Basrah Journal of Date Palm Research
Volume 23 Issue (1) 2024
BJDPR
ISSN: 1816-0379 online ISSN:3005-3722
مجلـــة البصـرة لأبحاث نخلـة التمـر
المجلد 23 العدد (1) 2024

Synthesis, Characterization of Coumarin-Chalcone Hybrid Compounds and Evaluation as Fungicidal Against Fungal Contamination of *In vitro* Culture of Date Palm *Phoenix dactylifera* L.

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Abstract

This study involved the synthesis and structural characterization of Coumarin-containing chalcone derivatives as a fungicide. Several spectroscopic techniques, including FTIR, 1H NMR, and 13C NMR, were used to confirm their structures. The coumarin-chalcone hybrid compounds were tested *in vitro* for their ability to reduce the main contaminating fungi that infest *in vitro* culture labs. According to the study's findings, the PDA medium supplemented with 1.5 and 2 mg/L concentrations of all synthesis of coumarin-chalcone derivatives showed no signs of contaminating fungi. While the concentration of 1.0 mg/L showed weak inhibition, compared to the growth of fungi in the control treatment (not treated with chalcone derivatives), in which the diameter growth of the contaminated fungi reached 9 cm (100%). In addition, it was found that, in comparison to the control, the cultures treated with chalcone derivatives over longer periods showed a trend toward decreased and delayed biomass production. Compared to controls, coumarin-chalcone hybrid treatments at 1.5 and 2.0 mg/L did not result in fungal contamination *in vitro* culture experiments. Furthermore, the result of the *in vitro* culture experiment of date palm found the multiplication rate of shoots enhanced in MS supplemented with coumarin-chalcone hybrids at a concentration of 1.5 mg/L increased considerably to 32.24%, compared with 12.33% in the control. The results of this investigation will offer practical recommendations for lowering or removing the risk of fungal contamination by applying Coumarin-Chalcone Hybrid as an environmentally friendly fungicide.

Keywords: Coumarin-Chalcon hybrid, Contamination fungal, Date palm, *In vitro* culture, anti-microbial.

Introduction

Iraq is one of the top ten countries in the world for date production, with the greatest forest of date palm trees (*Phoenix dactylifera* L.) before 1991 (Abass, 2013). A variety of techniques are used to propagate date palms, such as *in vitro* culture micropropagation, asexual propagation through offshoots, and sexual propagation through seeds (Aaouine 2003; Al-Khayri, 2007). By using micropropagation, it can produce a high number of plants, although contamination is the main issue with this method. The greatest difficulty faced by those who operate *in vitro* culture laboratories is fungal contamination, which causes increased turbidity, altered medium pH, and cell death (Hameed and Abass, 2006). Additionally, plant *in vitro* browning and degradation brought on by fungal toxins and enzymes have the most obvious adverse impacts on date palm *in vitro* cultures (Abass, 2013). As agriculture expands, various challenges related to crops growing have become increasingly apparent (Marianna and Octa´ vio, 2022). Therefore, it is crucial to explore new commercial pesticides that are inexpensive, environmentally friendly, highly effective, and low in toxicity (Jiang *et al*., 2020). Chalcones are bioactive plant metabolites that have several medical and biological advantages to human (Rudrapal *et al*., 2021). Natural chalcones are abundant in plants in the groups of Leguminosae, Asteraceae, and Moraceae (Wu *et al*., 2020). Chalcones and their derivatives possess oxidant enzymes (Bale *et al*., 2021; Zahrani *et al*., 2020) and demonstrate diverse biological activities against a broad spectrum of organisms, including fungi, bacteria, and viruses (Fu *et al*., 2020; Chen *et al*., 2020; Zhou *et al*., 2022). They can be utilized in the synthesis of biopesticides such as the registered fungicide Isobavachalcone, which is synthesized from the tonics of legumes (Guan *et al*., 2014). According to Wei *et al*. (2018) and Ding *et al*. (2018), indole is an aromatic heterocyclic alkaloid that is present in many mammals and marine organisms, in addition to natural plants like jasmine, oleander, citrus, croton root, and orange blossom (Liu *et al*., 2017; Lin and Tan, 2018). Both indoles and their derivatives have a variety of biological properties, including antibacterial and antiviral activities (Xie *et al*., 2020; Wang *et al*., 2021). Coumarin molecules is a combination of benzene and α-pyrone rings and are chemically poses with a group of phenolic compounds found in plants. Chromene, which is largely present as natural alkaloids, phenols, flavonoids, and anthocyanins, forms the structural core of many different types of polyphenols. Natural and synthetic chromene compounds are known to have significant biological activity (Doan and Tran, 2011; Lahsasni *et al*., 2014; Prasad *et al*., 2008). The synthesis of coumarin-chalcone hybrids has garnered attention due to their unique features and promising pharmacological properties including anticancer,

antimicrobial, antioxidant, and anti-inflammatory effects, therefore, researchers have successfully developed innovative methodologies to synthesize these compounds, which possess diverse biological activities and potential therapeutic applications (Xie *et al*., 2020; Wang *et al*., 2021; Zhang *et al*., 2013; Zahrani *et al*., 2020; Liu *et al*., 2016). Despite the notable advancements in this field, there are still areas of scientific research that warrant attention, such as the anti-microbial properties of coumarin-chalcone hybrids. Furthermore, the ongoing exploration of efficient and environmentally friendly synthetic approaches, coupled with the identification of novel targets for these compounds, is essential to enhance the therapeutic potential of coumarin-chalcone hybrids. Therefore, the synthesis of new hybrid molecules by hybridizing both coumarin and chalcone is required. In this study, new coumarin-chalcone hybrid compounds were prepared and evaluated for their activity against the important contamination fungi *in vitro* culture of date palms.

