International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 15-03-2025, Accepted: 15-04-2025, Published: 30-04-2025
Volume 10, Issue 4, 2025, Page No. 155-164

Toxicological, chemical profiling, biochemical, and histological impacts of some selected essential oils against *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae)

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Abstract
The toxicity of four plant-derived essential oils extracted from *Boswellia Carterii* Birdw., *Melissa officinalis* L, *Valeriana officinalis* L., and *Cymbopogon citratus* (DC.) Stapf. against 2nd and 4th instars of *Spodoptera littoralis* larvae at different time intervals and under laboratory conditions was assessed compared with Indoxcarb. Of the tested essential oils, *B. carterii* BCEO and *M. officinalis* MOEO were the most potent after 3 and 5 days of treatments with LC_{50} values (287.68 and 457.84 µg/ml) and (151.31 and 266.03µg/ml), respectively, against 2nd instar larvae, while against 4th instar larvae MOEO recorded the highest toxicity followed by BCEO after 3 and 5 days of treatments and LC_{50} values were (650.32 and 1239.01 µg/ml) and (132.94 and 306.09 µg/ml), respectively. The GC-MS technique was used to perform qualitative and quantitative phytochemical analyses. *B. carterii* BCEO riches with n-Octyl acetate (37.95%), E-Nerolidol (27.40%), Incensole (9.56%), and Isoincensole (4.30%), while *M. officinalis* MOEO riches with (-)-Spathulenol (23.94%), Caryophyllene oxide (19.09%), and β-Curcumene (10.63%). The effect of the promising essential oils (BCEO and MOEO) on some key enzymes was studied. A significant inhibition in the activity of glutathione S-transferase (GST) was observed by BCEO (47.58%) and MOEO (30.67%) in comparison to the control; also, MOEO recorded a significant inhibition on AChE activity of57.28%. Histological studies on the midgut revealed that BCEO and MOEO caused basement membrane separation, degeneration, and loss of some epithelial cell lining. The presented data announces the potential of designing two eco-friendly green insecticides from plant-derived essential oils.

Keywords: Essential oils, Toxicity, *Spodoptera littoralis*, phytochemical analyses, Biochemical parameters, Histology

Introduction

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is a highly destructive polyphagous pest. The larval stage is well-known as a notorious leaf consumer, almost infesting more than 100 different species of crops and vegetables, causing widespread economic losses [1, 2]. The management program, based mainly on insecticide usage and the long-term use of conventional synthetic insecticides, resulted in several negative impacts such as environmental pollution, insecticide resistance, hazards to human health, natural enemies, and beneficial insects [3].

The ecological concerns, resistance emergence, health risks, and satisfying the needs for quality-demanding crop products paved the way to develop sustainable, naturally occurring green insecticides and minimize the usage of harmful synthetic insecticides [4].

Plant-derived essential oils have been recognized as a perfect candidate and important natural source of new green pesticide generation. Essential oils are a considerable alternative to traditional pesticides because of their low toxicity for humans and wildlife, short residual persistence, rapid biodegradation, and renewable resources [5]. Several studies proved the bioactivity of essential oils as acute toxicants, feeding deterrents, growth retardants, ovicidal, ovipositional deterrents, and detoxifying enzyme inhibitors [6, 7].

Most plant essential oils are complex mixtures of terpenoids, and their bioactivity comes from how well the ingredients work together [8]. Mono- and sesquiterpenoids are rapid-acting neurotoxins potentially interacting with multiple target sites in insects, compared to synthetic pesticides, their application is less likely to lead to resistance [3, 7, 9]. Consequently, several effective extraction methods in terms of quality, cost, and environmental friendliness are being investigated [10].

As part of our ongoing screening program for plant-derived insecticides, four essential oils of plant origin were extracted using a hydrodistillation Clevenger-type apparatus and assessed for their insecticidal activity against 2nd and 4th instars of *S. littoralis* larvae under laboratory conditions. Phytochemical analysis of the most potent essential oils was evaluated using the GC-MS technique along with their effect on some key enzymes and histological effects against the midgut of 4th instar larvae of *S. littoralis.*

Materials and methods

Plant Materials

Valeriana officinalis L. VOEO (Caprifoliaceae) (roots), *Melissa officinalis* L. MOEO (Lamiaceae) (aerial parts) and *Boswellia Carterii* Birdw. BCEO (Burseraceae) (resin) were purchased from Mansoura's herbal marketplaces, while *Cymbopogon citratus* (DC.) Stapf CCEO (Poaceae) (aerial parts) was picked up from Mansoura University farm.

Insecticides (Indoxacarb): Kamfal 15% EC, Jiangsu Flag Chemical Industry Co.

Essential Oil Extraction

The four essential oils were hydrodistillated using a Clevenger-type apparatus for 8 hrs and dried over anhydrous sodium sulphate before storage in the dark at -20ºC.

Gas Chromatography-Mass Spectrometry

GC/MS experiments were carried out on a Varian GC interfaced to Finnegan SSQ 7000 mass selective detector (SMD) with ICIS V2.0 data system for MS identification of the GC components. DB-5 was the column that was used. The oven's temperature was set to be isothermal for three minutes at 50°C, then heated by 7°C per minute to 250°C and then isothermally for ten minutes at 250°C. A 70 eV ionization energy was established.

Insect rearing

A laboratory strain of cotton leaf-worm *S. littoralis* was reared in the Plant Protection Research Institute (A.R.C.) laboratory, Dokki, Giza, Egypt for several generations. The tested insect was reared in an incubator under carefully monitored conditions on fresh castor bean leaves, *Ricinus communis* L. Daily observations were conducted at 60-70% relative humidity and 22°C for the experiment.

