[Short title + Author Name - P&H title] 30 (2023) 103843

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journal homepage: www.sciencedirect.com

الجمعية السعودية لعلوم الحياة
SAUDI BIOLOGICAL SOCIETY

Original article

In vitro efficacy of *Boswellia carterii* resin extracts formulated as an emulsifiable concentrate against *Tetranychus urticae* and phytopathogenic fungi

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ARTICLE INFO

Keywords:
Frankincense resin
Tetranychus urticae
Antioxidant
Antifungal
Unsaponifiable matters
Fatty acids

ABSTRACT

Contemporary agriculture heavily relies on pesticides for pest eradication and disease management. Consequently, current study was carried out to assess the acaricidal/antifungal efficacy of emulsifiable concentrate (10 % EC) derived from *Boswellia carterii* (*B. carterii*) against adult females of *Tetranychus urticae* (*T. urticae*), and five fungal pathogens. The meticulous examination of the chemical constitution of the crude extracts derived from the resin of *B. carterii* was conducted through the employment of the venerable technique known as Gas-Liquid Chromatography (GLC). The formulated petroleum-ether extract (FPEE) and formulated ethyl-acetate extract (FEAE) of *B. carterii* at a concentration of 10 mg ml^{-1} exhibited notable antioxidant activity with rates of 62.0 % and 90.8 %, respectively. *In vitro*, the FEAE exhibited potent inhibition against all the tested phytopathogenic fungi at different concentrations, whereas FPEE showed comparatively less efficacy. Interestingly, at 4000 ppm concentration, FEAE completely ceased the mycelial growth compared with the control. Moreover, following a span of 72 h of intervention, FPEE exhibited a greater degree of toxicity towards mature females of the *T.* urticae. This was evidenced by the LC_{50} value of 422.52 parts per million (ppm) for FPEE, which surpassed the LC_{50} value of 539.50 ppm observed for FEAE. In summary, the present study indicates that *B. carterii* resin formulated as an emulsifiable concentrate (10 % EC) can offer a natural and effective alternative for integrated pest management, thereby reducing reliance on synthetic pesticides and offering a more environmentally sustainable strategy for pest control.

1. Introduction

Frankincense, a splendidly organic resin extracted from the majestic *Boswellia* trees of the esteemed Burseraceae family (Han et al., 2017), emanates from none other than the illustrious *Boswellia carterii* (*B. carterii*), which thrives in the arid and lofty realms of East Africa, China, and India (Frank et al., 2009). *Boswellia* trees offer abundant reservoirs of natural resins and various bioactive compounds. The essential oil found in resin comprises 5–9 % of its composition. Additionally, it contains alcohol-soluble resin comprising 65–85 % and water-soluble gum accounting for approximately 20 %, which is a blend of heteropolysaccharides, polysaccharides, arabinogalactans, glycoproteins and polymeric substances (Al-Yasiry and Kiczorowska, 2016).

Furthermore, the resin possesses a significant amount of non-volatile triterpenoic constituents, including ursane (β-boswellic acid), oleonane (α-boswellic acid), and lupine, which have been associated with a range

Peer review under responsibility of King Saud University.

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https://doi.org/10.1016/j.sjbs.2023.103843
Received 25 August 2023; Received in revised form 3 October 2023; Accepted 15 October 2023
Available online 20 October 2023

Table 1
Extraction yields (%) of *B. carterii* extracts.

Extract*	Plant Wt (g)	Extract Wt (g)	Yields (%)	Color of matter
PE	10	4.60	46.0	Yellow
EA	10	3.98	39.8	Yellow

* PE; petroleum-ether, EA; ethyl-acetate.

Table 2
Phytochemical screening of frankincense resin-extracts.

Phytochemical constitutes*	PE extract	EA extract
Saponins	–	+
Alkaloids	–	+
Flavonoids	–	+
Steroids	+	–
Tannins	+	–
Phenolic compounds	–	+
Glycosides	–	+

* PE; petroleum-ether, EA; ethyl-acetate, +; presence, –; absence.

Table 3
Total phenolic and flavonoid contents in *B. carterii* ethyl acetate.

Extract*	Total phenol (mg/g, GAE)	Total flavonoids (mg/g, QE)
EA	3.28	0.29

* EA; Ethyl-acetate, GAE; gallic acid equivalent, QE; quercetin equivalent.

of biological actions (Karlina et al., 2007; Al-Yasiry and Kiczorowska, 2016). Studies by Ahmed et al. (2015) and Sultan (2020) have verified the presence of fatty acids in the resin, such as oleic, myristic, palmitic, linoleic, arachidic, arachidonic, and lignoceric acids, which contribute to its antibacterial properties. Furthermore, Ayub et al. (2018) have elucidated the presence of phenolic compounds, such as thujene, camphene, β-pinene, myrcene, limonene, M−cymene, and *cis*-verbenol, which manifest antimicrobial properties.

The resin has a long history of use in traditional medicine for diverse purposes, including rheumatoid arthritis (Banno et al., 2006), and acknowledged properties such as antifungal, antibacterial, and anti-inflammatory effects (Huang et al., 2000). Recently, European countries have witnessed an upsurge in the popularity of frankincense resin due to its potential to manage persistent inflammatory problems (Mishra et al., 2020), promote skin health (Han et al., 2017), and exhibit anti-cancer activity (Frank et al., 2009; Swallah et al., 2020). Additionally, it's worth noting that the US Food and Drug Administration (FDA) has granted approval for its safe usage as a food additive (Raja et al., 2011).

The two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Tetranychidae), can infest a wide range of vegetable crops globally. It is found in tropical regions worldwide and has been identified in more than 3,877 plant species. This mite poses a significant economic threat to at least 150 plant varieties (Le Goff et al., 2009; Migeon et al., 2010; Islam et al., 2017). Its feeding behavior involves puncturing the leaf tissues and extracting the contents of individual plant cells. With a consumption rate of 18–22 plant cells per minute, it hampers photosynthesis, transpiration, leaf chlorophyll levels, and leaf nitrogen content, consequently diminishing plant growth and productivity (Kiran et al., 2017). Additionally, it is involved in transmitting various viruses, including potato virus Y, tobacco mosaic virus, and tobacco ring spot virus (Sarwar, 2020). The current climate change scenario will undoubtedly impact the role of spider mites as agricultural pests. In arid and warm climates, *T. urticae* accelerates its life cycle, increases its yearly generational count, and expands its range of host plants (Ximénez-Embún et al., 2017).

Fungi have a global reputation for causing diseases in plants. They can infiltrate plants through various means, such as natural openings like stomata and injuries resulting from pruning, harvesting, hail, insects, and mechanical damage. Prompt management actions are necessary to prevent tomato diseases from turning fatal. Fungi, including crown and root rot, fusarium wilt, early blight, rhizoctonia, phoma, and others cause most tomato diseases. These diseases significantly impede tomato production, leading to substantial economic losses (Sanoubar and Barbanti, 2017). *Botrytis cinerea* leads to grey mould, a destructive disease in strawberries. This pathogen negatively impacts the fruit at different stages, including in the field, during storage, transportation, and even in the market. The existence of grey mould is the primary cause for growers, shippers, and consumers to reject affected fruits, resulting in significant economic losses (Petrasch et al., 2019).

