OPEN ACCESS Plant Protection, 10 (05) 2026. 945-959 DOI:10.33804/pp.010.05.6257

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Available Online at EScience Press

Plant Protection

ISSN: 2617-1287 (Online), 2617-1279 (Print)

http://esciencepress.net/journals/PP

Research Article

Antifungal Activity and GC–MS Profiling of Date Palm (*Phoenix dactylifera* L.) Seed Extracts against *Alternaria alternata* and *Fusarium oxysporum* Associated with *In Vitro* Micropropagation

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ARTICLE INFO

Article history

Received: 28th August, 2026

Revised: 23rd October, 2026

Accepted: 26th October, 2026

Keywords

Micropropagation

Antifungal activity

Date palm seed extracts

GC–MS profiling

Alternaria alternate

Fusarium oxysporum

ABSTRACT

Fungal contamination is a major challenge in date palm micropropagation. This study characterized the chemical profiles of alcoholic seed extracts from four date palm cultivars and evaluated their *in vitro* antifungal activity against *Alternaria alternata* and *Fusarium oxysporum*. Gas chromatography–mass spectrometry (GC–MS) identified 131 compounds, with cultivar-dependent variations in chemical composition. Barhi contained the highest number of identified compounds (52), whereas Maktoom contained the lowest (18). Six compounds were common to all cultivars, and fatty acid ethyl esters were the predominant chemical class. Ethyl oleate was the most abundant common compound, while DDMP accounted for 12.01% and 7.84% of the matched peak area in Shukr and Khadrawi, respectively, but was absent from Barhi and Maktoom. Three-way analysis of variance revealed significant effects of cultivar, extract concentration, fungal species, and their interactions on mycelial growth inhibition ($P < 0.01$). Mean inhibition was highest for Maktoom (65.28%), followed by Shukr (39.12%), Khadrawi (35.65%), and Barhi (29.80%). Inhibition increased with extract concentration, reaching 54.50% at 15 mg/mL. Maktoom extract at 15 mg/mL produced the highest inhibition against *F. oxysporum* (80.37%) and *A. alternata* (77.60%). The cultivar ranking (Maktoum > Shukr > Khadrawi > Barhi) for antifungal activity corresponded descriptively with ethyl oleate abundance, although causality was not established. These findings highlight Maktoom seed extract as a promising candidate for further investigation as a potential antifungal treatment for managing fungal contamination in date palm micropropagation. Validation under tissue culture conditions and assessment of phytotoxicity are required.

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Introduction

Phoenix dactylifera L. belongs to the Arecaceae family and is an economically important fruit crop grown in large numbers in many parts of the world especially in dry and semi-dry climates. It plays a significant role in food security, rural livelihoods, and agricultural sustainability in areas with scarce water resources and challenging weather conditions. Date fruits are very rich in carbohydrates, including sugars, and contain minerals, vitamins and other nutritionally beneficial compounds (Rashid et al., 2021; Al-Mayahi, 2024). The production of good quality planting material is crucial for the

sustainable development of date palm production, because of its economic and nutritional significance. Traditional propagation methods are mostly based on seeds and vegetative offshoots. On the other hand, seed propagation results in high genetic variability and is not effective in maintaining the desirable qualities of elite cultivars, while offshoot propagation has limitations in availability, slow propagation rate and long establishment period (Abdelghaffar et al., 2023). Therefore, micropropagation using plant tissue culture has become a promising alternative approach to multiply genetically uniform and disease free planting material with the maintenance of desirable agronomic and commercial traits of selected cultivars (Al-Qatrani et al., 2021).

Although date palm micropropagation has great potential, microbial contamination of plant materials and culture media is often a major constraint for their successful application. Fungi and bacteria may contaminate explants and compete for nutrition, change the physical and chemical properties of the culture media, disrupt tissue growth and eventually lead to explant damage or death. The most common fungal genera causing contamination of plant tissue culture are *Alternaria*, *Cladosporium*, *Aspergillus*, *Epicoccum*, and *Penicillium*. They can affect the establishment and multiplication of cultures, and may cause significant economic losses (Abass, 2017; Esmaiel and Abdel-Galeil, 2026). Some of the contaminating fungi can release secondary metabolites and extracellular enzymes which also lead to tissue damage and inhibition of growth. Thus, the use of efficient and reliable strategies to control fungal contamination is crucial to improve the efficiency and economics of date palm micropropagation.

In plant tissue culture, microbial contamination can be suppressed by chemical fungicides and certain antimicrobial agents. But their efficacy might be restricted by negative effects on plant growth and physiology, phytotoxicity, and the possible emergence of antimicrobial resistance when used repeatedly. Their injudicious or unnecessary applications could also lead to issues associated with environmental and occupational safety. These restrictions have encouraged alternative production methods that are biologically derived, sustainable and ecofriendly, such as plant based compounds and value added products from agricultural by-products (Abass et al., 2016; Al-Sawad, 2025). The use of such materials can have a dual advantage not only by reducing agricultural wastes, but also by offering a pool of naturally-occurring bioactive compounds for the management plant diseases.

Seeds of date palm, generally known as date pits, are one of the most abundant by-products that are produced in the process of date processing and consumption. Although date pits are often discarded or utilized for low value applications, yet they are a rich source of a variety of phytochemicals, such as fatty acids, flavonoids, phenolic compounds, and other potentially bioactive components. Depending on extraction method and solvent employed, these compounds have antioxidant and antimicrobial properties. Extraction through the use of alcoholic solvents can promote the release of various moderately polar and relatively polar constituents that make the date pit extracts good candidates to study natural antifungal activity. Furthermore, due to variations in the genetic background, fruit traits, and other biological and environmental factors, the phytochemical profile of date pits can differ among cultivars. This variation can therefore affect the antifungal activity of extracts derived from different varieties of date palm.

Gas chromatography–mass spectrometry (GC–MS) is an efficient analytical method for the characterization of volatile and semi-volatile compounds found in plant extracts and for the comparison of their chemical compositions. This method, when used in conjunction with antifungal bioassays, can assist in the identification of compounds which might be correlated with differences in inhibitory activity among cultivars. The presence of a compound in an extract, however, does not necessarily mean that the compound is responsible for fungicidal activity and further testing might be necessary to prove the activity of each compound in the extract and to explain how each compound works. Chemical characterization coupled with biological evaluation thus could be used to identify promising cultivars of date palm for the preparation of plant-based antifungals.

Although there is a great interest in the antimicrobial properties of botanicals and agricultural by-products, the antifungal activities of alcoholic date pit extracts obtained from Barhi, Khadrawi, Maktoom and Shukr cultivars against *Fusarium oxysporum* and *Alternaria alternata* are not fully characterized. These fungi are of significance in plant health and can occur as contaminants in tissue cultures but their presence and effects are dependent on the tissue culture environment and production. Comparative assessment of these extracts may be useful in determining if

differences in chemical composition among cultivars are associated with differences in fungal growth inhibition and find candidate cultivars for further evaluation to be used as alternatives or as supplements to conventional fungicides.

Thus, the present study was undertaken to investigate the antifungal activities of alcoholic extracts of date pits of cultivars Barhi, Khadrawi, Maktoom and Shukr against *F. oxysporum* and *A. alternata* and to characterize the chemical constituents of alcoholic extracts of date pits by GC–MS. This study aimed to determine chemical profiles and inhibitory activities of four cultivars, and search for active extracts with potential antifungal properties, and to evaluate the possibilities of valorizing date palm pits as a natural source of bioactive substances for possible use in plant tissue culture and sustainable fungal contamination management.