Materials and Methods

Chemical experiment

Coumarin-Chalcones derivatives synthesis was conducted in the laboratories of department of chemistry, University of Basrah, Basra, Iraq.

General procedures of synthesis of Coumarin-Chalcones derivatives

The method given by Smit *et al*. (2015) was followed for the procedure of synthesis of Coumarin-Chalcones derivatives, which included the following procedures:

Synthesis 3-Acetyl-2H-chromen-2-one (1)

A mixture of salicylaldehyde (3 mol), ethylacetoacetate (3 mol), and piperidine (0.3 mol) was prepared by stirring at room temperature for 4 hours. The reaction progress was monitored by TLC (Thin-layer chromatography) using n-hexane: ethyl acetate (4:6) v/v as the mobile phase, R_f = 0.47. The mixture was filtered, and the precipitated product was made by recrystallizing ethanol to produce a yellow crystalline solid (95%) with melting point 119-120˚C. IR (KBr)(cm^{-1}): 2925 (CH aliphatic), strong band at 1739 (C= O lactone carbonyl of coumarin); 1678 (C=O, acetyl); 1600 (C = C, aromatic); 1209 (C-O). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.697(s,3H), 7.33(m,H-6/8`),7.62(m,H-5`/7`),8.47(s,H-4`); ^{13}C-NMR ($CDCl_3$, 400 Hz) δ: 116.4(C8`), 195.5(C9`),130(C-5`), 30(CH_3) ppm. MS: m/z 188 (M^+, 80%), 145 (100%), 94 (50%).

General Preparation of Coumarin-Chalcone derivatives.

One gram of 3-acetylcoumarin in 4 ml of 30% sodium hydroxide solution, a solution of 1 ml of substituted aromatic aldehyde (H, 4-$NHCOCH_3$, 4-Cl and 4-OCH_3) in 5 ml ethanol was added.

The red solution was allowed to stand for 24 hours, then diluted to 100 ml with cold water and acidified with diluted hydrochloric acid. The precipitate so formed was filtered, dried and recrystallized from glacial acetic acid.

Preparation of (E)-3-(3-phenylacryloyl)-2H-chromen-2-one (2).

Yield 90%; m.p. 260-262˚C, light yellow color; FT-IR (Fourier-transform infrared spectroscopy) (υ^{-1}; cm^{-1}) 1728($\upsilon_{C=O}$, lactone of coumarin);1660 ($\upsilon_{C=O}$, unsaturated ketone)1610($\upsilon_{C=C,}$ ethylene group); ^{1}H-NMR (DMSO-d_6, 400 Hz) δ 8.56(s,1H,C4`); 7.93 (d, J=15.8,C10`), 7.85(d.J=15.8, C11`) ,7.38-7.40(m, Ar-H). $^{13}$C-NMR(DMSO,d-6, 400Hz) δ 134.8-128.9(C1`-C6`,aromatic ring); 186.5(C9`), 123.9(C10`), 145.1(C11`) ; EIMS m/z 276 [M^{+}], 248, 231, 131, 103, 77.

Preparation of (E)-N-(4-(3-oxo-3-(2-oxo-2H-chromen-3-yl) prop-1-en-1-yl)phenyl) acetamide (3).

Yield 60% Mp.: 202-204 ͦC; FT-IR(υ^{-1}; cm^{-1}) 1618($\upsilon_{C=O}$,unsaturated ketone); H-NMR (400 MHz, DMSO-d6) δ 8.64(s, 1H);10.21(s,1H,NH); 7.9 (dd, J = 8Hz, 2H), 7.48 – 7.72 (m, 4H); 2.03(s,3H, $COCH_3$). ^{13}C-NMR (DMSO-d6,400 Hz) δ 186.52(C=O, $CONHCH_3$) ; 23.96(CH_3) ;186.94, 122.70, and 134.97 (CO-CH=CH), respectively. Mass m/z; 333.2.

Preparation of (E)-3-(3-(4-chlorophenyl) acryloyl)-2H-chromen-2-one (4).

Yield 78%, m.p.246-248˚C, light yellow color, FT-IR(υ^{-1};cm^{-1})1620($\upsilon_{C=O}$ unsaturated ketone),1742($\upsilon_{C=O}$, lactone of coumarin),1559($\upsilon_{C=C,}$ ethylene group); ^{1}HNMR(DMSO-d_6 400MHz) δ 8.6 (s, 1H,C4`), δ 7.95(d.,J=15.3,C10`), δ 7.82 (d, J= 15.3, C11`); δ 7.62 ( d,J=8.8); $^{13}$C-NMR (DMSO-d6,400 Hz) δ133.2-129.9( aromatic carbone ), 186.2(C`9) , 126.2(C10`), 140.5( C11`) . EIMS m/z; 310[M^{+}], 282, 275, 266, 247, 165, 137, 101.