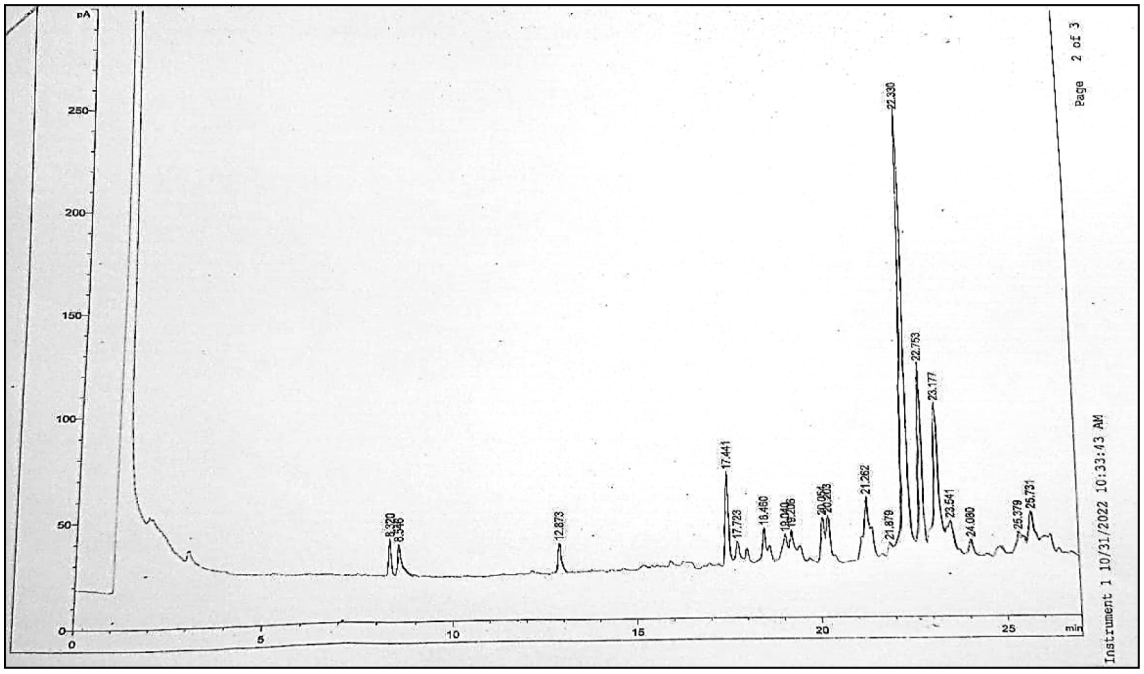
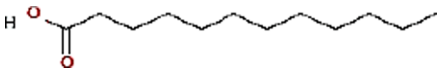
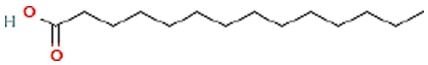
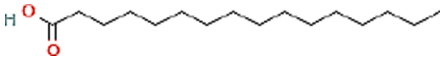
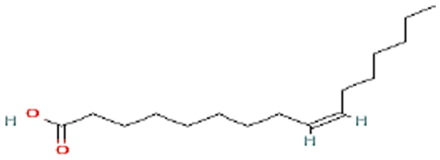
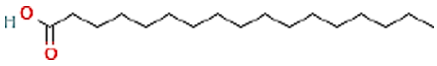
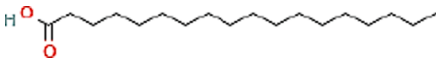
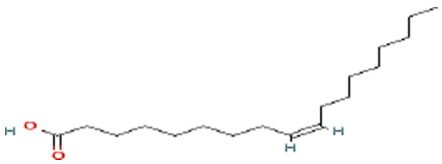
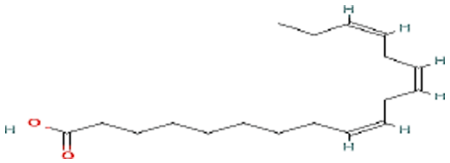
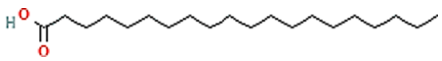
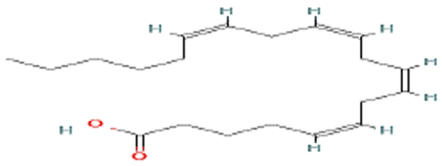


Fig. 1. GLC chromatogram of the saponified matters in *B. carterii* petroleum-ether crude extract.

Table 4
Type and percent of saponifiable compounds identified in *B. carterii* petroleum-ether crude extract by GLC.

Compounds*	Chemical Structure	Area (%)	Rt (min)
Lauric (C_{12})		3.2	8.5
Myristic (C_{14})		1.7	12.8
Palmetic (C_{16})		5.2	17.4
Palmitoleic ($C_{16.1}$)		2.1	18.4
9,12 hexadecadienoic ($C_{16.2}$)		1.7	19.2
Heptadecanoic (C_{17})		6.1	20.2
Stearic (C_{18})		7.9	21.2
Oleic ($C_{18.1}$)		36.5	22.3
Linoleic ($C_{18.2}$)		13.1	22.7
α-Linolenic ($C_{18.3}$)		16.0	23.1
Arachidic (C_{20})		1.3	24.0
Arachidonic ($C_{20.4}$)		5.2	25.7

* RT; Retention time obtained by chromatogram.

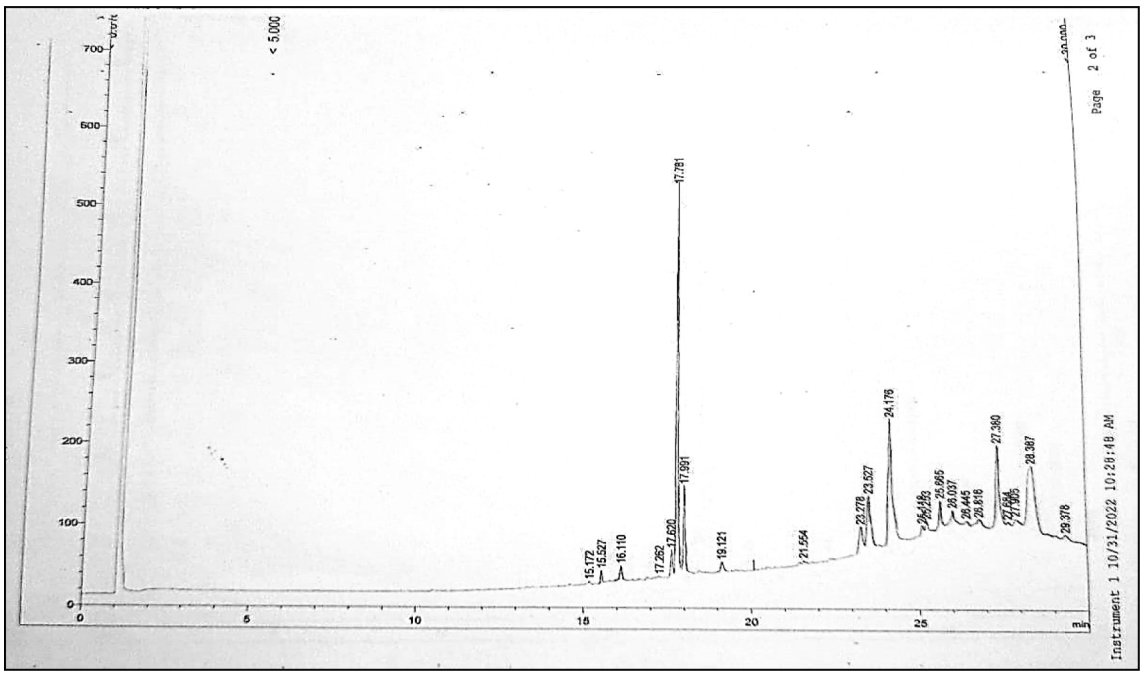


Fig. 2. GLC chromatogram of the unsaponified matters in *B. carterii* petroleum-ether crude extract.

Pesticides are artificial compounds employed for pest eradication. Their global consumption reaches approximately two million tons annually. Although pesticides can enhance agricultural productivity, their indiscriminate usage has detrimental impacts on soil quality, human and animal health, water purity, and the emergence of issues such as insect resistance, genetic alterations in plants, and toxic residues in food and animal feed. Therefore, advocating for using biopesticides instead of chemicals can benefit our planet and well-being. Biopesticides are naturally occurring plant-based substances that control pests through non-toxic and environmentally friendly mechanisms (Aktar et al., 2009; Hussien et al., 2022).

Essential oils are organic substances that possess intricate volatile properties that contribute to the unique scents of plants. They are produced as secondary metabolites and serve as a natural defense mechanism for plants against various threats such as bacteria, fungi, viruses, insects, and herbivorous (Akthar et al., 2014; Gaber et al., 2021; Abdel-Wahab et al., 2022). Recently, they have gained attention due to their diverse range of advantageous effects on pests and disease-causing organisms. Consequently, they are considered potential substitutes to synthetic chemical pesticides in crop protection and pest management applications. They are less harmful than pesticides and can quickly evaporate, leaving behind minimal residue (Isman, 2020; Hussien et al., 2022; Helmy et al., 2023; Yousef et al., 2023).

Hence, the current study aims to examine the efficacy of emulsifiable concentrate (10 % EC) formulations derived from *B. carterii* against two-spotted spider mites and fungal pathogens *in vitro*. Moreover, it is imperative to ascertain the active constituents of these substances through the utilization of chromatographic and spectroscopic techniques.

2. Materials and methods

2.1. Plant material

The *B. carterii* resin utilized in this study was acquired from authenticated Egyptian herbal shops. The plant material was finely crushed and kept in airtight plastic bags at normal room temperature until use.