Materials and methods

Collection of fruit samples

Fully ripe, undamaged dates of four varieties viz., Barhi, Khadrawi, Maktoom, and Shukr were collected from palm groves in district Al-Madina, north of city Basrah in Southern Iraq. The date seeds were removed from the fruit and thoroughly washed with running tap water, followed by sterile distilled water to remove any remaining fruit pulp and dissolved sugars, thereby eliminating any carbon source outside the pits that might interfere with biological tests to inhibit fungal growth (Krakowska-Sieprawska et al., 2022). The pits were dried on sterile filter paper at laboratory temperature (25 ± 2°C) for 24 h and then dried in an electric oven at 40°C for 6 h. Finally, they were ground into a fine powder for extraction. The powder was stored in dark glass containers in a refrigerator until extraction (Bouaziz et al., 2010; Krakowska-Sieprawska et al., 2022).

Preparation of the alcoholic extract

The alcoholic extract of the seeds of the four date varieties was prepared by mixing 100 g of each powder with 500 mL of 70% ethyl alcohol (1:5 w/v) (Lohvina et al., 2022). The mixture was thoroughly stirred and left to stand in the laboratory for 24 h at 25–30°C. The mixture was then filtered using Whatman No. 1 filter paper followed by a rotary evaporator at 40°C and reduced pressure to concentrate the filtrate. The crude extracts were collected in opaque glass bottles and stored in a refrigerator at 4°C until use (Elmastas et al., 2015).

Phytochemical profiling of seed extracts from four date palm cultivars by GC–MS

GC–MS analysis was carried out at the Basrah Oil Company Laboratory, by using an Agilent Technologies 7890B GC system coupled to an Agilent Technologies 5977A mass-selective detector (MSD) with electron ionization (EI) signal detector. Chromatographic separation was achieved using an HP-5ms capillary column (5% phenyl–95% methyl polysiloxane; 30 m × 250 µm × 0.25 µm film thickness). The oven temperature program was initiated at 40°C held for 5 min, then increased at a rate of 8°C/min to a final temperature of 300°C, which was held for 20 min. Helium was used as the carrier gas at a constant flow rate of 1 mL/min, with a purge flow of 3 mL/min. Sample injection (0.5 µL) was performed in pulsed splitless mode at an injector temperature of 290°C. The ion source temperature was set to 230°C, mass scan speed was 1,562 u/s (N = 2) and mass scan range was set to 44–750 m/z. All the obtained mass spectra were compared with the mass spectral libraries of NIST 2014 and NIST 2020 in accordance with the standard procedure (National Institute of Standards and Technology, 2020) for library-based identification of compounds in the GC–MS analysis of plant extracts (Krishnamoorthy and Subramaniam, 2014).

Testing the inhibitory efficacy of alcoholic extracts of date pits against *F. oxysporum* and *A. alternate*

Two fungal isolates *F. oxysporum* and *A. alternata*, known contaminants of tissue cultures, which were previously identified morphologically and molecularly were obtained from the Date Palm Research Center, University of Basrah. For each of the four date palm cultivars, 10 g of dry date seeds extract was suspended in 100 mL of sterile distilled water to prepare concentrated stock solutions; homogenized by sonication in an ultrasonic water bath for 15 min. The date palm seed extracts were tested for their antifungal activity using poisoned food technique (Grover and Moore, 1962). Sterile potato dextrose agar (PDA) medium was cooled to 45–50°C, and 5, 10, 15 mL aliquots of the stock suspension were aseptically mixed with 95, 90, and 85 mL of PDA media, respectively. In order to get the final concentrations of 5, 10 and 15 mg/mL respectively, all the mixtures were homogenized thoroughly. A negative control, the PDA medium, without extract was included. Mycelial discs measuring 5 mm in diameter were cut from the active margins of 7-day-old fungal cultures of *F. oxysporum* and *A. alternata*

and were inoculated at the center of each plate. The experiment followed a Completely Randomized Design (CRD) with three replicates. The plates were incubated at 25 ± 2°C. When the fungal mycelium in the control plates reached the edges of the dish, radial growth was measured along two perpendicular diameters, and percentage inhibition was calculated using the equation described by Vincent (1947):

$$\text{Inhibition (\%)} = \frac{R1 - R2}{R1} \times 100$$

Where: R1 = Diametric growth of fungal colonies in the control Petri dishes and R2 = Diametric growth of fungal colonies in treatment dishes.

Statistical analysis

A three-factor factorial experiment (4 × 3 × 2) was conducted under a completely randomized design (CRD) with three replicates (N = 72), following standard procedures for factorial agricultural experiments (Gomez and Gomez, 1984). The factors evaluated were date seed cultivar (Barhi, Khadrawi, Maktoom, and Shukr), extract concentration (5, 10, and 15 mg/mL), and fungal species *F. oxysporum* and *A. alternata*. The untreated control was excluded from the variance. Percentage inhibition data were arcsine-transformed according to the formula:

$$\theta = \arcsin \frac{\sqrt{(\text{inhibition\%}}}{100} \times \frac{180}{\pi}$$

to meet the assumptions of normality and homogeneity of variance required for ANOVA (Snedecor and Cochran, 1989), using R software (version 4.3.2; R Core Team, 2024) with the agricolae package (de Mendiburu, 2023). Means for significant main effects and interactions were compared using the Least Significant Difference (LSD) test (Gomez and Gomez, 1984). Back-transformed means were presented in the results for biological interpretation.

Results and discussion

GC-MS chemical profiling of four date palm cultivars

A total of 131 compounds were detected from alcoholic seed extracts of four cultivars of date palm (Barhi, Khadrawi, Maktoom and Shukr) using GC–MS. There were significant differences in the number of identified compounds and their total matched peak-area percentages among the tested cultivars (Table 1). The numbers of identified compounds were the highest (52) in Barhi with 34.67% of the matched peak area followed by Khadrawi, Shukr and Maktoom yielding 32, 29 and 18 compounds accounting for 49.90%, 61.67% and 46.75% of the total matched peak areas respectively. The total ion chromatograms (TICs) and the list of compounds are included in the Supplementary Information (Figures 1, 2, 3, 4 and Tables 2, 3, 4, 5).

Table 1: Number of GC-MS-identified compounds and cumulative matched peak area (Area%) of each date palm cultivar.