Preparation of (E)-3-(3-(4-methoxyphenyl) acryloyl)-2H-chromen-2-one (5).

Yield 85%; m.p.202-203 ˚C, bright yellow color, FT-IR(υ^{-1}; cm^{-1}) 1742($\upsilon_{C=O}$,lactone of coumarin),1618($\upsilon_{C=O}$ unsaturated ketone), 1558($\upsilon_{C=C,}$ ethylene group); ^{1}HNMR(DMSO-d_6 400MHz) δ ppm 8.54 (s, 1H,C4`); 7.79 (d, J = 15.8 Hz,C10`); 7.84(d, J = 15.8, C11`), 6.89-7.61(d.,J=8., 4H);$^{13}$CNMR (DMSO-$d_6$,400 Hz) δ 125.6 -130.8 (Aromatic carbone), 186.3(C9`), 121.6(C10`) , 140.4(C11`) and, 55 (OCH_3). EIMS m/z (%) 307[M+1], 306[M^{+}], 278,263, 161, 133,108.

Fungal isolates

The contaminated fungi of *in vitro* culture used in this investigation were obtained from a study by Ahmed (2023) in the Department of Plant Protection, College of Agriculture, University of Basrah. These isolates were recognized based on their morphology and molecular biology. They

have been entered into the National Center for Biotechnology Information (NCBI), and documented in the GenBank database with accession numbers (Table 1).

Table 1. The contaminating fungi isolates used in the study that were registered in NCBI

Fungal isolates	Base pair	GenBank accession number	Sequence identity(%)	GenBank accession number of organism
Alternaria alternata	574	UBAMAIOP090358	99.45%	(KU936229.1)
Aspergillus fumigatus	599	UBAMAFOP090360	100%	(MT267795.1)
Chaetomium globosum	599	UBAMCOP090361	99.31%	(MT742687.1)
Cladosporium ramotenelum	518	AMCOL589159	99.30%	(MF473247.1)
Fusarium solani	536	UBAMFOP090359	99.43%	(MG211160.1)
Fusarium luffae	517	AMFOL589160	98.06%	(MT448895.1)
Penicillum expanum	588	UBAMPOP090362	100%	(MT582774.1)

According to (Ahmed, 2023)

Fungicidal activity of chemical compounds assay

A sterilized PDA (Potato Dextrose Agar) medium was treated with 1, 1.5, and 2 g/L of four types of coumarin-chalcone derivatives (2, 3, 4, and 5), and then 20 mL was poured onto each Petri plate. Five-mm disks were taken from the edge of the plates from the mycelial growth colony of each tested fungus (5-day-old), and transferred to the center of each Petri dish's contained PDA medium supplemented with one type of coumarin-chalcone derivatives and incubated at 28±2 °C. Five replicates were used for each treatment, while Petri dishes with a PDA medium without any chemical compounds were used as a control. The colony growth diameter of each fungus was measured in millimeters after the completion of the mycelial growth for the control. The following formula was used to determine the rates of mycelial growth inhibition (GI%).

$$\% \textbf{ Growth inhibition} = (\mathbf{dc} - \mathbf{dt})/\mathbf{dc} \times \mathbf{100}$$

where dc = average of mycelial growth in control.

dt = average of mycelial growth in treatment.

(Sing and Tripathi, 1999).

Effect of coumarin-chalcone derivatives treatments on biomass production of contaminating fungi

Conidia suspensions were prepared by washing 7-day-old colonies of all contaminating fungi grown on potato dextrose agar (PDA) plates at 25 °C with sterile distilled water containing 0.1% Tween 80 (v/v). We determined the conidia count with a haemocytometer and set the inoculum concentration at 10^6 conidia per milliliter. A conidia suspension (10^6 conidia/mL) was inoculated into Erlenmeyer flasks containing 50 mL of defined broth culture medium (Jiménez *et al*., 2003). The broth culture medium composition was: 0.5 g/L malt extract, 1 g/L mycological peptone, 1 g/L KH_2PO_4, 0.3 g/L $MgSO_4.7H_2O$, 0.3 g/L KCl, 1 mL $CuSO_4.H_2O$ solution (0.005 g/L), 1 mL $ZnSO_4.7\ H_2O$ solution (0.01 g/L) and 20 g/L fructose. Coumarin-chalcone derivatives were added at 1.5 mg/L into each 50 mL of broth culture medium after 24 hours. While the control cultures (without coumarin-chalcone derivatives) were only inoculated with contaminating fungi. Incubation was conducted at 28±2°C and 180 rpm for 10 and 15 days at four replicates of each culture. After the incubation periods, aliquots were collected and the cultures were subsequently filtered through Whatman No. 1 filter paper (GE Healthcare) to separate the cell-free extract and determine biomass production.

Estimating biomass

Every fungus's mycelia were dried on Whatman No. 1 filter paper at 70°C until they reached a consistent weight. The mycelia's weight was calculated by deducting the filter paper's original weight from the total weight of the mycelia and filter paper. The fungal biomass was calculated by averaging four different samples.