2.2. Preparation of resin extracts

Fifty grams of dried powdered sample were subjected to separate maceration procedures using petroleum ether (60–80 °C) and ethyl acetate using an orbital shaker (150 rpm) at room temperature for 24 h. The resulting extracts were then filtered through Whatman No.1 filter paper. Residues were re-extracted twice with fresh aliquots of the same solvents. The filters of each solvent were evaporated at 40 °C using a rotary vacuum evaporator to obtain crude extracts of petroleum ether and ethyl acetate. The dried sample of each extract was weighed to determine the yield of soluble constituents and subsequently stored at 4 °C (Gupta et al., 2022).

2.3. Phytochemical screening of *B. carterii* crude extracts

The phytochemical screening of PE and EA derived from *B. carterii* was carried out according to (Iqbal et al., 2015).

2.4. Antioxidant activity evaluation

2.4.1. Determination of total phenol content

The content of total phenolic compounds in EA of *B. carterii* was determined using a colorimetric method described by (Velioglu et al., 1998), with gallic acid employed as the standard phenolic acid. The results were expressed in mg of gallic acid equivalent per gram of extract (mg GAE g^{-1}).

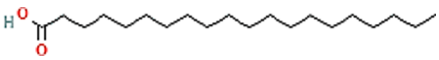
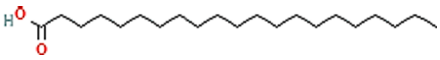
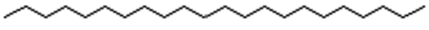
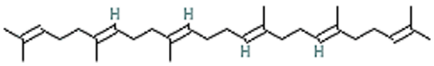
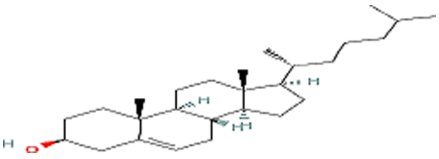
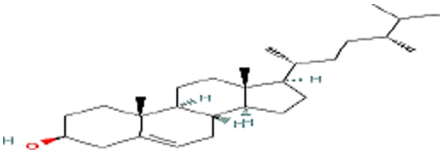
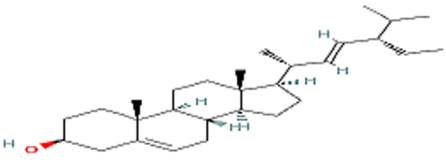
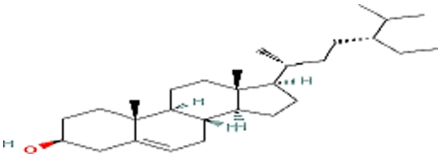
2.4.2. Determination of total flavonoid content

The content of total flavonoid compounds in EA of *B. carterii* was determined through a colorimetric method described by (Jiao and Wang, 2000), with quercetin employed as the standard flavonoid.

2.4.3. DPPH antioxidant assay

The antioxidant activity of *B. carterii* FPEE and FEAE were assessed for their ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals according to Liu et al. (2010). The DPPH was calculated according to Eq. (1):

Table 5
Type and percent of unsaponifiable compounds identified in *B. carterii* petroleum-ether crude extract by GLC.

Compounds*	Chemical Structure	Area (%)	Rt (min)
n.Nonadecane (C_{19})		1.0	15.5
n.Eicosanoic (C_{20})		1.2	16.1
n.Heneicosanoic (C_{21})		26.3	17.7
n.Docosane (C_{22})		0.90	19.1
n.Tetracosane (C_{24})		0.6	21.5
n.Pentacosane (C_{25})		7.4	23.5
Squalene (C_{30})		17.1	24.1
n.Heptacosane (C_{27})		2.5	25.2
Cholesterol like compound (C_{27})		4.8	25.6
Campesterol (C_{28})		8.0	26.0
Stigmasterol (C_{29})		14.7	27.3
β.sitosterol (C_{29})		15.5	28.3

* RT; Retention time obtained by chromatogram.

Table 6
Physicochemical properties of *B. carterii* crude extracts as an active ingredient.

Extract*	Solubility % (W/V)			Free acidity as H_2SO_4
	Water	Acetone	Xylene	
PE	insoluble	100	83	0.80
EA	insoluble	100	71	3.43

* PE; petroleum-ether, EA; ethyl-acetate.

Table 7
Physicochemical properties of surfactants used for the preparation of *B. carterii* as an emulsifiable concentrate.

Surfactants *	HLB	CMC	Free acidity as H_2SO_4	Surface tension (Dyne cm^{-1})
Tween 80	>13	0.5	0.50	39.2
PEG 600ML	>13	0.3	0.88	30.6

* HLB; hydrophilic-lipophilic balance, CMC; critical micelle concentration, PEG 600ML; polyethylene glycol 600 monolaurate.

Table 8
Storage stability of *B. carterii* resin extracts prepared as an emulsifiable concentrate (10% EC).

Storage	Physicochemical properties		Extract	
			Petroleum Ether	Ethyl Acetate
Before storage	Spontaneity (%)	Hard	100	100
		Soft	100	100
	Emulsion stability	Hard	pass	pass
		Soft	pass	pass
	Foam (cm^3)	Hard	4	3
		Soft	4	3
	Free acidity as % H_2SO_4		0.39	0.69
After storage	Spontaneity (%)	Hard	100	100
		Soft	100	100
	Emulsion stability	Hard	pass	pass
		Soft	pass	pass
	Foam (cm^3)	Hard	4	3
		Soft	4	3
	Free acidity as % H_2SO_4		0.49	0.78

Table 9
Physicochemical properties of *B. carterii* resin as emulsifiable concentrate formulation (10% EC) and used at a field dilution rate (0.5%).

Extract*	Physicochemical properties			
	Surface tension (dyne cm^{-1})	Viscosity (cm $poise^{-1}$)	Electrical conductivity (μ mhos)	pH
FPEE	37.02	1.40	317	6.90
FEAE	37.02	1.48	323	6.80

* FPEE; formulated petroleum-ether extract, FEAE; formulated ethyl-acetate extract.

Table 10
Antioxidant activity (%) and DPPH scavenging activity of *B. carterii* FPEE and FEAE.

Extract/ Standard *	Concentration (mg/ml)	Optical Density	Antioxidant Activity (%)	DPPH * (IC_{50} mg/ml)
FPEE	2	0.72	10.3	8.68
	4	0.63	21.4	
	6	0.56	30.0	
	8	0.44	44.9	
	10	0.30	62.0	
FEAE	2	0.60	24.8	4.15
	4	0.47	41.6	
	6	0.31	61.0	
	8	0.17	78.5	
	10	0.07	90.8	
AA	0.1	0.58	27.5	0.19
	0.2	0.48	40.4	
	0.3	0.24	70.6	
	0.4	0.10	87.0	
	0.5	0.06	92.0	

* FPEE; formulated petroleum-ether extract, FEAE; formulated ethyl-acetate extract, AA; ascorbic acid, DPPH; 2,2-diphenyl-1-picrylhydrazyl, IC_{50}; half-maximal inhibitory concentration.

$$\textbf{DPPH scavenging activity}\ (\%) = \frac{A_0 - A_1}{A_0} \times 100 \quad (1)$$

where: A_0; the absorbance of DPPH, A_1; the absorbance of the sample

2.5. Chemical composition determinations using gas-liquid chromatography (GLC)

2.5.1. Preparation of unsaponifiable matters (USMs) and fatty acids (FAs)

The BE of *B. carterii* (0.5 g) was refluxed with 0.5 N alcoholic sodium hydroxide for 2 hrs in a boiling water bath for saponification. The saponified extract underwent a cooling process, followed by its combination with an equivalent volume of distilled water, and subsequent extraction using diethyl ether. The diethyl ether extract was dehydrated using anhydrous sodium chloride, and the remaining residue was weighed and preserved for further analysis (Firsta et al., 2020). The USMs (%) was calculated according to Eq. (2):

$$\textbf{USMs}\ (\%) = \frac{\text{USMs}}{\text{Weight of petroleum ether extract}} \times 100 \quad (2)$$

The alkaline aqueous remaining solution was acidified with hydrochloric acid to liberate the FAs, which were separated using diethyl ether. The diethyl ether extract was dehydrated by evaporation, and the residue was subjected to methylation with acidified methanol for 1 hr. The methylated fatty acids were extracted with diethyl ether. The fatty acids methyl esters were identified by the GC apparatus.

2.5.2. Identification/separation of USMs and FAs by GLC technique

The measurements were performed at the National Research Center, Giza, Egypt. The identification of USM and FAs were performed using a Gas Chromatograph (Agilent Technologies 6890 N) by comparing the sample retention time with standards (Adams, 2007).