Cultivar (Sample)	Compounds Identified	Sum of matched area (%)
Barhi	52	34.67
Khadrawi	32	49.90
Maktoom	18	46.75
Shukr	29	61.67

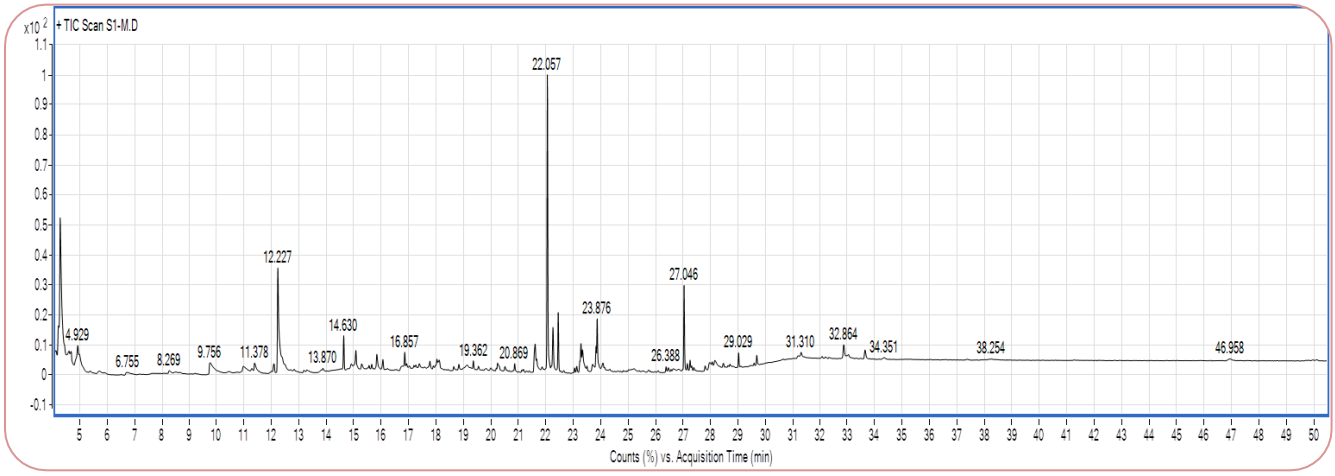


Figure 1. Total ion chromatogram of the alcoholic pit extract of Barhi cultivar.

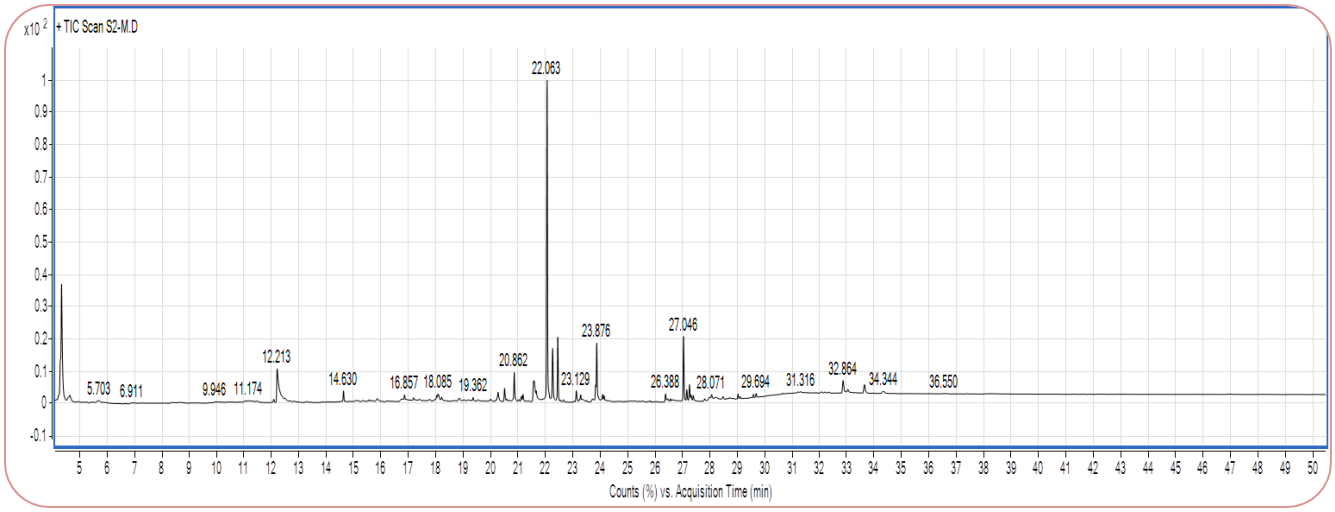


Figure 2. Total ion chromatogram of the alcoholic pit extract of Khadrawi cultivar.

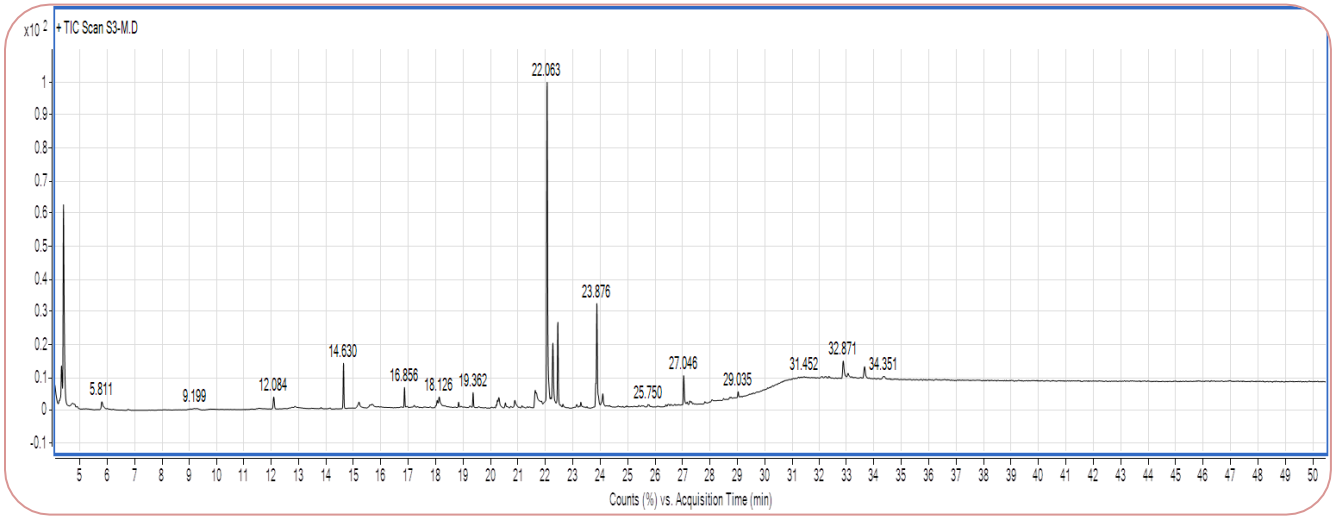


Figure 3. Total ion chromatogram of the alcoholic pit extract of Maktoom cultivar.