Activity of chalcone derivative compounds on micropropagation of date palm

An experiment was conducted to evaluate the activity of chalcone derivative compounds against contaminating fungi and on the morphological characteristics of explants. Firstly, 5-year-old offshoots of the Hallawi cultivar of Phoenix were used to create clonal material. The leaves and fiber sheath were removed using a saw and knife. The explants or shoot tips were then soaked in an aqueous solution containing 250 mg/L of ascorbic acid and 250 mg/L of citric acid for one hour. The shoot tips were treated with a 20% commercial sodium hypochlorite solution, along with a few drops of Tween 20, for 20 minutes. After this, the shoot tips were washed with sterile distilled water twice. Following the sterilization process, the shoot tip was divided into four fragments, each of which was about 3 cm^3 in size. MS medium (Murashige & Skoog, 1962) (CAISSON LABORATORIES INC. 1740 RESEARCH PARK WAY,NORTH

LOGAN,UT84341 TOLL FREE 877.840.0500, USA) was supplemented with Coumarin-chalcone derivatives at 1.5 mg/L into each 50 mL. The MS medium without any addition of Coumarin-chalcone derivatives served as control. The PH of the media was adjusted to 5.8 with NaOH (0.1M) or HCL (0.1M) before autoclaving. 50 milliliters of the specified medium were added to each culture jar. The culture jars, or media, were then autoclaved for 20 minutes at 121±2 °C. *In vitro* cultural explants, or shoot tips, were placed straight into these culture jars once they had cooled and solidified. Cool white fluorescent lights were used to maintain a 16-hour photoperiod, 23±2°C temperature, and 2000 lux of light intensity at the plant level in all cultures. Twenty replications were made for each treatment. After three months of planting the explants and the emergence of vegetative growth from the calli, the following parameters were calculated: percentage of contamination and browning of cultures, percentage of response to cultures on shoot formation, and the number of proliferated shoots.

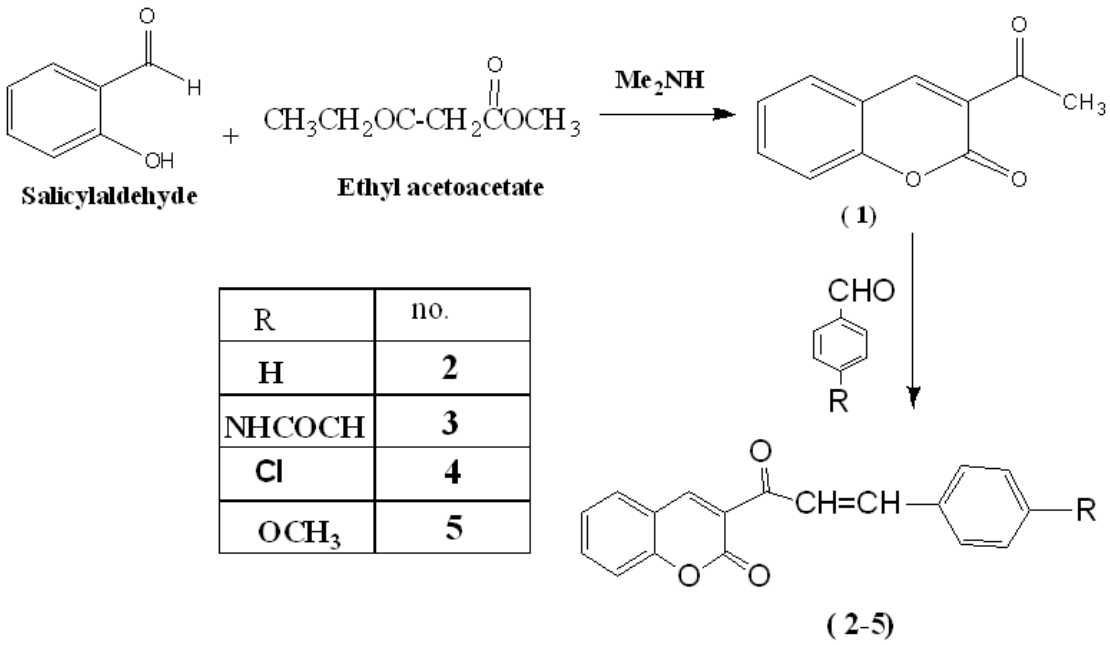
Data analysis

Treatments were distributed in all experiments according to a completely randomized design (C.R.D), with four replications for each treatment, and the means were compared using the least significant difference (LSD) test (*P-value* ≤ 0.05), the data were analyzed by applying SPSS (Statistical Package for the Social Sciences) V.22.

Results

Prepared coumarin -chalcone derivatives

Initially,3-acetyl-2H-chromen-2-one(1) was prepared through the mixing of 2-hydroxybenzaldehyde with ethyl acetoacetate in the presence of dimethyl amine as a catalyst, which was characterized by the IR absorption of the (3-$COCH_3$) group, which appears at position 1739.7 cm^{-1} belonged to the carbonyl of the coumarin structure (lactone group), and the appearance band at 1678 cm^{-1} referred to the carbonyl of the 3-acetyl group. Finally, coumarin-chalcone derivatives were prepared through the reaction of 3-acetylcoumarin with substituted aromatic benzaldehydes (4-H, 4-$NHCOCH_3$, 4-Cl, and 4-OCH_3). The preparation of coumarin-chalcone hybrid derivatives (2–5) was carried out by the Claisen-Schmidt reaction of the appropriate aldehyde derivatives and 3-acetylcoumarin in the presence of a base (NaOH), according to Scheme 1.