2.6. Preparation of B. carterii extracts as an emulsifiable concentrate (10 % EC) formulation

2.6.1. Tested materials

a) Active ingredient: Resin crude extracted by petroleum-ether (PE) and ethyl-acetate (EA).
b) Surface active agents: Potassium salt and polyethene glycol 600 monolaurate are supplied by the Egyptian Starch, Yeast & Detergents Co., Canal El Mahmoudeya St., Moharram Bey, Alexandria, Egypt. Tween 80 supplied by EL-Gomhoria Chemical Company, Cairo, Egypt.

2.6.2. Physicochemical properties of formulation basic constituents

2.6.2.1. Active ingredient.

a) The solubility or miscibility was calculated following an Eq. (3) developed by (Nelson and Fiero, 1954).

$$\textbf{Solubility}\ (\%) = \frac{W}{V} \times 100 \quad (3)$$

where: W; weight of active ingredient, V; volume of solvent required for complete solubility.

b) Acidity/Alkalinity was determined following (WHO, 1979) guidelines.

2.6.2.2. Surface active agents.

a) Surface tension was measured using a Du-Nouy tensiometer (ASTM D-1331, 2001).

b) Hydrophilic-lipophilic balance (HLB) was determined according to (Lynch and Griffin, 1974).

c) Critical micelle concentration (CMC) was calculated using (Osipow, 1964).

d) Acidity/Alkalinity was determined as previously mentioned.

2.6.3. Formulation of B. carterii extracts

The emulsifiable concentrate (10 % EC) of *B. carterii* was prepared

Table 11
In vitro antifungal activity of *B. carterii* formulated as an emulsifiable concentrate on some fungal pathogens.

Fungi*	Extracts	Mycelial growth inhibition (%) at concentration (ppm)				EC_{50}	EC_{90}	Slope value
		1000	2000	3000	4000			
R. solani	FPEE	11 ± 0.00	32 ± 0.98	53 ± 1.70	67 ± 3.21	2826.71	8193.29	2.77+/-0.33
	FEAE	36 ± 1.61	53 ± 1.70	68 ± 1.37	100 ± 0.00	1544.41	4413.31	2.81+/-0.31
S. rolfsii	FPEE	19 ± 1.6	33 ± 0.00	44 ± 0.37	66 ± 0.64	3003.17	13621.93	1.95+/-0.31
	FEAE	41 ± 1.61	47 ± 0.00	89 ± 0.00	100 ± 0.00	1426.81	4663.46	2.49+/-0.39
F. solani	FPEE	0	0	0	0	–	–	–
	FEAE	28 ± 3.21	41 ± 1.34	55 ± 0.37	70 ± 1.70	2312.62	12052.89	1.79+/-0.29
F. oxysporum	FPEE	0	0	0	0	–	–	–
	FEAE	20 ± 1.70	31 ± 1.85	43 ± 0.64	59 ± 1.61	3374.90	18829.26	1.71+/-0.30
F. semitectium	FPEE	31 ± 1.61	42 ± 1.70	52 ± 1.28	70 ± 1.61	2321.54	15209.36	1.57+/-0.29
	FEAE	41 ± 1.61	54 ± 1.34	68 ± 2.31	83 ± 2.89	1478.00	7505.06	1.82+/-0.29
B. cinerea	FPEE	29 ± 1.70	40 ± 2.31	50 ± 0.00	77 ± 0.64	2305.28	11025.99	1.91+/-0.30
	FEAE	32 ± 0.98	52 ± 1.28	70 ± 1.61	100 ± 0.00	1617.18	4172.38	3.11+/-0.32
A. alternata	FPEE	30 ± 1.61	45 ± 2.59	65 ± 0.98	81 ± 1.61	1874.33	7186.39	1.86+/-0.29
	FEAE	31 ± 2.06	54 ± 1.34	83 ± 0.74	100 ± 0.00	1540.61	3583.26	3.50+/-0.34
Phoma sp.	FPEE	0	20 ± 1.70	23 ± 0.74	31 ± 1.70	5752.78	18837.85	2.49+/-0.43
	FEAE	0	11 ± 0.00	18 ± 1.48	22 ± 0.00	7304.52	23947.37	2.49+/-0.50

* Values shown in the table are means ± standard error (n = 3), following Duncan's multiple range test. FPEE; formulated petroleum-ether extract, FEAE; formulated ethyl-acetate extract, EC_{50}; half maximal effective concentration, ppm; parts per million.

following (Rao et al., 2000). A certain quantity of extract was obtained, dissolved in an appropriate solvent, and an emulsifier was introduced. The mixture was stirred for 1 h, after which it was transferred to a measuring flask and filled with the same solvent to achieve volume of 10 mL. The flask was shaken to homogenize the solution, and the physicochemical properties of the formulation were conducted.

a) Emulsion stability test was performed according to (CIPAC MT 36.3, 2003).
b) The storage stability test was conducted as per (CIPAC MT 46.3, 2000).

2.6.4. Physicochemical properties of spray solution at field dilution rate (0.5 %)

a) Surface tension was determined, as mentioned before.
b) Viscosity was assessed using a viscometer according to (ASTM D-2196, 2005).
c) Electrical conductivity was determined as per (CIPAC MT 75.3, 2000) using a conductivity meter (Thermo Orion 115A + USA).
d) pH was measured as per (CIPAC MT 75.3, 2000) using a pH meter (Hanna pH 211).

2.7. In vitro antifungal activity of formulated B. carterii extracts

2.7.1. Fungal pathogens tested

The fungal pathogens *Rhizoctonia solani, Sclerotium rolfsii, Fusarium oxysporum, Fusarium semitectum, Fusarium solani, Alternaria alternata, Botrytis cinerea,* and *Phoma* sp. were acquired from the Department of Fungicides, Bactericides, and Nematicides at the Central Agricultural Pesticides Laboratory. The strains were cultured on a potato dextrose agar (PDA) medium under controlled conditions at a temperature of 25 ± 2 °C to ensure their viability and stability for subsequent investigations.

2.7.2. In vitro antifungal activity

In vitro, the FPEE and FEAE of *B. carterii* at different concentrations (1000, 2000, 3000 and 4000 ppm) were used to study their efficacy against fungal pathogens using food-poisoned technique (Schmitz, 1930). Uniform® 390SE (Azoxy-strobin + mefenoxam, 650 mL $200L^{-1}$) was used as a reference control at different concentrations (40, 81, 162 and 325 ppm). Both extracts were individually combined with 50 mL of sterilized Potato Dextrose Agar (PDA) medium and evenly distributed into three Petri dishes, serving as replicates. Following this, a fungal culture disc with a diameter of 6 mm was introduced precisely at the central region of every Petri dish. A zero treatment was prepared for each fungus utilized as a control. The dishes were subjected to incubation at a temperature of 25 ± 2 °C. The subsequent evaluation involved measuring the radial expansion of the fungus, which was performed once the mycelia of the control had nearly enveloped the entire surface area of the Petri dishes. The percentage of inhibition of fungal growth was determined using the following equation (4):

$$\textbf{The fungal growth inhibition}(\%) = \frac{C - T}{C} \times 100 \quad (4)$$

where: C; hyphal growth (mm) under control, T; hyphal growth (mm) under treatment

2.8. Toxicological effect of formulated B. carterii extracts on two-spotted spider mite

2.8.1. Rearing of two-spotted spider mites (TSSM)

The two-spotted spider mite (TSSM), *Tetranychus urticae* was reared at the Central Agricultural Pesticides Laboratory, Bioassay department, Agricultural Research Center, Dokki, Giza, Egypt, under lab conditions at 25 ± 2 °C, 65 ± 5 % RH, and a photoperiod of 16 light: 8 Dark h according to the procedure of Dittrich (1962).

2.8.2. In vitro acaricidal activity

To assess the toxicity of FPEE and FEAE at different concentrations (62.5, 125, 250, 500, 1000, 2000 and 3000 ppm) on *T. urticae,* the leaf disc-dip technique was used (Siegler, 1947). Additionally, control discs were dipped in tap water only. A reference control, Abamectin (Vertimec® 1.8 % EC) was utilized at concentrations of 2.25, 4.5, 18, 36 and 54 ppm. Mortality (%) were counted utilizing the methodology established by Abbott (1925) following the Eq. (5). The calculation of the toxicity index for the tested compounds was performed in accordance with the methodology described by Sun (1950) following the Eq. (6).

$$\textbf{Mortality}\ (\%) = \frac{T - C}{100 - C} \times 100 \quad (5)$$

$$\textbf{Toxicity index} = \frac{LC_{50}\ \text{of the most toxic compound}}{LC_{50}\ \text{of other compounds}} \times 100 \quad (6)$$

where: T; mortality (%) of treatment, C; mortality (%) of control.