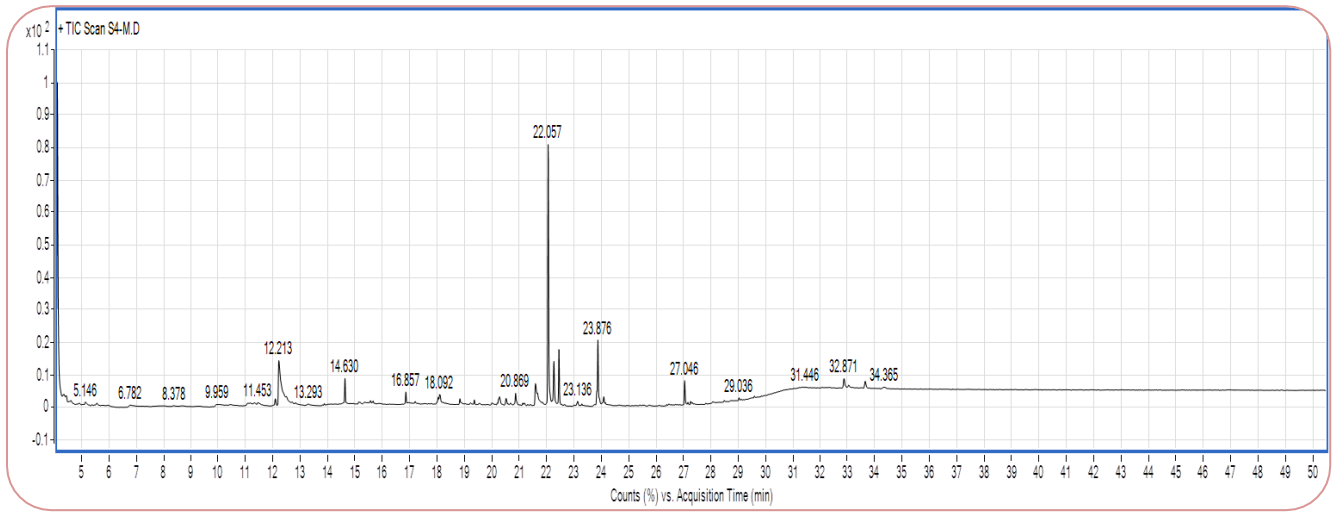


Figure 4. Total ion chromatogram of the alcoholic pit extract of Shukr cultivar.

Table 2. GC-MS compound list for the alcoholic seed extract of Barhi cultivar.

N.	PK #	RT (min)	Area (%)	Compound	Functional group(s)
1	2	4.229	0.49	Methyl-methoxy-hydroxymethyl-amine	Amine / ether / alcohol
2	4	4.451	0.83	2-Propenoic acid, ethenyl ester	Ester (unsaturated)
3	8	4.997	0.93	Glycine, N-methyl-N-methoxycarbonyl-, nonyl ester	Ester / carbamate
4	10	5.684	0.31	Oxime-, methoxy-phenyl-_	Oxime / aryl ether
5	11	5.935	0.31	Maleic anhydride	Anhydride
6	12	6.757	0.81	Methylenecyclopropanecarboxylic acid	Carboxylic acid
7	13	7.626	0.45	4-Cyclopentene-1,3-dione	Ketone (diketone)
8	14	8.272	0.53	Oxime-, methoxy-phenyl-_	Oxime / aryl ether
9	16	9.758	2.83	Propanoic acid, anhydride	Anhydride
10	18	10.972	1.25	Furaneol	Furanone (cyclic enol/ketone)
11	19	11.288	0.27	Phenol, 2-methoxy-	Phenol / ether
12	20	11.385	0.96	Propanoic acid, anhydride	Anhydride
13	21	11.999	0.18	5-Oxotetrahydrofuran-2-carboxylic acid, ethyl ester	Ester / lactone
14	25	12.494	0.18	Benzenamine, 2,4-dimethyl-	Aromatic amine
15	26	13.179	0.22	2-Butanamine, 2-methyl-	Amine
16	29	14.907	0.44	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	Hydrocarbon (diene)
17	30	15.074	1.51	Terpinyl formate	Ester
18	31	15.287	0.39	Phenol, 2-methoxy-4-(1-propenyl)-, (Z)-	Phenol / ether
19	32	15.555	0.19	1R,2c,3t,4t-Tetramethyl-cyclohexane	Hydrocarbon (alkane)
20	33	15.839	2.28	Methyleugenol	Aromatic ether
21	34	16.060	0.47	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4Z,9S*)]-	Hydrocarbon (sesquiterpene alkene)
22	36	17.262	0.19	Dodecanoic acid, methyl ester	Ester
23	37	17.381	0.29	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-	Aromatic ether (methylenedioxy)
24	38	17.774	0.37	Nerolidol 2	Alcohol (terpenoid)
25	39	17.898	0.19	8-Acetoxycarvotanacetone	Ester / ketone
26	40	18.032	0.50	1-Dodecanol	Alcohol
27	41	18.114	0.24	Undecane, 5,5-dimethyl-	Hydrocarbon (alkane)
28	42	19.058	0.31	.beta.-l-Arabinopyranoside, methyl	Glycoside (acetal) / polyol
29	43	19.105	0.19	Propane, 2-methoxy-2-methyl-	Ether
30	45	20.241	0.41	1-Undecanol	Alcohol
31	47	20.867	0.29	Sebacic acid, di(2-hexyl) ester	Ester (diester)
32	49	21.615	0.70	Hexadecanoic acid, methyl ester	Ester
33	53	22.267	1.17	Hexadecanoic acid, ethyl ester	Ester
34	55	23.129	0.24	1-Dodecanol	Alcohol
35	56	23.237	0.23	Chloroacetic acid, dodec-9-ynyl ester	Ester / alkyl halide / alkyne
36	57	23.283	1.34	cis-12-Octadecenoic acid methyl ester	Ester (unsaturated)
37	58	23.339	1.67	Dodecanoic acid, 2,3-dihydroxypropyl ester	Ester / diol (monoacylglycerol)
38	59	23.712	0.42	1-Nonylcycloheptane	Hydrocarbon (alkane)
39	60	23.833	0.76	Linoleic acid ethyl ester	Ester (unsaturated)
40	61	23.873	2.62	(E)-9-Octadecenoic acid ethyl ester	Ester (unsaturated)
41	62	24.068	0.31	Trans-1-methyl-2-nonyl-cyclohexane	Hydrocarbon (alkane)
42	63	25.220	0.18	Tetradecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Ester / diol (monoacylglycerol)
43	65	26.641	0.27	1H-Indene, 1-hexadecyl-2,3-dihydro-	Hydrocarbon (polycyclic)
44	67	27.163	0.26	1H-Indole, 5-methyl-2-phenyl-	Heterocyclic amine (indole)

45	68	27.262	0.46	1-Propene, 3-(2-cyclopentenyl)-2-methyl-1,1-diphenyl-	Hydrocarbon (alkene)
46	69	27.973	0.63	3-Indol-1-yl-propionic acid, methyl ester	Ester / heterocyclic amine (indole)
47	70	28.168	0.67	Bicyclo[5.2.1]decan-10-one	Ketone
48	71	29.031	0.49	3,7-Dimethyl-1-phenylsulfonyl-2,6-octadiene	Sulfone
49	72	29.695	0.39	(9Z,12Z)-(E)-3,7-Dimethylocta-2,6-dien-1-yl octadeca-9,12-dienoate	Ester (unsaturated)
50	74	31.313	0.42	Rac-glycerol-1,3-dilaurate	Ester (diacylglycerol)
51	75	32.871	1.58	.gamma.-Sitosterol	Alcohol (sterol)
52	77	33.648	1.05	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]-	Hydrocarbon (sesquiterpene)

N = (52 compounds); cumulative matched peak area = 34.67%.

Table 3. Complete GC-MS compound list for the alcoholic seed extract of Khadrawi cultivar.

