palm. Mean comparisons were performed using the Tukey-Kramer test at the $p < 0.05$ significance level.

3 RESULTS AND DISCUSSION

3.1 FUNGAL ISOLATION AND MOLECULAR CHARACTERIZATION

Fungal isolation from in vitro-cultured date palm explants revealed three distinct species, which were subsequently identified by both morphological assessment and DNA-based analysis as *A. alternata*, *C. globosum*, and *N. osmanthi* (Figure 1).

On potato dextrose agar (PDA), *A. alternata* formed colonies exhibiting dark green to black pigmentation. The fungus expanded rapidly, reaching a diameter of 9 cm within 7 days when maintained in complete darkness at 25 °C. Conidial development was abundant, forming chains of oval, ellipsoid, or subspherical spores. The initial 1–2 conidia in the chain were typically elongated, measuring between 25–30 µm in length and 5–9 µm in width, with 4–7 transverse septa and minimal to no longitudinal septation.

In *C. globosum*, colonies initially appeared white on PDA but turned pale yellow by day 7. These colonies eventually produced olive-green perithecia. The perithecial hairs were brown, tapering towards the tips, and measured 3–5 µm in thickness. Ascospores were single-celled, lemon-shaped, gray-green to brown, and measured between 6–8 µm in width and 10–12.5 µm in length.

N. osmanthi initially formed white colonies on PDA, which darkened to black over time. After 14 days at 25 °C, colony growth reached the full diameter of the Petri dish, which was 9 cm. Conidia were formed individually at the tips of clear vesicles located at the ends of the conidiophores. These conidia were large, spherical, smooth, and black.

Species confirmation was achieved through phylogenetic analysis of DNA sequences. Sequence comparison showed 0 % uncorrected p-distance between sample R_1 and *A. alternata*, 0 % between R_2 and *N. osmanthi*, and 0.02 % between the sample and *C. globosum*, respectively. Phylogenetic tree construction confirmed these identifications, with bootstrap support values of 100 %, 65 %, and 100 % for each respective grouping (Figure 2).

Fungal occurrence differed between callus and shoot tissues. *A. alternata* was the most prevalent, detected in 8.83 % of callus samples and 8.3 % of shoot samples. *C. globosum* and *N. osmanthi* were found only in shoot tissues at frequencies of 5.79 % and 2.12 %, respectively, with no presence recorded in callus tissues (Figure 3).

These fungi being present in tissue cultures suggest they may have contaminated the explants during handling or may have originated from endogenous sources. Their ability to persist in controlled environments highlights their prevalence on MS medium. *A. alternata* appeared as the dominant fungus among the isolated ones. This could be possibly due to its rapid growth, enzyme secretion capabilities, and competitive advantage in culture.

This is consistent with field and laboratory studies

documenting *A. alternata* as a major pathogen affecting date palms. It is associated with diseases such as inflorescence rot (Al-Zayat, 2002; Alasadi & Al-Sadoon, 2011; Alasadi, 2024), leaf spot (Alasadi et al., 2014; Alasadi, 2024), and fruit rot (Al-Sharidi & Al-Shahwan, 2003; Alasadi et al., 2006; Alasadi, 2024). Prior investigations, including those by Al-Mayahi et al. (2010), also isolated *A. alternata* and *C. globosum* from tissue-cultured 'Barhi' date palms, reinforcing the idea of their adaptability to in vitro conditions. The latest outcomes showed similarity with a previous study. This shows that these fungi have the ability to live after surface sterilization. As well as, to adapt under tissue culture conditions.

Importantly, this study is the first to report the isolation of *N. osmanthi* from date palm explants.

Interestingly, fungal colonies displayed noticeable differences in growth patterns and morphology when cultured on Murashige and Skoog (MS) medium compared to PDA. Mycelial expansion on MS medium was limited, suggesting the nutrient composition might not favor fungal proliferation. The presence of complex nutrients or inhibitory compounds in MS could suppress fungal growth, as supported by previous research (Ibrahim et al., 2015; Francisco et al., 2019; Nastasi et al., 2020). Islam and Ohga (2013) further proposed that elevated concentrations of vitamins and hormones may trigger the activity of growth-inhibiting enzymes or create osmotic conditions that hinder fungal metabolism.