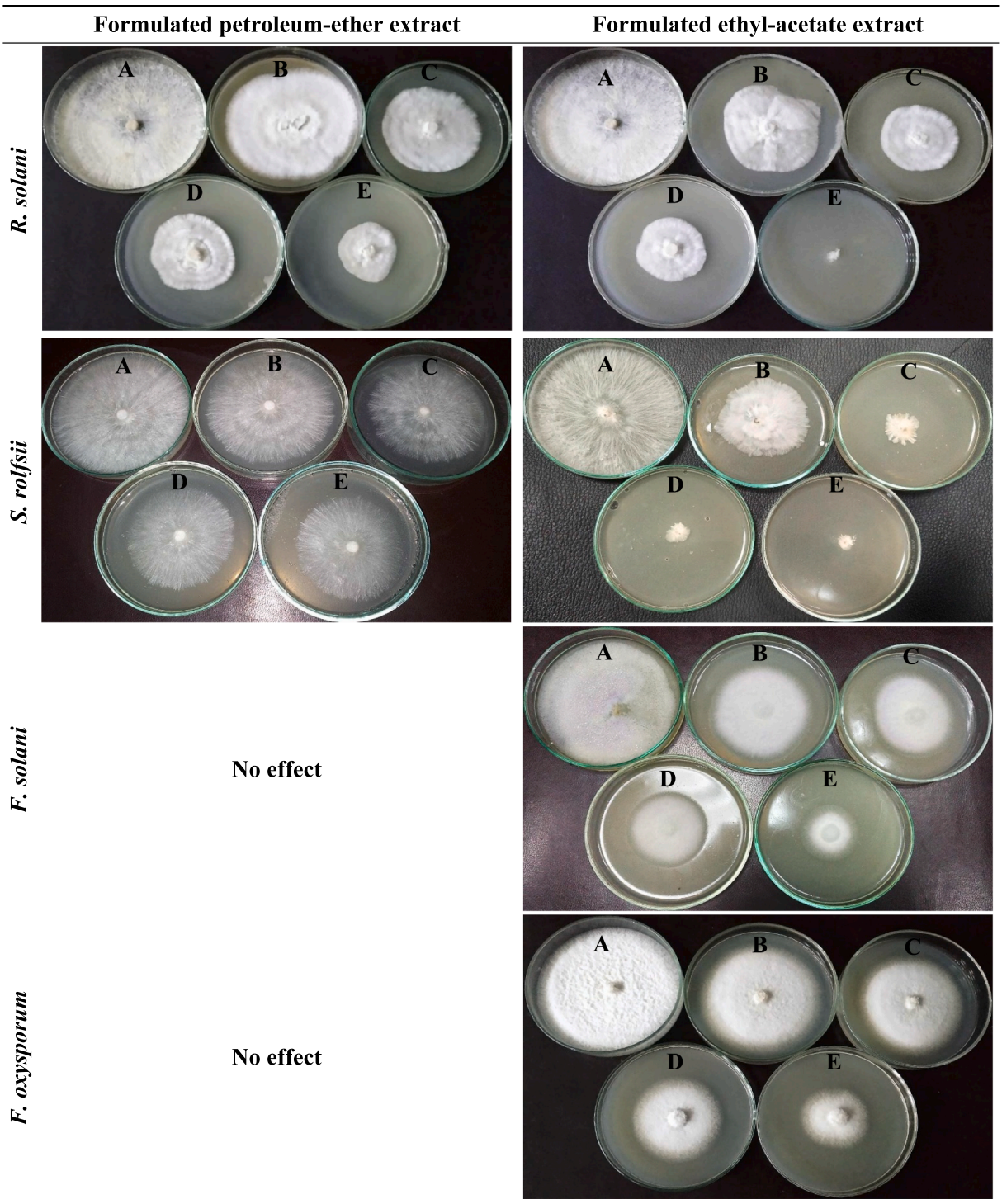


Fig. 3. *In vitro* antifungal activity of *B. carterii* formulated as an emulsifiable concentrate on some fungal pathogens. A; Control, B; 1000 ppm, C; 2000 ppm, D; 3000 ppm, and E; 4000 ppm.

2.9. Statistical analysis

All experiments were conducted in a completely randomized design (CRD) with three replications for each treatment. The statistical analysis of all data was conducted utilizing the principles of analysis of variance (ANOVA) and Duncan's multiple-range test (Duncan, 1955). The formula proposed by (Abbott, 1925) was employed to ascertain the corrected mortality rate of mites. The LC_{50} and LC_{90} values for various formulations were determined by logarithmically transforming each concentration and its corresponding probit value, which represents the inhibition percentage (Finney, 1971; Lei and Sun, 2018).

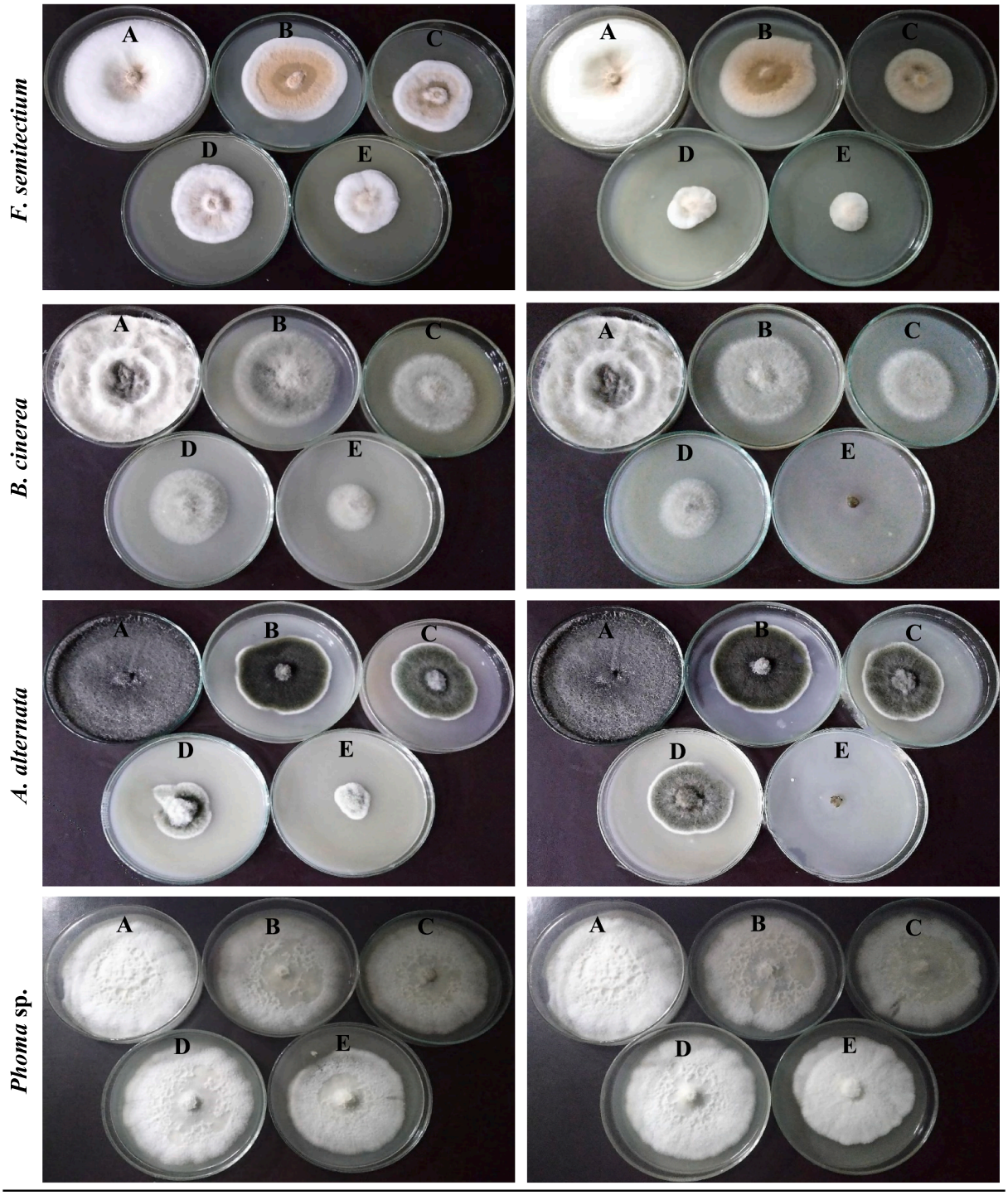


Fig. 3. (*continued*).