N.	PK #	RT (min)	Area (%)	Compound	Functional group(s)
1	2	4.293	0.78	Bis(N-methoxy-N-methylamino)methane	Amine / ether (aminal)
2	3	4.341	16.16	4,6-Heptadiyn-3-one	Ketone / alkyne
3	5	4.923	0.31	Hexa-1,3,5-triyne	Alkyne (hydrocarbon)
4	6	5.239	0.36	Propanedioic acid, amino-, diethyl ester	Ester / amine
5	7	5.704	0.63	Oxime-, methoxy-phenyl-_	Oxime / aryl ether
6	8	6.889	0.32	Methylenecyclopropanecarboxylic acid	Carboxylic acid
7	9	8.370	0.45	Oxime-, methoxy-phenyl-_	Oxime / aryl ether
8	10	9.985	0.77	Benzeneacetic acid, 1,1-dimethylethyl ester	Ester (aromatic)
9	11	11.046	0.40	2,4,5-Trihydroxypyrimidine	Heterocyclic amine / polyol
10	13	11.452	0.25	Propanoic acid, anhydride	Anhydride
11	15	12.212	7.84	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP)	Ketone / cyclic enol (pyranone)
12	18	15.563	0.26	1R,2c,3t,4t-Tetramethyl-cyclohexane	Hydrocarbon (alkane)
13	19	15.861	0.34	Methyleugenol	Aromatic ether
14	22	18.034	0.46	1-Undecanol	Alcohol
15	23	18.084	0.58	Dodecanoic acid, ethyl ester	Ester
16	27	20.274	0.59	Tetradecanoic acid, ethyl ester	Ester
17	29	20.863	2.26	Sebacic acid, di(2-hexyl) ester	Ester (diester)
18	31	21.182	0.57	1-Dodecanol	Alcohol
19	34	21.617	0.33	Hexadecanoic acid, methyl ester	Ester
20	38	22.268	2.80	Hexadecanoic acid, ethyl ester	Ester
21	40	23.130	0.86	1-Hexadecanol	Alcohol
22	41	23.285	0.47	cis-12-Octadecenoic acid methyl ester	Ester (unsaturated)
23	42	23.834	0.68	Linoleic acid ethyl ester	Ester (unsaturated)
24	43	23.874	4.82	(E)-9-Octadecenoic acid ethyl ester	Ester (unsaturated)
25	44	24.091	0.48	Octadecanoic acid, ethyl ester	Ester
26	45	24.146	0.37	1-Tricosene	Hydrocarbon (alkene)
27	47	26.676	0.23	1H-Indole-3-acetic acid, ethyl ester	Ester / heterocyclic amine (indole)
28	50	27.258	1.28	1-Propene, 3-(2-cyclopentenyl)-2-methyl-1,1-diphenyl-	Hydrocarbon (alkene)
29	55	29.030	0.25	Supraene	Hydrocarbon (triterpene)
30	56	29.695	0.26	2,6-Octadien-1-ol, 3,7-dimethyl-, propanoate, (Z)-	Ester
31	57	32.866	1.71	.gamma.-Sitosterol	Alcohol (sterol)
32	58	33.647	2.03	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	Alcohol (triterpenoid)

N = (32 compounds); cumulative matched peak area = 49.90%.

Table 4. Complete GC-MS compound list for the alcoholic pit extract of Maktoom cultivar.

N.	PK#	RT (min)	Area (%)	Compound	Functional group(s)
1	2	4.339	3.45	4,6-Heptadiyn-3-one	Ketone / alkyne
2	3	4.413	18.56	4,6-Heptadiyn-3-one	Ketone / alkyne
3	4	4.588	0.34	2-Propenoic acid	Carboxylic acid
4	6	5.814	1.03	Oxime-, methoxy-phenyl-_	Oxime / aryl ether
5	10	12.863	1.40	l-Norvaline, N-ethoxycarbonyl-, dodecyl ester	Ester / carbamate
6	13	15.199	0.79	Benzene, 2-(1,3-butadienyl)-1,3,5-trimethyl-	Hydrocarbon (aromatic/diene)
7	16	18.042	0.82	(S)-(+)-6-Methyl-1-octanol	Alcohol
8	17	18.125	0.98	Butanoic acid, 2-methyl-, methyl ester	Ester
9	19	20.251	0.93	1-Dodecanol	Alcohol
10	20	20.294	0.54	Tetradecanoic acid, ethyl ester	Ester
11	22	20.885	0.52	Sebacic acid, di(3-hexyl) ester	Ester (diester)
12	27	22.276	3.73	Hexadecanoic acid, ethyl ester	Ester
13	29	23.840	0.77	Linoleic acid ethyl ester	Ester (unsaturated)
14	30	23.879	8.55	(E)-9-Octadecenoic acid ethyl ester	Ester (unsaturated)
15	31	24.095	0.75	Octadecanoic acid, ethyl ester	Ester
16	34	29.036	0.26	3,7-Dimethyl-1-phenylsulfonyl-2,6-octadiene	Sulfone
17	37	32.875	1.62	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	Alcohol (sterol)
18	38	33.654	1.71	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-9-(phenylsulfonyl)-, (E,E)-	Sulfone / alcohol

N= (18 compounds); cumulative matched peak area = 46.75%.

Table 5. Complete GC-MS compound list for the alcoholic pit extract of Shukr cultivar.

N.	PK#	RT (min)	Area (%)	Compound	Functional group(s)
1	1	4.122	24.60	4,6-Heptadiyn-3-one	Ketone / alkyne
2	2	4.172	0.40	2-Propenoic acid, ethenyl ester	Ester (unsaturated)
3	3	4.237	0.65	Propane, 1,1-dimethoxy-	Acetal / ether
4	6	4.619	0.28	Oxime-, methoxy-phenyl-_	Oxime / aryl ether
5	8	5.145	0.69	Glycine, N-methyl-N-methoxycarbonyl-, nonyl ester	Ester / carbamate
6	9	5.573	0.37	Oxime-, methoxy-phenyl-_	Oxime / aryl ether
7	10	5.989	0.93	Maleic anhydride	Anhydride
8	11	6.808	0.88	2-Furanmethanol (Furfuryl alcohol)	Alcohol (furan)
9	12	8.365	0.57	4-Ethylbenzoic acid, tridec-2-ynyl ester	Ester / alkyne
10	13	10.464	0.37	Benzeneacetaldehyde	Aldehyde (aromatic)
11	14	11.325	0.37	2-Acetyl-5-methylfuran	Ketone (furan)
12	15	11.456	0.43	Propanoic acid, anhydride	Anhydride
13	17	12.216	12.01	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP)	Ketone / cyclic enol (pyranone)
14	18	12.494	0.27	Benzenamine, 2,4-dimethyl-	Aromatic amine
15	21	15.183	0.53	Naphthalene, 1,2-dihydro-4,5,7-trimethyl-	Hydrocarbon (aromatic, dihydronaphthalene)
16	22	15.362	0.43	2,5-Pyrrolidinedione, 3-(1-aminoethylidene)-4-methyl-	Imide / enamine
17	23	15.567	0.37	1R,2c,3t,4t-Tetramethyl-cyclohexane	Hydrocarbon (alkane)
18	26	18.038	0.63	1-Dodecanol	Alcohol
19	27	18.091	0.65	Dodecanoic acid, ethyl ester	Ester
20	28	18.119	0.60	Decane, 3,8-dimethyl-	Hydrocarbon (alkane)
21	30	20.245	0.53	1-Dodecanol	Alcohol
22	31	20.280	0.39	Tetradecanoic acid, ethyl ester	Ester
23	33	20.869	0.76	Sebacic acid, di(2-hexyl) ester	Ester (diester)
24	37	22.271	2.38	Hexadecanoic acid, ethyl ester (Ethyl palmitate)	Ester
25	39	23.839	0.28	Linoleic acid, ethyl ester	Ester (unsaturated)
26	40	23.876	5.33	(E)-9-Octadecenoic acid ethyl ester	Ester (unsaturated)
27	41	24.093	0.42	Octadecanoic acid, ethyl ester	Ester
28	44	32.876	1.45	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	Alcohol (sterol)
29	45	33.649	4.10	1-Formyl-2,2,6-trimethyl-3-cis-(3-methylbut-2-enyl)-5-cyclohexene	Aldehyde (terpenoid)

N = (29 compounds); cumulative matched peak area = 61.67%.