3.2 EFFECT OF VARYING PH LEVELS ON FUNGAL RADIAL GROWTH AND MYCELIUM BIOMASS

The growth behavior of *A. alternata*, *C. globosum*, and *N. osmanthi* was found to be significantly influenced by the pH of the culture medium. The most notable effect

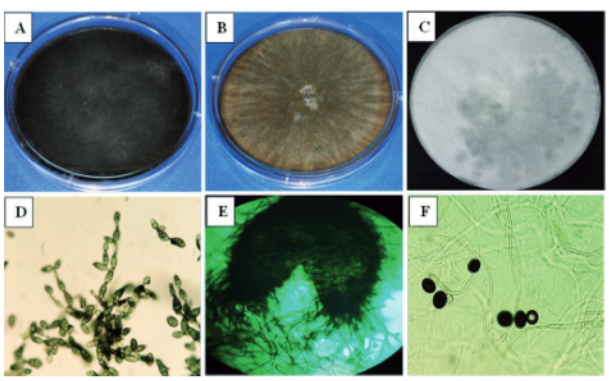

Figure 1: Fungal growth on PDA medium. (A) *Alternaria alternata*. (B) *Chaetomium globosum*. (C) *Nigrospora osmanthi*. (D) Conidial chains of *A. alternata*. (E) Ascomata and ascospores of *C. globosum*. (F) Conidia of *N. osmanthi*.

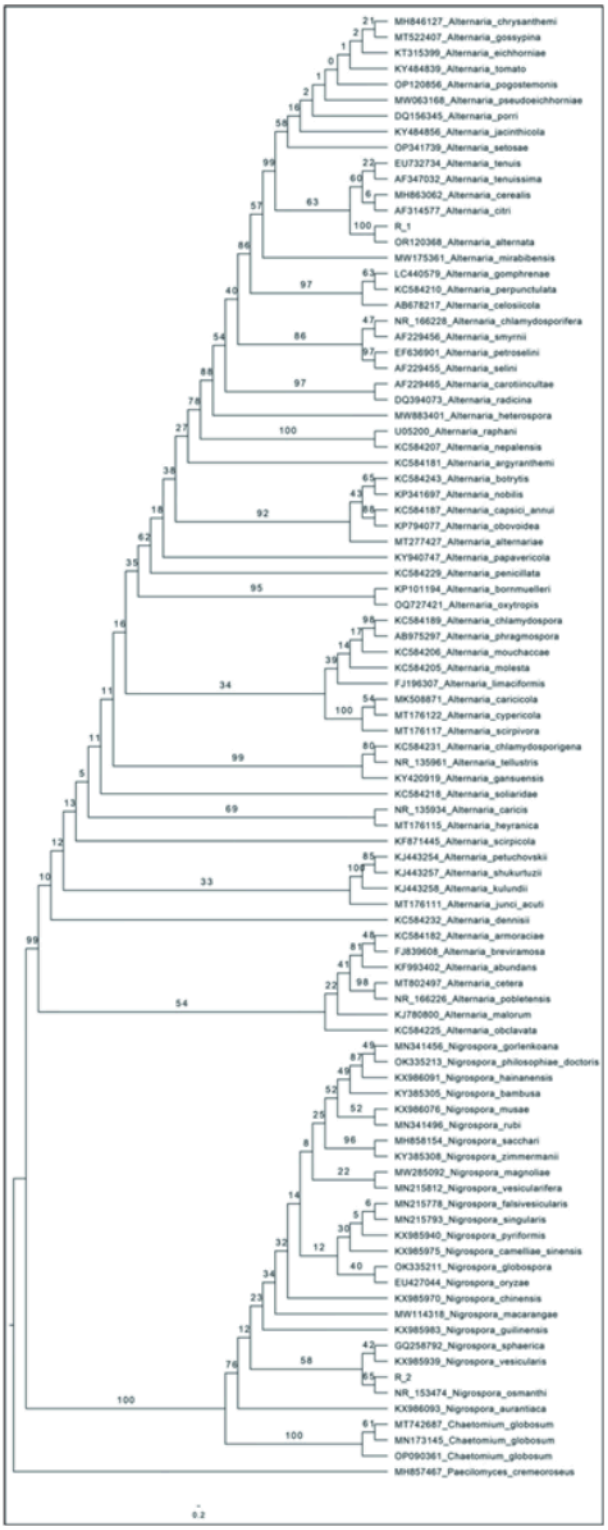

Figure 2: Phylogenetic tree derived from the concatenated nDNA dataset (ITS1, 5.8S, and ITS2) using Maximum Likelihood (ML) analysis with 1000 bootstrap iterations. Bootstrap values are shown along the branches to represent the ML support.