3. Results

3.1. Chemical composition of B. carterii resin crude extracts

This work evaluated the efficacy of *B. carterii* resin as an emulsifiable concentrate formulation against *Tetranychus urticae* and some phytopathogenic fungi (tomato fungal diseases and grey mould mulberry) *in vitro*. As shown in Table 1, we obtained 46.0 % and 39.8 % yield of yellowish resin crude extracted by petroleum-ether (PE) and ethyl-acetate (EA), respectively.

The phytochemical screening of EA obtained from *B. carterii* revealed the existence of saponins, alkaloids, flavonoids, phenolic compounds and glycosides. On the other hand, in *B. carterii* PE only steroids and tannins were identified (Table 2).

3.2. Total phenolic and flavonoids contents in B. carterii ethyl-acetate

Phenolic and flavonoids are secondary metabolites with redox properties contributing to their antioxidant capabilities. Total phenol and flavonoids content in *B. carterii* EA were assessed using a colorimetric technique (Table 3). The cumulative phenolic and flavonoid contents in *B. carterii* EA were recorded 3.28 mg GAE g^{-1} and 0.29 mg

Table 12
In vitro antifungal activity of Uniform® (a reference control) on some fungal pathogens.

Fungi*	Mycelial growth inhibition (%) at concentration (ppm)				EC_{50}	EC_{90}	Slope value
	40	81	162	325			
R. solani	31 ± 1.70	48 ± 1.85	91 ± 1.28	100 ± 0.00	64.80	170.62	3.05+/- 0.29
S. rolfsii	64 ± 1.61	72 ± 0.00	88 ± 0.64	100 ± 0.00	45.41	153.84	2.42+/- 0.28
F. solani	44 ± 0.00	78 ± 0.98	100 ± 0.00	100 ± 0.00	45.17	98.32	3.79+/- 0.44
F. oxysporum	35 ± 1.85	57 ± 2.31	77 ± 0.37	88 ± 0.74	64.16	335.88	1.78+/- 0.22
F. semitectium	33 ± 0.74	54 ± 1.34	87 ± 1.70	100 ± 0.00	63.64	179.68	2.84+/- 0.23
B. cinerea	64 ± 1.61	76 ± 1.85	86 ± 1.61	100 ± 0.00	27.71	151.15	1.74+/- 0.26
A. alternata	26 ± 1.85	48 ± 1.85	65 ± 0.98	100 ± 0.00	81.28	267.06	2.48+/- 0.24
Phoma sp.	0	0	0	0	–	–	–

* Values shown in the table are means ± standard error (n = 3), following Duncan's multiple range test. EC_{50}; half maximal effective concentration, ppm; parts per million.

QE g^{-1}, respectively.

3.3. Identification/separation of USMs and FAs by GLC

The saponification procedure was conducted on a 0.5 g sample of *B. carterii* PE, yielding 12.6 % saponified matters (SMs) and 87.2 % unsaponifiable matters (USMs). Analysis using gas–liquid chromatography (GLC) identified 12 compounds in the saponified matters (SMs) (Fig. 1 and Table 4). The chromatographic profile of the SMs indicated a notable concentration of unsaturated fatty acids, constituting 69.4 % of the total content, while saturated fatty acids accounted 30.6 %. The most abundant compounds observed were oleic acid (36.5 %), followed by α-linolenic acid (16 %) and linoleic acid (13.1 %), whereas arachidic acid had the lowest presence at 1.3 %. These findings imply that *B. carterii* PE contains a substantial quantity of volatile components, with oleic acid being the most prominent and arachidic acid being the least abundant.

Fig. 2 and Table 5 display the chemical composition of unsaponifiable substances as determined by GLC analyses, confirming the presence of eight hydrocarbons (57.0 %) and four sterols (43.0 %). Among the components, the highest area under the curve was observed for n. henicosanoic (26.3 %), followed by squalene (17.1 %), β.sitosterol (15.5 %), and stigmasterol (14.7 %). Conversely, the lowest area under the curve was found for n.Docosane (0.9 %) and n.Tetracosane (0.6 %), indicates the most prominent and least abundant constituents in *B. carterii* PE, respectively.

3.4. Characterization of formulation components

Table 6 displays the physicochemical characteristics of *B. carterii* PE and EA. The *B. carterii* PE demonstrated insolubility in water but solubility in xylene (83 %) and complete solubility in acetone (100 %), with an acidity level of 0.80. On the other hand, the *B. carterii* EA exhibited similar properties of insolubility in water but solubility in xylene (71 %) and complete solubility in acetone (100 %), showcasing the highest acidity level (3.43).

The results presented in Table 7 describe the physical and chemical characteristics of the surfactants utilized in the emulsifiable concentrate formulation of *B. carterii* resin. Both surfactants, namely Tween 80 and polyethene glycol 600 monolaurate (PEG 600ML) possessed hydrophilic-lipophilic balance values exceeding 13 and exhibited weakly acidic properties equivalent to 0.50 (Tween 80) and 0.88 (PEG 600ML), comparable to sulfuric acid. Additionally, both surfactants demonstrated low surface tension values, with Tween 80 and PEG 600ML measuring 39.2 and 30.64 dyne cm^{-1}, respectively. Furthermore, the critical micelle concentration for Tween 80 and PEG 600 ML was determined to be 0.5 and 0.3 %, respectively.

The data in Table 8 provides an overview of the physical and chemical characteristics of emulsifiable concentrate (10 % EC) formulations. These formulations were subjected to three days of storage at a temperature of (54 ± 3 °C). The results indicate that the new formulations successfully passed in tests assessing the stability and spontaneity of the emulsions. No indications of oil separation, precipitation, or cream separation were observed in either soft or hard water. Before storage, the newly developed emulsifiable concentrates exhibited a mild acidity level, producing minimal foam in both types of water. However, there was a slight increase in free acidity for both formulations after accelerated storage.

Table 9 presented the physicochemical properties of *B. carterii* resin extracts formulated into an emulsifiable concentrate (10 % EC) and used at a field dilution rate (0.5 %). The formulated extracts exhibited characteristics such as low surface tension, high viscosity, high electrical conductivity, and low pH. The surface tension (dyne cm^{-1}) of FPEE and FEAE was 37.02 %. The viscosity (cm $poise^{-1}$) of FPEE and FEAE were 1.40 and 1.48, respectively. The electrical conductivity (μ mhos) of FPEE and FEAE were 317 and 323, respectively. Additionally, the pH of FPEE and FEAE were 6.90 and 6.80, respectively.

3.5. Antioxidant activity of B. carterii formulated as an emulsifiable concentrate

The antioxidant potential of *B. carterii* formulated as an emulsifiable concentrate at various concentrations, was determined by neutralizing the stable DPPH radical cation presented in Table 10. Compared to ascorbic acid (used as a reference), the findings demonstrate that the antioxidant activity (%) of FPEE and FEAE was increased with increasing concentration. At higher concentrations, FPEE (10 mg ml^{-1}), FEAE (10 mg ml^{-1}) and ascorbic acid (0.5 mg ml^{-1}) exhibited an antioxidant activity (%) of 62 %, 90.8 %, and 92 %, respectively. In the DPPH assay, FPEE displayed low reducing power capacity, while FEAE showed good concentration-dependent activity. The FPEE displayed a favorable IC_{50} value of 8.68 mg ml^{-1}, FEAE demonstrated a moderate activity of 4.15 mg ml^{-1}, while ascorbic acid exhibited an excellent IC_{50} value of 0.19 mg ml^{-1}.