Six compounds were common to all four varieties. These included: (E)-9-Octadecenoic acid ethyl ester, hexadecanoic acid ethyl ester, linoleic acid ethyl ester, sebacic acid di (2-hexyl) ester, 1-dodecanol, and methoxyphenyl oxime (Table 6). (E)-9-Octadecenoic acid ethyl ester was the most abundant common compound, comprising 2.62% in the Barhi variety and 8.55% in the Maktoom variety.

All identified compounds were classified into 14 chemical classes. The composition and relative abundance of these classes varied considerably across the four cultivars. The most abundant chemical class across all varieties consists of fatty acids and their ethyl esters, followed by hydrocarbons and terpenoids, while nitrogen-containing compounds and phenolic compounds were present in moderate proportions. Phytosterols, sugars/glycosides, carboxylic acids/anhydrides, lactones, furanones, and other compounds were present in lower concentrations and showed greater variation among the varieties. Terpinol was found solely in Barhi with 1.51% of matched peak area. Phytosterols like triterpenoids and γ-sitosterol such as 9,19-cyclolanost-24-en-3-ol were other compounds of interest. These differences suggest that there are significant variations in the chemical compositions of the four cultivars. It is the most abundant in the Khadrawi cultivar, at a total of 3.74%, whereas the furanone/lactone compound DDMP (4H-Pyran-4-one, 2,3-dihydro-3,5- dihydroxy-6- methyl-) was among the individual compounds with the 3, 5 highest relative abundance, reaching 12.01% in the Shukr variety and 7.84% in the Khadrawi variety, while it is absent in the Barhi and Maktoom varieties.

Table 6. Core compounds common to all four cultivars.

Compound	Formula	Barhi	Khadrawi	Maktoom	Sukkari
(E)-9-Octadecenoic acid, ethyl ester (Ethyl oleate)	C20H38O2	2.62	4.82	8.55	5.33
Hexadecanoic acid, ethyl ester (Ethyl palmitate)	C18H36O2	1.17	2.80	3.73	2.38
Linoleic acid, ethyl ester	C20H36O2	0.76	0.68	0.77	0.28
Sebacic acid, di(2-hexyl) ester*	C22H42O4	0.29	2.26	0.52	0.76
1-Dodecanol	C12H26O	0.74	0.57	0.93	1.16
methoxy-phenyl oxime	C8H9NO2	0.84	1.08	1.03	0.65

Note: In Maktoom cultivar, sebacic acid, di(2-hexyl) ester was identified as the ester structural isomer. Values represent Area% from GC-MS analysis.

Analysis of variance for mycelial growth inhibition

The three-way ANOVA performed on the arcsine-transformed inhibition data showed that the main effects of cultivar, concentrations, and fungal species were all highly significant ($P < 0.01$) (Table 7). The two-way interactions, cultivar × concentration, and cultivar × fungal species were also highly significant ($P < 0.01$), as was the three-way interaction cultivar × concentration × cungal species ($P = 0.0003$). The only non-significant source of variation was the concentration × fungal species interaction ($P = 0.471$), indicating that the pattern of response to increasing extract concentration was statistically similar for both fungal species when the cultivar was not considered. The low coefficient of variation (CV = 3.89%) indicates a high degree of experimental precision.

Effect of cultivar, concentration, and fungal species on inhibition

Inhibition of mean mycelial growth was significantly different among the four cultivars when averaged across all extract concentrations and fungal species ($LSD_{0.01}$ = 1.410) (Table 8) Maktoom showed significantly the highest mean inhibition of 65.28% compared to Shukr, Khadrawi, and Barhi which showed mean inhibitions of 39.12%, 35.65%, and 29.80% respectively. There were significant differences among all the four cultivars.

Inhibition of mycelial growth increased significantly with extract concentration ($LSD_{0.01}$ = 1.221), growing from 31.06% at 5 mg/mL to 41.83% at 10 mg/mL and 54.50% at 15 mg/mL. The difference in susceptibility was also highly significant between the two fungal species ($LSD_{0.01}$ = 0.997), with *A. alternata* showing higher mean inhibition of 45.29% than that of *F. oxysporum* which showed an inhibition of 39.64%.

Cultivar × concentration × fungal species interaction

The significant three-way interaction ($P = 0.0003$) indicated that the effects of concentration and cultivar on inhibition were not identical for the two fungal species. Table 9 and Figure 5 present the means (original percentage scale) for all 24 treatment combinations, with $LSD_{0.01}$ = 3.454 (angular scale) for pairwise

comparison among any two of the 24 combination means. The greatest inhibition was observed for the Maktoom cultivar at 15 mg/mL against both *F. oxysporum* (80.37%) and *A. alternata* (77.60%), which did not differ significantly from each other but were significantly higher than all other combinations (Figure 6). The lowest inhibition was observed for the Barhi at 5 mg/mL against *F. oxysporum* (16.60%). This indicates that the effect of concentration on inhibition depended on both the fungal species and the cultivar.

Relationship between fatty acid ethyl ester content and inhibition

The mean mycelial growth inhibition (Maktoom > Shukr > Khadrawi > Barhi) was found to be the same as the rank order of the matched peak-area percentage of the most abundant common fatty acid ethyl ester (Table 10). This pattern was observed in comparison to overall chemical richness: Barhi was found the richest in terms of number of identified compounds (52) and showed the lowest mean antifungal activity, while Maktoom had the lowest number of identified compounds (18) but the highest mean activity. The highest individual matched peak-area percentage was found for compound DDMP in Shukr (12.01%) and Khadrawi (7.84%) while this compound was not detected in Maktoom. The abundance of DDMP was not the only factor accounting for the antifungal activity as it was not present in the most active cultivar.

Table 7. ANOVA for the arcsine-transformed mycelial growth inhibition rate (%) as affected by cultivar, extract concentration, and fungal species.

Source of Variation	SS	df	MS	F-value	P-value	Sig. (0.01)
Cultivar (A)	4801.184	3	1600.395	643.57	<0.0001	**
Concentration (B)	2448.862	2	1224.431	492.39	<0.0001	**
Fungal species (C)	218.114	1	218.114	87.71	<0.0001	**
A × B	69.251	6	11.542	4.64	0.0009	**
A × C	213.124	3	71.041	28.57	<0.0001	**
B × C	3.806	2	1.903	0.77	0.4708	ns
A × B × C	80.037	6	13.339	5.36	0.0003	**
Error	119.363	48	2.487			
Total	7953.741	71				

** Significant at $P < 0.01$; ns = not significant at $P > 0.01$. SS = sum of squares; df = degrees of freedom; MS = mean square; CV = 3.89%. $LSD_{0.01}$ (angular scale, df = 48, MSE = 2.487): Cultivar = 1.410; Concentration = 1.221; Fungal species = 0.997; Cultivar × Concentration × Fungal species = 3.454.