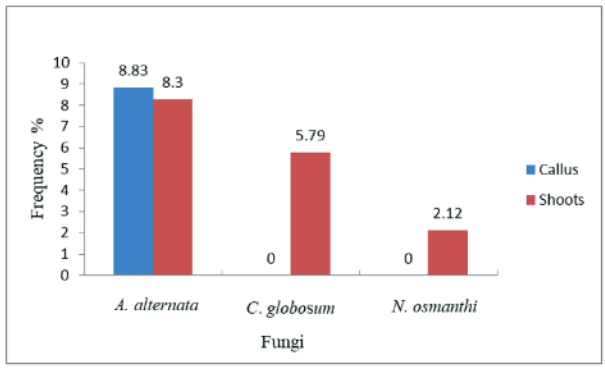

Figure 3: Distribution frequency of fungal isolates observed on callus and shoot tissues.

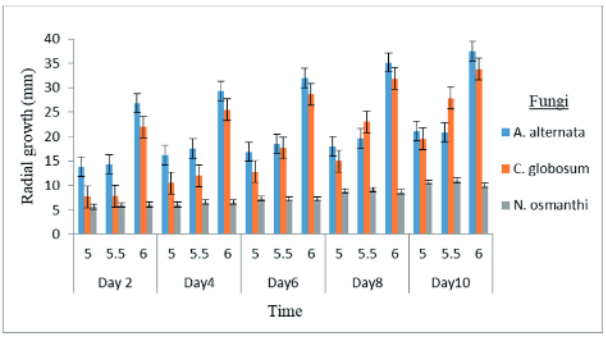

Figure 4: Impact of varying pH levels on the radial growth of fungi. The pH levels (5, 5.5, and 6) were tested with three replicates. The bars represent the standard error of the mean.

on radial growth was observed at pH 5 among the tested pH levels (5, 5.5, and 6). Of the three species, *N. osmanthi* exhibited the greatest sensitivity to changes in pH, whereas *A. alternata* and *C. globosum* showed comparatively stable growth. All three species displayed progressive radial growth over the incubation period (Figure 4).

These results point out the differences of phsioplogical mechanaisms among species based on thier responses to the pH.

On Murashige and Skoog (MS) medium, *A. alternata* developed dense yellow mycelium that rose above the agar and expanded irregularly. In contrast, *C. globosum* formed a cottony colony with white mycelia and embedded yellowish pigmentation in the agar, and exhibited irregular growth. *N. osmanthi*, however, produced sparse white aerial hyphae, with notably slower surface expansion across the medium. These growth characteristics were documented under different pH conditions supplied in the MS medium (Figure 5).

Measurements of fungal biomass through dry mass confirmed the impact of pH on fungal development. Analysis of variance revealed highly significant differences ($p < 0.0001$) among the pH treatments. In the case of *N. osmanthi*, growth was most pronounced at pH 6, yielding a dry mass of 0.167 g, compared to 0.037 g at pH 5.5 and 0.028 g at pH 5. Similarly, *A. alternata* produced more biomass at pH 5.5, registering 0.476 g, but this was lower than the values observed at pH 5 (0.397 g) and pH 6 (0.259 g). Interestingly, *C. globosum* showed the lowest dry mass at pH 5.5 (0.166 g), while higher values were recorded at pH 5.0 (0.212 g) and pH 6.0 (0.312 g) (Figure 6).

What influences radial growth and biomass accumulation in fungal cultures is pH. Although all three fungal species grew across the pH range tested, growth rates and mycelial mass varied with pH, suggesting that pH influences nutrient uptake or enzyme activity.

The effects of pH on fungal physiology have been evaluated by numerous studies. For instance, Opgenorth and Endo (1983) reported that *Fusarium oxysporum* f.sp. *apii* grew optimally within a pH range of 4.6–7, whereas growth was markedly suppressed at pH 2. Shabana et al. (2000) found the optimal pH for *Neochetina eichhorniae* Warner was around 7, though conidial production