3.6. In vitro efficacy of B. carterii formulated as an emulsifiable concentrate

3.6.1. Antifungal activity

In vitro, the FPEE and FEAE at different concentrations (1000, 2000, 3000, and 4000 ppm) were tested for their ability to hinder the mycelial growth of fungal pathogens. Uniform® 390SE served as a reference control, with different concentrations (40, 81, 162 and 325 ppm). The antifungal activity of *B. carterii* resin extracts formulated as an emulsifiable concentrate on *R. solani*, *S. rolfsii*, *F. solani*, *F. oxysporum*, *F. semitectium*, *B. cinerea*, *A. alternata* and *Phoma* sp. were summarized in (Table 11 and Fig. 3) in comparison to the performance of Uniform® 390SE as illustrated in (Table 12 and Fig. 4). The FPEE displayed antifungal activity against tested fungal pathogens at different concentration, except for *F. solani* and *F. oxysporum*. The greatest suppression of fungal growth was observed against *R. solani*, *S. rolfsii*, *F. semitectium*, *B. cinerea*, and *A. alternata,* particularly at 4000 ppm concentration. Similarly, FEAE also exhibited antifungal activity against all tested

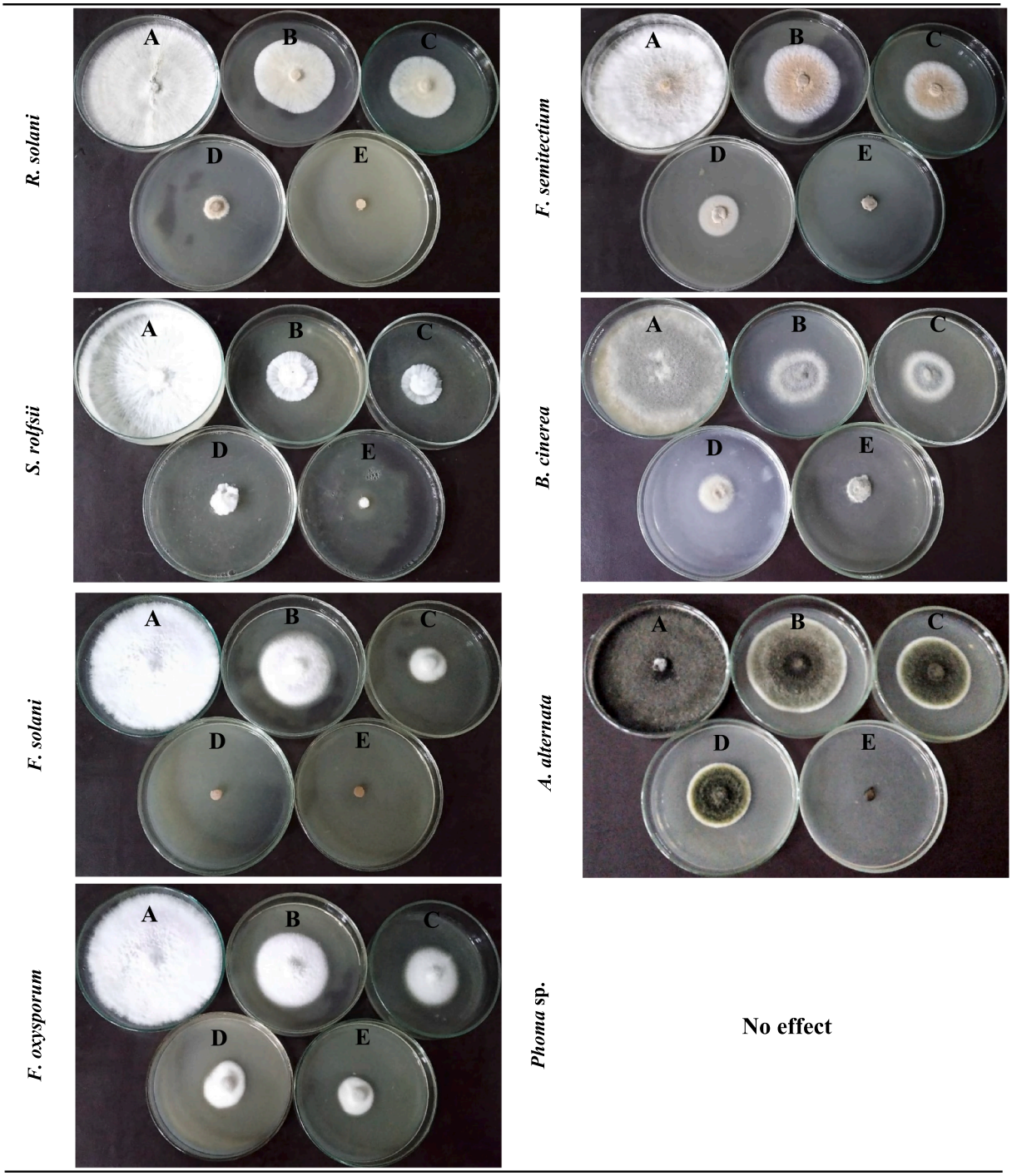


Fig. 4. *In vitro* antifungal activity of Uniform® (a reference control) on some fungal pathogens. A; Control, B; 40 ppm, C; 81 ppm, D; 162 ppm and E; 325 ppm.

Table 13
In vitro acaricidal activity of *B. carterii* formulated as an emulsifiable concentrate and Abamectin (a reference control) on *T. urticae*.

Treatments*	LC_{50} (ppm) (95 % Fiducial limits)	Slope ± S.E	Toxicity index
Abamectin	6.66 (2.04–12.51)	1.23 ± 0.12	100
FPEE	422.58 (252.77–654.65)	0.89 ± 0.14	1.58
FEAE	539.50 (408.11–698.11)	1.92 ± 0.25	1.23

* FPEE; formulated petroleum-ether extract, FEAE; formulated ethyl-acetate extract, LC_{50}; lethal concentration 50, ppm; parts per million, S.E.; standard error.

fungal pathogens. At a concentration of 4000 ppm, it completely ceased the growth of *R. solani*, *S. rolfsii*, *B. cinerea*, and *A. alternata*. Interestingly, *Phoma* sp., unlike the other tested fungal pathogens, appeared resistant or more tolerant to both extracts.

In vitro, Uniform® 390SE (a reference control) at different concentrations (40, 81, 162, and 325 ppm) was used to assess its ability to inhibit fungal pathogens (Table 12 and Fig. 4). The fungicide exhibited suppressive effects against all tested fungal pathogens, except for *Phoma* sp. At a concentration of 325 ppm, the application of Uniform® 390SE effectively and entirely inhibited the mycelial proliferation of various fungal species. Furthermore, it displayed a notable 89 % inhibition against *F. oxysporum*, while *Phoma* sp. did not exhibit any response to the

fungicide at different concentrations.

3.6.2. Acaricidal activity

The acaricidal activity of FPEE and FEAE at various concentrations (62.5, 125, 250, 500, 1000, 2000 and 3000 ppm) was evaluated for their toxicity against the adult females of *T. urticae* using leaf disc-dip technique. Abamectin (Vertimec® 1.8 % EC) with concentrations (2.25, 4.5, 18, 36 and 54 ppm) was used as a reference control (Table 13). Concerning LC_{50} level, FPEE displayed the most potent acaricidal effect on *T. urticae,* with an LC_{50} value of 422.58 ppm and a corresponding toxicity index of 1.58 after 72 h of treatment. Conversely, FEAE exhibited moderate toxicity against *T. urticae*, with an LC_{50} value of 539.50 ppm and a toxicity index 1.23 after 72 h of treatment. These findings indicate that FPEE is 1.28 times more effective than FEAE against *T. urticae* adult female.

4. Discussion

Modern agriculture extensively depends on pesticides to eradicate pests and disease control. However, their indiscriminate application negatively affects soil, water, and the overall ecosystem, thereby impacting animal and human health (Aktar et al., 2009). Recently, plant extracts and biostimulants have gained considerable attention as environmentally friendly remedies for plant diseases due to their natural properties, antimicrobial activities, ease of decomposition in the environment, and non-harmfulness to plants, animals and human health (Martínez, 2012; Abo-Elyousr et al., 2019; Ashmawy et al., 2020; Sánchez-Montesinos et al., 2021).

Current work was mainly conducted to assess the efficacy of *B. carterii* resin formulated as an emulsifiable concentrate (10 % EC) against *T. urticae* and some phytopathogenic fungi (tomato fungal diseases and grey mould mulberry). Our findings showed that *B. carterii* PE and EA yields were 45.9 % and 39.7 %, respectively (Table 1). In contrast, *B. dalzielii* yielded 16.08 %, 3.37 % and 0.03 % when methanol, hexane and ethyl-acetate were used as solvents (Kohoude et al., 2017). Additionally, Jansen et al. (2010) observed that *B. dalzielii* leaves produced yields of 4.7 %, 24.7 % and 33.4 % when subjected to solvents such as dichloromethane, methanol and water-based decoction, respectively.

The phytochemical screening of *B. carterii* PE and EA revealed the presence of various substances, including saponins, alkaloids, flavonoids, phenolic compounds, glycosides, steroids and tannins (Table 2). Additionally, total phenol and flavonoids in *B. carterii* EA were 3.28 mg GAE g^{-1} and 0.29 mg QE g^{-1}, respectively (Table 3). These findings align with a previous study by Kohoude et al. (2017), who identified higher levels of phenolic (315.97 g GAE/kg dry mass) and flavonoid (37.19 g QE/kg dry mass) contents in methanol extract of *B. dalzielii* leaves. Further, previous research has reported that medicinal plants containing phenolic and flavonoid compounds exhibit antioxidative, antibacterial, antiviral, and anticancer properties (Tungmunnithum et al., 2018). The specific type and quantity of these compounds vary across different plant parts and are influenced by the choice of solvent for extraction (Vuddanda et al., 2016).