Table 8. Mean mycelial growth inhibition (%) for the main effects of cultivar, extract concentration, and fungal species (angular and original percentage scales).

Factor	Level	Mean (angular, °)	Mean (original, %)	LSD grouping (0.01)
Cultivar	Maktoum	54.19	65.28	a
	Shukr	38.54	39.12	b
	Khadrawi	36.52	35.65	c
	Barhi	32.77	29.80	d
Concentration	15 mg/mL	47.80	54.50	a
	10 mg/mL	40.18	41.83	b
	5 mg/mL	33.53	31.06	c
Fungal species	*A. alternata*	42.24	45.29	a
	F. oxysporum	38.76	39.64	b

Means within a factor followed by different letters differ significantly at P < 0.01 (LSD test). LSD0.01 (angular scale): Cultivar = 1.410, Concentration = 1.221, Fungal species = 0.997.

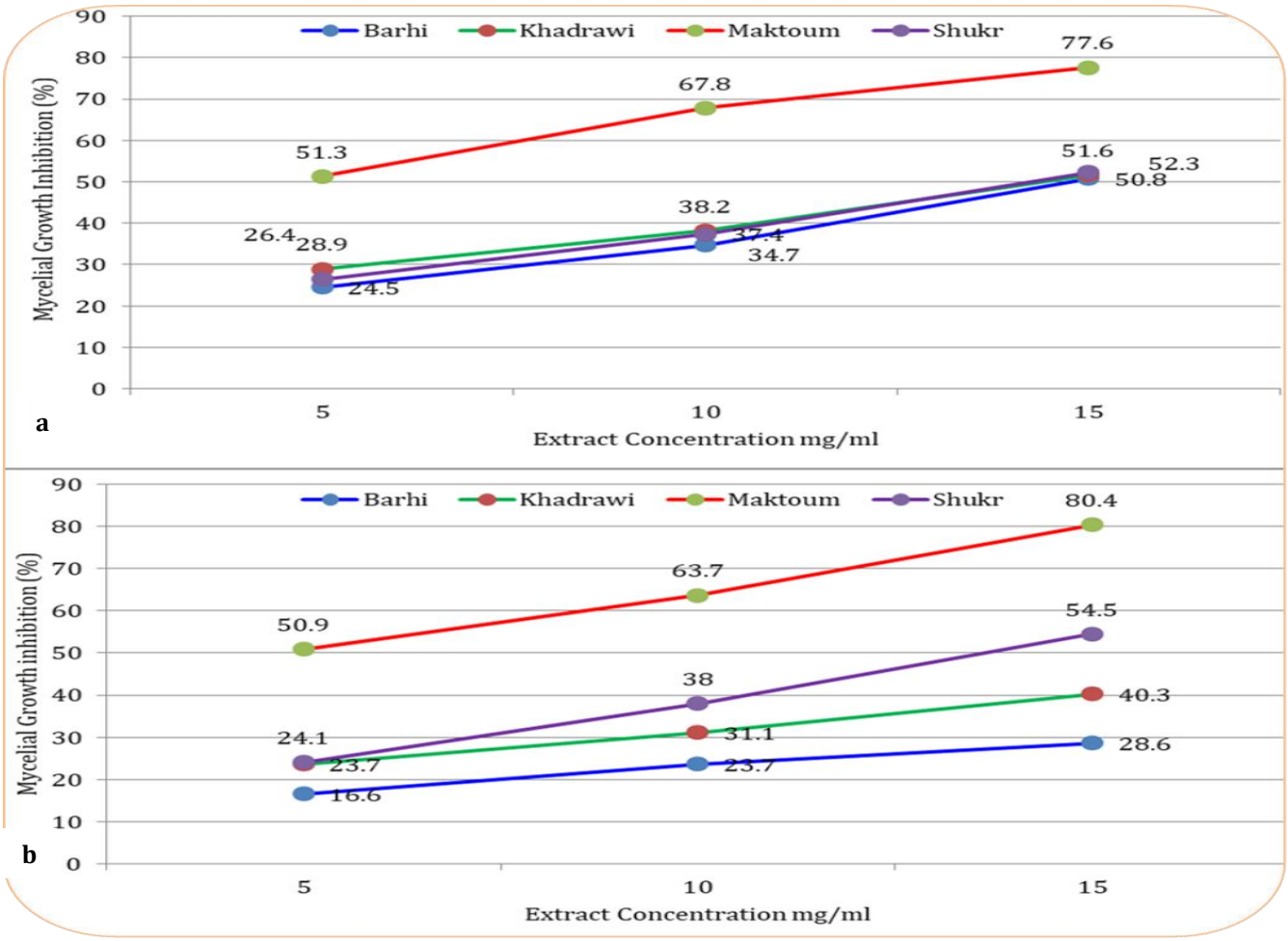


Figure 5. Cultivar × concentration ×fungal species interaction on mycelial growth inhibition (a) *A. alternate* (b) *F. oxysporum.* $LSD_{0.01}$ (angular scale) = 3.454 for comparing any two of the 24 mean values.

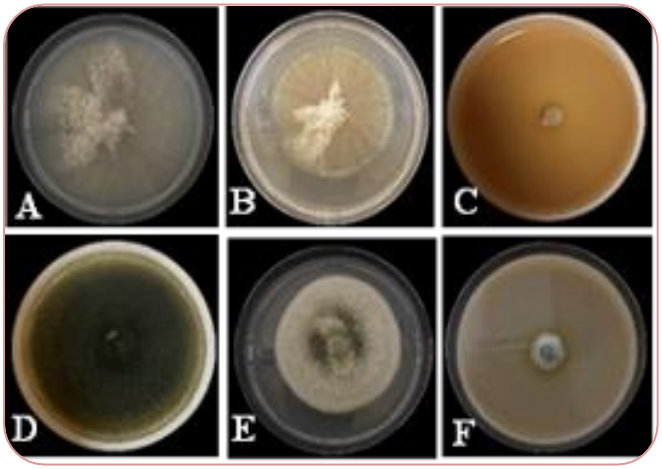


Figure 6. Plates showing mycelial growth inhibition of *Alternaria alternata* (A-C) and *Fusarium oxysporum* (D-F) by Phoenix dactylifera seed extracts. (A and D) Untreated control; (B and E) Maktoom extract at 15mg/mL; (C andF) Barhi extract at 15mg/mL.

Cultivars were ranked from highest to lowest mean mycelial growth inhibition. Ethyl oleate followed the same rank order of inhibition across the four cultivars. Ethyl palmitate showed slightly different pattern in Khadrawi exhibiting a higher abundance than Shukr.

The results of GC–MS showed significant variations in the chemical profiles of alcoholic extracts of the seeds of the four cultivars of date palm. Regardless of these variations, fatty acid ethyl esters, in particular, ethyl palmitate, ethyl oleate, and linoleic acid ethyl ester, were the most prominent compounds identified across the cultivars. This finding is in line with the previous studies that stated that date palm seed lipids contain rich amounts of oleic acid and C12–C18 fatty acids (Ibourki et al., 2021). The differences in number and relative richness of the identified compounds, where Barhi showed the highest number of phytochemicals and Maktoom the lowest, further underscores the cultivar-

dependent variations in the phytochemical profiles of seeds. Similar differences have also been previously reported in the chemical compositions of date palm seeds (Ismail et al., 2025).

Table 9. Mean mycelial growth inhibition (%) for the cultivar × concentration × fungal species interaction (original percentage scale).