R	no.
H	2
NHCOCH	3
Cl	4
OCH_3	5

Scheme 1: Synthesis 3-Acetylcoumarin and Chalcone derivatives.

Identification of Coumarin-chalcone hybrid derivatives (2–5) using FT-IR, ^{1}H-NMR, 13CNMR, and mass spectrometry analysis.

The first derivative (2), was characterized by the symmetric protons (5H) of the aromatic ring at 7.71–7.31 ppm. Coumarin appeared at 7.8–8 ppm. The ethylene of the chalcone moiety (-CO-CH=CH-) appeared at 7.96 and 7.87 ppm as signals, respectively, while the proton at position 4 of the coumarin structure was at 8.59 ppm. Physical-chemical properties of coumarin-chalcone derivatives are explained in Table (2).

Table 2. Physical-chemical properties of coumarin-chalcone derivatives

Parameter	Comp.2	Comp.3	Comp.4	Comp.5
Totalenergy (kcal/mol)	- 73710.42232	-90770.54488	-80665.2818	-83928.0172
Dipole moment (debye)	4.054	9.784	6.039	7.761
Clog P	1.93	1.54	3.21	2.44
Heat of formation	- 23.88575658	-71.531583	-34.6218297	-66.283852
Volume (cm^3)	804.33	931.58	852.13	885.34
Electronic chemical potential (μ)	-5.2937775	-5.1257725	-5.392843	-5.23418
Chemical hardness (η)	3.9818975	3.76038	3.953724	3.861164
Electrophilicity (ω)	3.518935409	3.493469240	3.67789400	3.547717
E_{HOMO} (ev)	-9.275675	-8.886155	-9.346567	-9.095344
E_{LUMO} (ev)	-1.311882	-1.36539	-1.439119	-1.373016
ΔE (ev)	- 7.963793	-7.520765	-7.907448	-7.722328

Potential antifungal properties of chalcone derivatives

The findings revealed that adding various concentrations of chalcone derivative compounds (2, 3, 4, and 5) to the culture medium was effective in reducing the radial growth mycelium colony of all the tested contaminating fungi in the medium. No significant difference was observed among the concentrations of chalcone derivative compounds in the culture medium for suppressing fungal contamination, but these treatments were significantly more effective than the control (without chalcone derivatives). Using the chalcone derivative compound in the culture medium at 1.5 and 2 mg/L showed excellent activity inhibition against these contaminating fungi at around 100%. While adding the chalcone derivative compound at the concentration (1 mg/L) to the culture medium inhibited radial growth for a range from 44.44 to 52.12% for all tested contamination fungi compared with the control treatment. (Fig. 1 and Table 3).

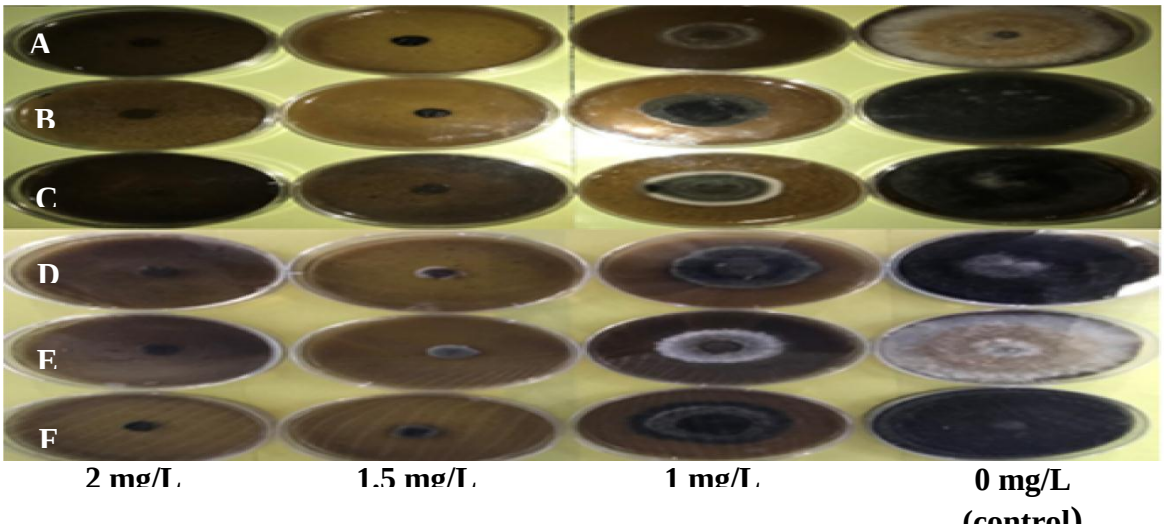


Fig. 1. *In vitro* antifungal activities of chalcone derivative compounds (No. 2) against contamination fungi (A: *Ch. globasum*, B: *As. fumigatus*, C: *Cl. ramotenelum*, D: *P. expanum*, E: *F. solani*, F: *Al. alternata)* at different concentrations (same results as those of the other compounds).

Table 3. Effectiveness of Chalcone derivative compounds against fungal contamination at different concentrations *in vitro*.