Analysis of *B. carterii* PE using GLC revealed the presence of 24 compounds in both the saponified and unsaponified fractions (Figs. 1 & 2, and Tables 4 & 5). Numerous studies have explored the composition of *B. carterii* oil and have reported varying components. In the study conducted by Kubmarawa et al. (2006), a total of 29 compounds were discerned within the essential oil of *B. dalzielii*. Notably, the prevailing constituents were a-Pinene, accounting for 45.7 % of the composition, and a-terpinene, representing 11.5 % of the mixture. Another study by Van Vuuren et al. (2010) identified various components in the essential oils of *B. carterii*, encompassing α-pinene (2.0–64.7 %), α-thujene (0.3–52.4 %), β-pinene (0.3–13.1 %), myrcene (1.1–22.4 %), sabinene (0.5–7.0 %), limonene (1.3–20.4 %), p-cymene (2.7–16.9 %) and β-caryophyllene (0.1–10.5 %). Furthermore, Kohoude et al. (2017) unveiled a remarkable array of 50 distinct chemical compounds within the essential oil of *B. dalzielii.* Amongst these compounds, 3-carene (27.72 %) and a-pinene (15.18 %) were observed. Furthermore, the methanol extract was found to contain 2,5-dihydroxy acetophenone and b-D-xylopyranose.

Results displayed in Tables 6–9 depict the physicochemical properties of PE and EA extracts from *B. carterii*. These extracts exhibit insolubility in water but can be effectively dissolved in xylene and acetone. This suggests that both extracts can be effectively used in the formulation of emulsifiable concentrates (Abd-Alla and Hamouda, 2021). The emulsifiable concentrate formulation of *B. carterii* resin employs surfactants that possess qualities suitable for serving as dispersing agents during the creation of these emulsifiable concentrates as emulsifiers (Abd-Alla and Hamouda, 2021). Table 9 outlines the physicochemical properties of *B. carterii* resin extracts formulated into emulsifiable concentrate (10 % EC) and applied at a 0.5 % field dilution rate. The observations underscore that the resulting spray solution exhibits low surface tension, high viscosity, high conductivity, and a diminished pH value. The augmentation of the pesticide spray solution's efficacy in pest control is achieved through the reduction of surface tension, thereby facilitating its superior wetting, spreading, and adhesion to the surfaces of treated plants (Pereira et al., 2016; Safar et al., 2022). The manipulation of pH levels, coupled with the augmentation of electrical conductivity within the spray solution, serves to expedite the deionization process of pesticides. This, in turn, fosters their deposition and penetration into the surfaces subjected to treatment, ultimately amplifying their retention and overall efficacy (El-Sisi et al., 2011). The heightened viscosity of the spray solution also contributes to its enhanced biological efficacy by mitigating pesticide drift and enhancing adhesion to treated surfaces (Spanoghe et al., 2007).

The antioxidant potential of the formulated *B. carterii* was evaluated by neutralizing the stable free radical DPPH (Table 10). At higher concentrations, FPEE (10 mg ml^{-1}), FEAE (10 mg ml^{-1}) and ascorbic acid (0.5 mg ml^{-1}) exhibited an antioxidant efficacy (%) of 62 %, 90.8 %, and 92 %, respectively. Compared to the potent antioxidant effect of ascorbic acid, FPEE exhibited limited capability in DPPH reduction, whereas FEAE demonstrated a moderate activity level. These results could be attributed to the high content of phenolic and flavonoid components in FEAE. These compounds may act as electron donors, interacting with free radicals to convert them into more stable substances, thereby terminating the radical chain reaction. In light of these findings, it can be inferred that FEAE holds significant potential as an antioxidant (Ruberto and Baratta, 2000; Mothana et al., 2011; Prakash et al., 2014).

The efficacy of *B. carterii* resin extracts against a diverse array of fungal strains has been presented in (Tables 11 & 12, and Figs. 3 & 4). The results indicated that the FPEE showed strong antifungal activity against most of the tested fungal pathogens, except for *F. solani* and *F. oxysporum*. The highest inhibition of mycelial growth was observed in *R. solani* (67 %), *S. rolfsii* (66 %), *F. semitectium* (70 %), *B. cinerea* (77 %), and *A. alternata* (81 %) at a concentration of 4000 ppm. The findings of the FEAE experiment revealed a striking pattern, effectively impeding the mycelial expansion of at a concentration of 4000 ppm. However, *Phoma* sp. exhibited more resilience to growth inhibition caused by both extracts. Chemical analysis of *B. carterii* EA resin crude extracts revealed the presence of various secondary metabolites, such as flavonoids and phenolics, known for their antioxidant properties due to their redox abilities. Furthermore, the composition of *B. carterii* PE using GLC shows predominant components comprising oleic acid (36.5 %), α-linolenic acid (16 %), linoleic acid (13.1 %), n.henicosanoic (26.3 %), squalene (17.1 %), β.sitosterol (15.5 %), and stigmasterol (14.7 %). These findings are consistent with previous studies (Camarda et al., 2007; Goñi et al., 2009; El-Nagerabi et al., 2013; Prakash et al., 2014; Sadhasivam et al., 2016; Stupar et al., 2016; Chaurasia and Gharia, 2017; Venkatesh et al., 2017; Powers et al., 2018; Raveau et al., 2020), suggesting that the compounds present in *B. carterii* extracts have the potential to disrupt enzymatic reactions within fungal hyphae, thus impacting their

metabolism and growth. This interference likely affects various aspects of fungal development, potentially leading to mechanisms such as cytoplasm retraction and hyphal wall disintegration.

The acaricidal activity of *B. carterii* FPEE and FEAE was assessed against adult females of *T. urticae* using the leaf disc-dip method (Table 13). Concerning LC_{50} level, FPEE exhibited higher toxicity against *T. urticae* (LC_{50} = 422.58 ppm) compared to FEAE (LC_{50} = 539.50 ppm) after 72 hrs of treatment. This could be attributed to the presence of various volatile components comprising oleic acid (36.5 %), α-linolenic acid (16 %), linoleic acid (13.1 %), n.henicosanoic (26.3 %), squalene (17.1 %), β.sitosterol (15.5 %), and stigmasterol (14.7 %) within FPEE, which were the most dominant. These compounds have the potential to block mite spiracles, leading to suffocation. Moreover, they can penetrate cell membranes, accumulate in the cytoplasm, cause cellular dehydration, and induce DNA condensation in the nucleus. Our findings align with a study conducted by Roh et al. (2011), who assessed the impact of *B. carterii* oil as an acaricide on two-spotted spider mites in a controlled laboratory setting. They recorded a 24.8 % mortality rate among mites and a reduction in egg laying, indicating a deterrent effect of the oil compared to the control.

In a study by Yoon and Tak (2018), the repellent and acaricidal properties of frankincense oil on adult two-spotted spider mites *T. urticae* were documented. Similarly, Kiran et al. (2017) found that essential oil from *B. carterii* is a potent insecticide against *Callosobruchus chinensis* and *C. maculates*, demonstrating no negative impact on seed germination. Furthermore, the remarkable efficacy of frankincense nanoemulsion in combating 2nd instar larvae of the spiny bollworm *Earias insulana* (Metayi et al., 2022). Their findings unequivocally demonstrate the enduring inhibitory effects on larvae, pupae, adult longevity, and reproductive capacity. According to Sabtharishi and Naveen (2017), Jankowska et al. (2018), Boate and Abalis (2020) and Yang et al. (2020), the toxicity exhibited by plant extracts might be attributed to their fatty acid components. The toxic effect of these fatty acids is due to their ability to penetrate the insect body covering and disrupt the lipoprotein matrix of the insect cellular membranes. Consequently, this disruption leads to the release of cellular contents, ultimately leading to cellular dehydration and death.

5. Conclusions and future perspective

The present study assessed the antifungal and acaricidal activities of *B. carterii* FPEE and FEAE *in vitro*. Our findings suggest that FEAE holds promise to serve as a natural antifungal remedy for preventing tomato fungal diseases and mulberry grey mould disease. Furthermore, FPEE exhibited specific capabilities in combating *T. urticae*, indicating its potential as an effective acaricidal agent. Nonetheless, further studies are necessary to assess the antifungal/acaricidal activities of *B. carterii* extracts *in vivo* and to deepen our comprehension of how these extracts impede fungal mycelia growth at a molecular level.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors also thank the Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2023R182), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

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