Cultivar	Concentratio	Fungal species	Mean
Barhi	5 mg/mL	*A. alternata*	24.47
	5 mg/mL	*F. oxysporum*	16.60
	10 mg/mL	*A. alternata*	34.67
	10 mg/mL	*F. oxysporum*	23.67
	15 mg/mL	*A. alternata*	50.80
	15 mg/mL	*F. oxysporum*	28.60
Khadrawi	5 mg/mL	*A. alternata*	28.93
	5 mg/mL	*F. oxysporum*	23.70
	10 mg/mL	*A.a alternata*	38.23
	10 mg/mL	*F. oxysporum*	31.13
	15 mg/mL	*A. alternata*	51.57
	15 mg/mL	*F. oxysporum*	40.33
Maktoom	5 mg/mL	*A. alternata*	51.30
	5 mg/mL	*F. oxysporum*	50.93
	10 mg/mL	*A. alternata*	67.80
	10 mg/mL	*F. oxysporum*	63.70
	15 mg/mL	*A. alternata*	77.60
	15 mg/mL	*F. oxysporum*	80.37
Shukr	5 mg/mL	*A. alternata*	28.40
	5 mg/mL	*F. oxysporum*	24.13
	10 mg/mL	*A. alternata*	37.40
	10 mg/mL	*F. oxysporum*	38.00
	15 mg/mL	*A. alternata*	52.30
	15 mg/mL	*F. oxysporum*	54.47

$LSD_{0.01}$ (angular scale) = 3.454, for comparing any two of the 24 Cultivar × Concentration × Fungal species means.

One interesting result was the relationship between the ranking of the cultivars (Maktoum > Shukr > Khadrawi > Barhi) with respect to antifungal activity and the relative abundance of ethyl oleate in these cultivars. Among the four cultivars, maktoom showed the highest mean inhibition as well as the highest relative abundance of this compound. On the other hand, Barhi possessed the maximum number of identified compounds and the least antifungal activity. This pattern indicates that antifungal efficacy is related to the amount of specific bioactive compounds, their interactions, or the chemical makeup itself rather than by the amount of the identified compounds. The presence of ethyl esters of fatty acids such as palmitic and oleic acid derivatives has been reported in other plant and algal extracts and were found to have antifungal activity (Walters et al., 2004; Pourakbar et al., 2021), further substantiating their potential role in the present observed antifungal activity. The presence of these compounds in the GC–MS analysis, however, does not prove that these compounds have any individual antifungal activity or that they actually caused the observed inhibition.

Table 10. The relationship between fatty acid ethyl ester abundance and mycelial growth inhibition across the four cultivars was ranked from highest to lowest inhibition.

Cultivar	Mean inhibition (%)	Ethyl oleate (%)	Ethyl palmitate (%)
Maktoom	65.28	8.55	3.73
Shukr	39.12	5.33	2.38
Khadrawi	35.65	4.82	2.80
Barhi	29.80	2.62	1.17

A formal Pearson correlation analysis was not done as only four cultivars were used in the comparison; an assessment with four observations would have low statistical power to detect a correlation and might be greatly affected by the individual cultivars. Therefore, the association of abundance of ethyl oleate with antifungal activity must be viewed as a descriptive correlation, not as a statistical one. Bioassay-guided fractionation in conjunction with a larger sample of cultivars, and quantitative chemical profiling, would help to elucidate whether ethyl oleate or other components is directly responsible for antifungal activity (Tang et al., 2021).

The pattern of distribution of DDMP also indicated that the presence of a large major peak in the GC–MS chromatogram does not necessarily reflect antifungal potency. Even though DDMP showed the highest single peak in Shukr and Khadrawi, it was not found in the most active cultivar, Maktoom. This outcome contradicts

a direct correlation between abundance of DDMP and antifungal activity. However, without specific chemical analysis and direct bioassays, its potential contribution cannot be conclusively ruled out.

Antifungal activity was also dependent on the combination of these factors as shown by the significant three way interaction among cultivar, extract concentration and fungal species (P = 0.0003). Maktoom extract at 15 mg/mL showed the highest inhibition against both fungal species giving 77.50% against *A. alternata* and 80.37% against *F. oxysporum*. Based on these findings, it can be concluded that extracts from date palm seeds have potential as an antifungal agent in date palm micropropagation to control fungal contamination, specifically those extracted from Maktoom palm seeds. Nonetheless, the activity observed was determined under *in vitro* conditions, and should not be construed as proof of efficacy or safety in real tissue culture systems.

The highest level of inhibition detected in the present study should be interpreted with caution when compared to the efficacy of fungicides reported earlier. In previous studies, mancozeb, carbendazim and propiconazole have proven to be highly active antifungal agents in *in vitro* conditions (Taware et al., 2014; Kumari et al., 2021; Patel et al., 2022). The experimental conditions, however, differed from that of the present study and no commercial fungicide was used as a positive control. Therefore, it is not scientifically appropriate to compare the percentages of inhibition between the extracts of date palm seeds and these fungicides. However, the antifungal activity observed in this study warrants further study, especially regarding fungicide resistance and differences in the effectiveness of fungicides for fungal species (Hu and Chen, 2021; Corkley et al., 2022).

Conclusion

This study, demonstrated *in vitro* inhibition of *Alternaria alternata* and *Fusarium oxysporum* by alcoholic extracts of date palm seeds from four date palm cultivars (Barhi, Khadrawi, Maktoom, and Shukr). Cultivar extract concentration and fungal species significant affected mycelial growth inhibition and the three-way interaction was significant (P = 0.0003). Maktoom showed the highest mean inhibition across concentration and fungal species 65.28% while Barhi showed the lowest 29.80%. At 15 mg/mL Maktoom extract produced the greatest inhibition of 80.37% against *F. oxysporum* and 77.60% against *A. alternata*. The cultivar level inhibition ranking paralleled the ranking of ethyl oleate or other extract constituents. Compound specific assays or bioassay guided fractionation are needed to test their contributions. Maktoom seed extract is therefore a candidate for further investigation, its efficacy, phytotoxicity and compatibility should be evaluated under actual date palm micropropagation conditions before practical application is considered.

Future recommendations

Future studies should focus on the bioassay guided fractionation, quantification of potentially active compounds in the extracts and the determination of the total flavonoid and phenolic contents to elucidate the chemical basis of the observed antifungal effects. Additional experiments are also needed to evaluate phytotoxicity, effects on explant regeneration, and effectiveness in real date palm tissue culture. The use of suitable commercial fungicide controls and standardized assay conditions would assist in comparison with known antifungal drugs. Together these investigations would contribute towards practical application of date palm seed extracts as potential alternative or complementary agents in plant tissue culture system for the management of fungal contamination.

Authors' Contributions

MJK and LKA conceptualized and designed the study. MJK collected the samples and prepared alcoholic extracts from date palm seeds. LKA evaluated the antifungal activity of the extracts and drafted the manuscript. Both authors critically reviewed and approved the final version of the manuscript and agreed to take full responsibility for the accuracy, integrity, and content of the work.

Research Funding

This research did not receive any grant from funding agencies.

Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 9: Industry, Innovation and Infrastructure
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

Policy Addressed

The study supports sustainable plant health management, agricultural waste valorization, and green biotechnology through the potential use of date palm seed extracts for fungal contamination management in micropropagation.

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