Chalcone derivative compounds	Concentration (mg/L)	The average percent of radial growth inhibition(%)				
		A. fumigatum	*F. solani*	*Al. alternata*	*C. globasum*	*P. expanasum*
2	1	44.48 d*	46.66 c	46.66 c	47.12 c	44.22 d
	1.5	100 a	100 a	100 a	100 a	100 a
	2	100 a	100 a	100 a	100 a	100 a
3	1	46.88 c	46.66 a	50.00 b	50.24 b	46.44 c
	1.5	100 a	100 a	100 a	100 a	100 a
	2	100 a	100 a	100 a	100 a	100 a
4	1	50.00 b	48.88 c	48.66 c	52.12 b	48.44 c
	1.5	100 a	100 a	100 a	100 a	100 a
	2	100 a	100 a	100 a	100 a	100 a
5	1	44.44 d	48.44 c	48.66 c	48.33 c	46.88 c
	1.5	100 a	100 a	100 a	100 a	100 a
	2	100 a	100 a	100 a	100 a	100 a

*Average based on four replicates. Means followed by different letters (in the same column) indicate significant differences by the LSD test ($p \leq 0.05$).

As part of the investigation into how chalcone derivatives affect biomass production, Table 4 displays the biomass that the infecting fungi create at several incubation times (10^3 conidia/mL). After 10 and 15 days, respectively, control cultures generated the highest biomass, which ranged from 0.30 to 0.38 g after 10 days and 0.36 to 0.48 g after 15 days. whereas treatments with chalcone derivatives only produced 0.16 to 0.21 g after 10 days and 0.12 to 0.24 g after 15 days of all contaminated fungi. The LSD test revealed a significant difference ($p \leq 0.05$) in biomass output between the treatments supplemented with chalcone derivative chemicals and the control group. In cultures supplemented with chalcone derivatives for longer periods, we observed decreased and delayed biomass production.

Table 4. Biomass production by contaminated fungi in defined liquid culture media in the absence (control) and presence (treatment) of chalcone derivative compounds the recommended dose (1.5 mg/ L) in different incubation periods.

Chalcone derivative	Period (day)	Biomass of contaminating fungi (g)									
		A. fumigatum		*F. solani*		*Al. alternata*		*C. globasum*		*P. expanasum*	
		Cont.	Treat.	Cont.	Treat	Cont.	Treat.	Cont.	Treat	Cont.	Treat.
2	10	0.30 a*	0.16 b	0.32 a	0.18 b	0.32a	0.20 b	0.32a	0.18 b	0.36 a	0.12 b
	15	0.36 a	0.15 b	0.40 a	0.12 b	0.44a	0.15 b	0.38a	0.22 b	0.44 a	0.16 b
3	10	0.37 a	0.20 b	0.36a	0.18 b	0.36a	0.18 b	0.34a	0.18 b	0.36 a	0.12 b
	15	0.40 a	0.17 b	0.42a	0.10 b	0.48a	0.12 b	0.44a	0.20 b	0.48 a	0.18 b
4	10	0.36 a	0.21 b	0.38a	0.20 b	0.34a	0.20 b	0.36a	0.18 b	0.38 a	0.14 b
	15	0.40 a	0.16 b	0.46a	0.12 b	0.44a	0.12 b	0.48a	0.24 b	0.44 a	0.18 b
5	10	0.32 a	0.17 b	0.36a	0.18 b	0.36a	0.18 b	0.36a	0.20 b	0.36 a	0.14 b
	15	0.36 a	0.15 b	0.48a	0.12 b	0.48a	0.10 b	0.48a	0.18 b	0.48 a	0.20 b

***** Average based on four replicates. Means followed by different letters (in the same line) indicate significant differences by the LSD test ($p \leq 0.05$).

Effects of different concentrations of chalcone derivative compounds on *in vitro* culture experiments

The present study showed that applying chalcone derivative compounds in different concentrations (1.5 and 2.00 mg/ L) is useful in controlling fungal contamination when supplemented with MS medium and applied to *in vitro* cultures of date palms. The results showed that the cultures significantly responded to the various additional concentrations of chalcone derivative compounds in the MS medium. The MS medium with added 1.5 and 2.0mg/L concentrations suppresses the fungal contamination by about 100%, while the control treatment recorded about 18.33% of fungal contamination of MS media. Furthermore, the dosage of 2.0 mg/L, there was a significant browning reaction observed, with the intensity of the reaction being the highest on the scale (80.6%). The treatments mentioned (1.5mg/L) showed an average percentage of 2.11% of cultures with less browning, as compared to the control which recorded a 24.44% browning reaction. The growth response of clonal cells differed strongly between the explants treated with the lower concentrations of chalcone derivative compounds (1.5 mg/L) compared with cultures treated with a high concentration (2.0 mg/L). Cultures failed to develop in MS medium at higher concentration, turning brown and dying. The MS medium with 1.5 mg/L recorded the greatest numbers of somatic embryogenesis compared to the MS medium without supplementation (control). The maximum number of somatic embryos was recorded about 32.24, while the MS medium in control was about 12.33 (Fig. 2 and Table 5).