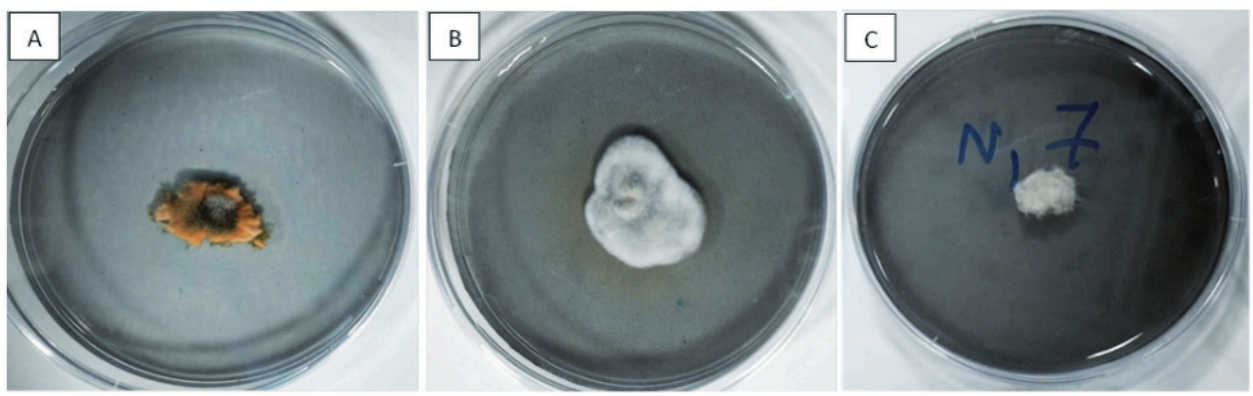

Figure 5: Growth patterns observed in the radial growth of (A) *A. alternata*, (B) *C. globosum*, and (C) *N. osmanthi* cultivated on MS medium for 10 days at 25 °C.

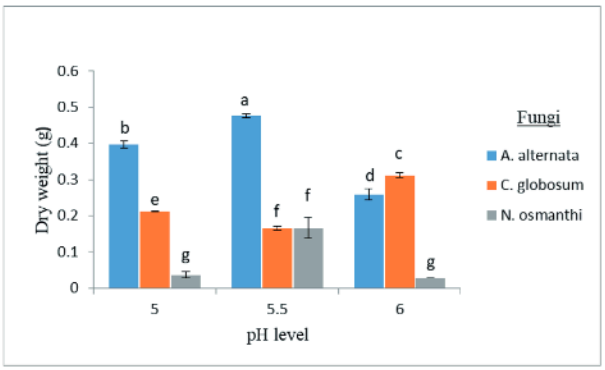

Figure 6: Impact of various pH levels on fungal biomass. Data points sharing the same letter show no significant difference. The bars represent the standard error of the mean.

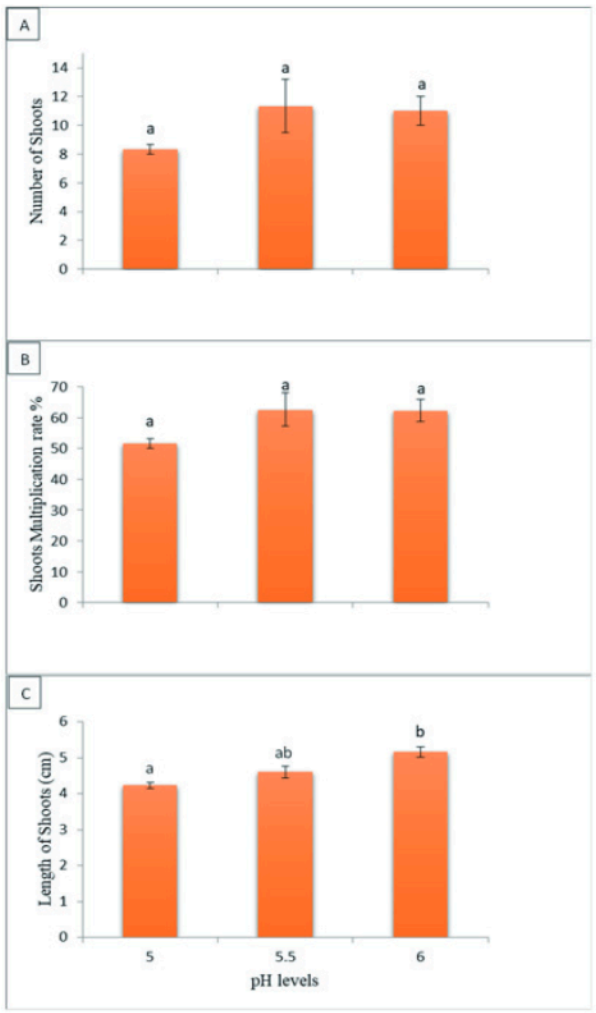

Figure 7: Influence of culture medium pH on (A) Shoot count, (B) Shoot multiplication rate, and (C) Shoot length (cm). Data points sharing the same letter are not significantly different, while different letters indicate statistically significant differences. The bars represent the standard error of the mean.