Fig.2. Effects of different concentrations of chalcone derivatives on somatic embryogenesis of *in vitro* culture experiments

Table 5. The effect of different concentrations of chalcone derivative on multiplication of somatic embryogenesis of *in vitro* culture of date palm, contamination fungi (%), and browning (%) in vitro.

Concentration of chalcone derivative (mg/L)	number of somatic embryos	% of contamined fungi	% of browning
0.00 (Control)	12.33 b*	18.28 a	24.44 b
1.50	32.24 a	0.00 b	2.11 c
2.00	0.66 c	0.00 b	60.80 a

***** Average based on four replicates. Means followed by different letters (in the same column) indicate significant differences by the LSD test ($p \leq 0.05$).

Discussion

Using the Hyperchem 8.0 program for molecular representation, the chemical parameters calculated using semi-empirical (PM3) are: total energy (E), electronic chemical potential (μ), chemical hardness (η), and electrophilicity (ω). These compounds' antifungal properties can be linked to their capacity to damage the cell wall or membrane of fungi, obstruct the fungi's enzymes or metabolic processes, or cause oxidative stress in fungi (Gupta and Jain, 2016).

The precise mode of action may vary depending on the derivative's chemical composition and the particular fungus that is intended to kill (Boek *et al*., 2005). For instance, it has been demonstrated that coumarin-chalcone compounds with a -4-hydroxy substituent can stop the growth of *Aspergillus niger* and *Candida albicans* by triggering cell lysis and rupturing the fungal cell wall. Chalcones suppressing the enzymes of chitin synthases and β (1,3)-glucan, which catalyze the manufacture of chitin and β(1,3)-glucan polymers of the fungal cell wall, respectively (Reddy *et al*., 2010). Several fungi, such as *Candida albicans*, *Cryptococcus neoformans*, and *Fusarium solani*, have been found to be responsive to coumarin-chalcone derivatives that contain a -4-amide substituent (Gupta and Jaian's, 2016). These derivatives are capable of impeding the growth of fungi by interfering with their enzymes or metabolic processes, such as the ergosterol biosynthesis pathway, which is vital for the integrity of fungal cell membranes. However, the antifungal efficacy of coumarin-chalcone compounds with a-4-Chloro substituent may be diminished due to the electron-withdrawing nature of the chlorine atom, as noted in the study of Chavan *et al*. (2016). Depending on the chemical composition of the derivative, coumarin-chalcone derivatives containing a -4-methoxy substituent may or may

not possess antifungal activity. Some derivatives with a -4-methoxy substituent have been found to have low antifungal activity against *Candida albicans* and *Aspergillus niger*, while others exhibit no antifungal activity at all, according to studies by Chavan *et al*. (2016) and Silva *et al*. (2020). This study showed that the application of different concentrations of chalcone derivative compounds (1.5 and 2.0 mg/L) to the MS medium significantly reduced the contaminated fungi compared with the control (no added chalcone derivative compounds). The antimicrobial activity of chalcone derivative compounds may be due to several factors, such as the permeation of CH_3O groups released from chalcone derivative compounds into the cell membrane. This destroys the morphology of the cell membrane through chalcone derivative compounds attaching to the cell wall, resulting in cell death. The current study's findings will help to prevent the fungal contamination *in vitro* culture and produce huge quantities of micro propagation cultures as well as provide valuable suggestions to lower or eliminate the risk of contamination. The increase numbers of somatic embryogenesis in agreement with the resulting research of Hassan *et al*. (2020) demonstrates that, in comparison to the untreated samples, all treated plants with chalcone derivative compounds exhibit significant development in the shoot and an increase in the number of leaves. Similar to the resulting study by Kalambe *et al*. (2015), who documented the beneficial effect of substituted chalcones on some agricultural plants, the treated plants' increased growth is being seen. The fact that substituted chalcones function as flavonoids in plants and have a variety of functions, including suppressing auxin (the primary growth hormone) inhibitors, who is accounts for their beneficial effects. Four novel chalcone derivatives were created and synthesized in an effort to locate the bio-fungicides. The activity of the biological compound of chalcone derivatives was produced by refining the system's structure. In the following, the relative surveys on *in vitro* anti- contaminated fungi were used to determine the practical usefulness of compound chalcone derivatives. This study suggests that chalcone derivatives have a better inhibitory effect on contaminating fungi, paving the way for additional supplementary research on novel bio fungicides. There are many previous studies that have been conducted on the use of chalcone derivative compounds in eliminating contamination problems caused by microorganisms such as fungi and bacteria. Zheng *et al*. (2015) found that synthesized chalcone derivative compounds are highly effective against the following plant pathogenic fungi: *Sclerotinia sclerotiorum, Helminthosprium maydis, Botrytis cinerea, Rhizoctonia solani,* and *Gibberella zeae.* The results of the study by Kuttithodi *et al*. (2022) indicated the synthesized chalcone derivative compounds have strong antioxidant properties in terms of DPPH and ABTS