peaked at pH 5.6 and 9.7. Likewise, Sekar et al. (2017) observed optimal growth of *F. graminearum*Schwabe at neutral pH compared to more acidic or alkaline conditions. Fungi have evolved various mechanisms to thrive in fluctuating pH environments, such as secreting extracellular enzymes or organic acids to adjust the medium's chemistry. Verhoeff et al. (1988) demonstrated that *Botrytis cinerea* Pers. modulated its environment by producing acids, including citric, malic, and succinic, during growth. Fungal species are known to tolerate both acidic (Dix and Webster, 1995) and alkaline environments (Yamanaka, 2003). Aleandri et al. (2007) noted that during growth of *F. culmorum* on an acidified yeast extract medium, the culture pH shifted from 4 to 8.7, coinciding with ammonia release, suggesting a fungal strategy to optimize conditions. Furthermore, Landraud et al. (2013) reported that *Magnaporthe oryzae* (T.T. Hebert) M.E. Barr produced more conidia at pH 8, whereas germination occurred more readily at acidic pH.

The results of recent study align with thses studies which conform that pH is an important factor control the growth of fungi, its morphology, and production of biomass.

To sum up, the results show that pH has an important role in regulating responses fungal growth physiological and characteristics. Also, noticeable variation among fungal species.

3.3 IMPACT OF MEDIUM PH ON SHOOT MULTIPLICATION OF DATE PALM 'BARHI' UNDER IN VITRO CONDITIONS

This investigation demonstrated that different pH levels influenced the growth response of date palm shoots cultivated in vitro. Statistical analysis indicated that shoot count and multiplication rates were not significantly affected by pH variations, suggesting no substantial differences among the tested pH levels in this regard (Figure 7). However, as shown in Figure 8, shoot proliferation was successful on Murashige and Skoog (MS) medium at all evaluated pH levels. Interestingly, shoot length was significantly affected by pH ($p < 0.009$), with the longest shoots developing at pH 6 (5.1 cm), followed by pH 5.5 (4.6 cm) and pH 5 (4.2 cm).

Regarding the biochemical composition, the pH of the culture medium had a significant impact on amino acid concentrations and carbohydrate. The highest amino acid content was showed under acidic conditions, while carbohydrate levels increased gradually with increasing pH. (Figure 9).

The unchanged number of shoots and multiplication frequency across pH levels suggest that the shoot explants were resilient and adaptable to the various in

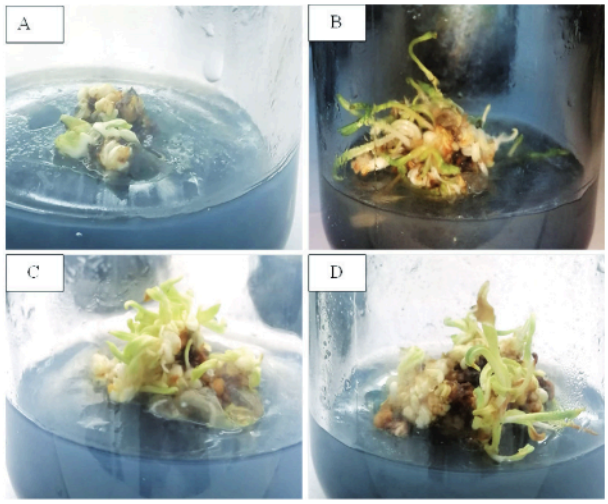

Figure 8: Shoot growth in tissue culture of the Barhi cultivar on MS medium. (A) Initial shoot formation. (B) Shoot growth and development on MS medium at pH 5. (C) Shoot growth and development on MS medium at pH 5.5. (D) Shoot growth and development on MS medium at pH 6.

vitro conditions. This aligns with findings by Chen et al. (2014), who reported that certain Douglas-fir genotypes could sustain growth under different pH environments due to inherent tolerance mechanisms. It is also likely that distinct growth stages have differing optimal pH requirements. For instance, Nesius and Fletcher (1973) found that pH levels between 5.2 and 5.4 were ideal for cellular division, whereas a slightly higher pH range (5.5–6) favored general cell expansion. Similarly, Ebrahim and Ibrahim (2000) identified pH 5.7 as optimal for promoting shoot multiplication and leaf differentiation in *Maranta leuconeura* E. Morren tissue cultures.