radical-scavenging potentials as well as the ability to reduce ferric. Silva *et al*. (2020) found that the chalcones synthesized from the 2-hydroxy-3,4,6-trimethoxyacetophenone isolated from *Croton anisodontus* with benzaldehyde and its derivatives were able to have cytotoxic and antifungal activities. Their results showed that the chalcone(E)-3-(4-fluorophenyl)-1-(2-hydroxy-3,4,6-trimethoxyphenyl)prop-2-en- one demonstrated highly effective against human colon carcinoma (HCT-116) cells after 72 h of incubation and was able to promote the reduction of fungal cells between the periods of 4 to 8 h and 100% inhibition. According to Prasad (2008), chalcones possess antifungal properties due to an unsaturated reactive keto function. Some phenolic synthetic chalcones also exhibit antifungal properties (Tsuchiya *et al*., 1994; Sato *et al*., 1994). Chalcones function by inhibiting the enzymes that produce the (1,3)-glucan and chitin polymers present in the fungal cell wall, respectively (Lahtchev *et al*., 2008; Reddy *et al*., 2010). According to the early findings, it may be useful to research the antifungal activity of chalcone derivatives by looking at the design and synthesis of these compounds. Additionally, it is encouraging and advantageous for future research into the development of new and improved friendly fungicides in agricultural science. More effort needs to be made, though. In order to discover new fungicides with potent effects and minimal toxicity, it is obvious that a number of chalcone derivatives should be further synthesized for screening and analyzing quantitative structure-activity relationships. In general, coumarin-chalcone derivatives with substituted aromatic benzaldehydes have antifungal activity that varies depending on the molecule's chemical structure and the kind of fungus being treated. These compounds have the potential to be turned into brand-new antifungal medications that might be used to treat fungal infections, a major worldwide health issue.

Conclusion

This study involved the synthesis and structural characterization of many coumarin-containing chalcone derivatives. The antifungal potential of coumarin-chalcone hybrids was investigated *in vitro* after their synthesis. According to the outcomes of biological experiments, the target compounds have good antifungal properties. The results of this study show that using chalcone derivative compounds at different concentrations (1.5 and 2 mg/L) helps to prevent microbial contamination during *in vitro* establishment on PDA medium, but the concentrate at 1 mg/L is less effective at reducing mycelial growth when compared to the control treatment. No fungal contamination was observed in the MS medium supplemented with 1.5 and 2.0 mg/L concentrations of coumarin-chalcone hybrids treatments compared with the control (coumarin-

chalcone hybrids). In addition, the multiplication rate of somatic embryos enhanced in MS supplemented with coumarin-chalcone hybrids at 1.5 mg/L increased considerably to 32.24%, compared to the control, which was 12.33%. According to reports, coumarin derivatives have a wide range of biological characteristics. Chalcone and coumarin can be combined to create novel compounds that may have fungicidal activity.

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تحضير وتوصيف مركبات هجين الكومارين–الكالكون وفعاليته كمبيد الفطريات ضد فطريات التلوث وتأثيره على ظاهرة الاسمرار وتكون الاجنة في مختبرات زراعة الأنسجة لنخيل التمر (*Phoenix dactylifera* L)

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الخلاصة

تضمنت هذه الدراسة التوليف والتوصيف الهيكلي لمشتقات الكالكون المحتوية على الكومارين. تم استخدام العديد من التقنيات الطيفية والتي شملت اختبارات 1H MNR , FITR و13CNMR لتشخيص تركيبها الكيميائي. اختبرت مركبات الهجين الكومارين كالكون في قدرتها على تثبيط نمو الفطريات الرئيسية الملوثة للزراعات النسيجية مختبريا. اظهرت نتائج الدراسة ان الوسط الزرعي PDA المعقم والمعاملة بتراكيز مختلفة من مشتقات الكالكون والملوثة بالفطريات اظهرت تثبيطا تاما لهذة الفطريات عند التركيزين 1.5و2.0 ملغم/لتر, في حين اظهر التركيز1.0 ملغم/لتر تثبيطا ضعيفا، مقارنة بنمو الفطريات بمعاملة المقارنة(غير المعاملة بمشتقات الكالكون) والتي بلغ النمو القطري للفطريات الملوثة 9 سم (100%). كما وجد ان معدل انتاج الكتلة الحيوية في المعاملات المضافة اليها مشتقات الكالكون عند التركيز 1.5 ملغم/لتر يتناقص فيها انتاج الكتلة الحيوية مع زيادة فترات الحضن اثبتت اختبار زراعة الانسجة ان اوساط MS المعاملة بمركبات الكالكون عند التركيزين 1.5 و 2.0 ملغم /لتر لم يلاحظ وجود اي تلوث فطري فيها مقارنة بمعاملة المقارنة(غير المعاملة بمركبات الكلكون) والتي بلغت نسبة التلوث الفطري فيها 18.28%. كما انخفض معدل ظاهرة الاسمرار بالبراعم الجسدية الى 2.11% عند التركيز 1.5 ملغم /لتر في حين ارتفعت هذة النسبة في التركيز 2.0 ملغم /لتر الى 60% وعند معاملة المقارنة 24.44%. بالاضافة الى ذلك فقد ازدادت النسبة المئوية للبراعم الناشئة الى 32.24% عند معاملة التركيز 1.5 ملغم /لتر بينما كانت في معاملة التركيز 2.0 ملغم/لتر والمقارنة 7.68 و12.33% على التوالي.

الكلمات المفتاحية: مركبات الكالكون ، الفطريات الملوثة، الزراعة النسيجية، نخيل التمر، مضادة الميكروبات.