pH influenced shoot elongation more strongly than other morphological characteristics. The longer shoots observed at higher pH levels may result from changes in ionic interactions between nutrients and the culture medium. Pasqua et al. (2002) suggested that callus formation dominated at pH 6, possibly due to improved phosphate uptake. Additionally, de Klerk et al. (2008) demonstrated that auxin absorption increased at pH levels below 5.3, potentially reducing root development. Thus, medium pH can function similarly to plant hormones, modulating morphogenic responses (George et al., 2008). As for the accumulation of amino acids, the highest concentration was found in tissues grown at pH 5 (50.6 mg 100 g^{-1}), and at pH 5.5 lower amounts were observed (14.7 mg 100 g^{-1}), and at pH 6 (7.03 mg 100 g^{-1}). A more acidic medium may favor amino acid synthesis or retention, possibly through enhanced enzyme activity or nutrient availability, because of the decline in amino acid content at higher pH. In contrast, carbohydrate accumulation increased as the medium became less acidic, reaching its the highest levels at pH 6. This implies that a mildly acidic to neutral pH environment supports carbohydrate synthesis and storage, likely due to elevated photosynthetic efficiency and energy utilization.

Changes in pH influence how the plant directs its metabolic activity, which is shown in the data. Acidic conditions seem to favor amino acid synthesis, while a higher pH encourages carbohydrate buildup. The results show that depending on whether you're targeting certain physiological traits or focusing on biochemical processes, adjusting pH in tissue culture can really matter.

To our knowledge, no previous studies have specifically addressed the effect of culture medium pH on morphological and biochemical traits of date palm shoots in vitro. As a result, this study provides a extensive physiological context and high spot the novelty of this work by discussing the current results with studies conducted on other plant species.

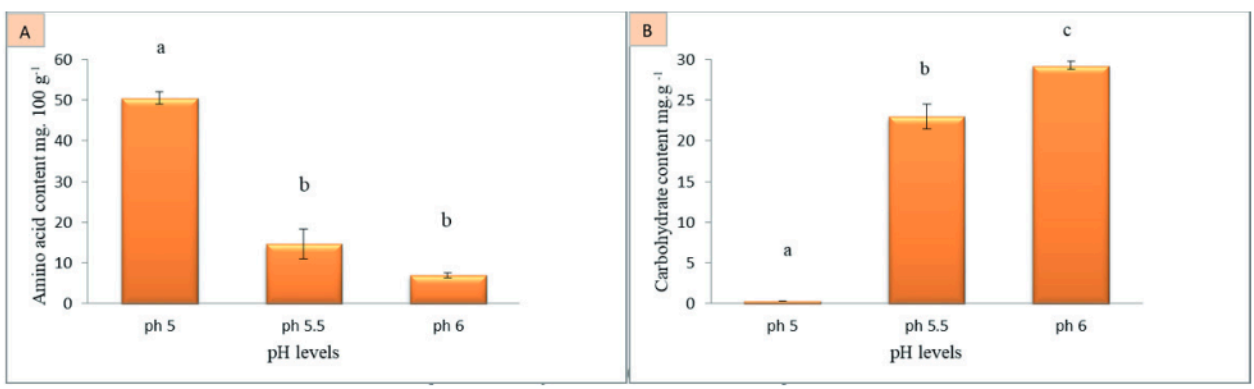

Figure 9: (A) Amino acid concentration in the leaves. (B) Carbohydrate concentration in the leaves. Values with the same lowercase letter indicate no significant difference at p = 0.05. The bars represent the standard error of the mean.

4 CONCLUSION

In this study, three fungal contaminants *A. alternata*, *C. globosum*, and *N. osmanthi* were isolated in date palm explants cultured in vitro. To identify the fungi, physical traits and molecular methods were used. The study mainly looked at how pH and other environmental factors influenced their growth. The fungi growing differently at certain pH levels really showed how much the environment affects their behavior and physiology. While pH levels did not significantly affect the number of shoots or the rate of multiplication, differences in shoot length were observed among specific pH treatments and pH influnced the biochemical compostion of leaves, particularly amino acids and carbohydrates. This means continuing research into how environmental factors can change fungal growth and shoot development in the long term. More effective strategies for controlling fungal contamination in date palm in vitro could be developed using the information from this study. The results show that the pH an important tool to control the fungal contamination and to improve the performance of date palm tissue culture.

Data Availability

All data generated and analyzed in this study are original and presented in this manuscript.

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