Bioassay

Newly hatched neonates of *S. littoralis* were fed on spinach leaves for two generations, and the leaf dipping method was used for 20 seconds just before drying by air. The essential oils of *B. carterii*, *M. officinalis*, *V. officinalis*, and *C. citratus* at six concentrations (5000, 2500, 1250, 625, 312.5, and 156.25 µg/ml) as well as Indoxacarb at five concentrations (20, 10, 5, 1, and 0.5 µg/ml) were evaluated for their effectiveness on the 2nd and 4th larvae instars in terms of the insect's mortality compared to the control. Each treatment was replicated four times with 10 larvae each. parallel control of 10 untreated larvae was performed by four replicates as well. The mortality percentage was measured after one day and every 48 hours until the 5th day.

Biochemical analysis

To test the enzyme activity, the LC50 of the most toxic essential oils was used. Alive adult individuals from the 4th instar of *S. littoralis* were transferred and treated with each selected essential oil along with the standard insecticide. The live individuals were collected, weighed, and stored at 4 °C after 24 h exposure. Untreated samples were employed as controls. All treated samples were sent to the Plant Protection Research Institute analysis unit, A.R.C. to assay the pest's enzyme activities of ALP [11], ACP [12], AST and ALT [13], AChE [14], and GST [15].

Histopathological studies

The histological effects of the most potent essential oils and Indoxacarb at their LC_{50} concentration were applied to the 4th instar larvae using the leaf dipping technique. Two of the survived larvae and untreated larvae were taken after five days of treatment and saved in 10% formalin solution. Tissue processing, sectioning, and staining were conducted [16] (Gaaboub *et al*. 2012) in Faculty of Medicine, Mansoura University, Egypt.

Data analysis

Mortality percentages were determined after one, three, and five days of initial application and corrected by using Abbotts' equation [17]. The median lethal concentration LC_{50}, LC_{90} and slope values were estimated using probit analysis according to Finney [18]. Toxicity index was computed for different essential oils by comparing these materials with the most effective one using Sun's equation [19].

Results

Bioassay

Four selected essential oils; *B. carterii* BCEO, *M. officinalis* MOEO, *V. officinalis* VOEO, and *C. citratus* CCEO besides a recommended insecticide Indoxacarb were assessed for their toxicity against 2nd and 4th instars of *S. littoralis* larvae under laboratory conditions. All EOs even if they act as an antifeedant from the first day, have the potential to cause larval mortality after three days, whereas Indoxacarb causes observed mortality after one day. Data observed from Table 1 indicated that the highest concentrations of *M. officinalis*, *B. carterii*, *V. officinalis*, and *C. citratus* recorded the highest total mortality rate against the 2nd instar larvae, respectively. Consequently, the lowest concentrations of *B. carterii*, *M. officinalis*, *V. officinalis*, and *C. citratus* had the lowest total mortality rate. On the other hand, the 4th instar larvae showed significant total mortality percent at the highest and lowest concentrations of *M. officinalis*, *B. carterii*, *V. officinalis*, and *C. citratus*, respectively. A further increase in all essential oil concentrations resulted in high mortality in larvae at any time for both instars.

Tables 2 and 3 displayed the toxicity of tested essential oils and Indoxacarb against *S. littoralis* 2nd instar larvae after 3 and 5 days of exposure. Probit analysis revealed that the selected essential oils showed high larvicidal activity after 3 days. Indoxacarb insecticide had the highest larvicidal efficacy among all tested toxicants. *B. carterii* essential oil was the most potent followed by *M. officinalis*, *C. citratus*, and *V. officinalis*, respectively, with LC_{50} values ranging from 287.68 to 742.98 µg/ml and LC_{90} values from 2362.41 to 17091.81 µg/ml, as shown in Table 2. Of the tested essential oils in Table 3, *B. carterii* showed the highest larvicidal potency against *S. littoralis* 2nd instar larvae after 5 days of exposure, followed by *M. officinalis*, *C. citratus*, and *V. officinalis*, respectively. The tested EOs demonstrated clear larvicidal effects after 5 days, with LC_{50} and LC_{90} falling between 151.31 and 412.11 µg/ml and 1683.55 and 6198.50 µg/ml, respectively.

Table 1: Efficacy of some selected essential oils against the 2nd and 4th instars larvae of *S. littoralis* under laboratory condition

Tested compounds	Concentrations (µg/ml)	Mortality after days post treatments					
		2nd instar			4th instar		
		1 day	3 days	5 days	1 day	3 days	5 days
B. carterii	5000	0	82.50	95.00	5.00	72.50	90.00
	2500	0	75.00	92.50	0	60.00	82.50
	1250	0	67.50	85.00	0	50.00	75.00
	625	0	57.50	77.50	0	35.00	62.50
	312.5	0	50.00	65.00	0	27.50	50.00
	156.25	0	40.00	50.00	0	17.50	40.00
M. officinalis	5000	0	85.00	97.50	10.00	82.50	92.50
	2500	0	75.00	92.50	2.50	70.00	87.50
	1250	0	62.50	85.00	0	55.00	80.00
	625	0	52.50	67.50	0	47.50	65.00
	312.5	0	37.50	55.00	0	37.50	62.50
	156.25	0	30.00	40.00	0	27.50	52.50
V. officinalis	5000	5.00	80.00	87.50	7.50	70.00	85.00
	2500	0	67.50	77.50	0	60.00	77.50
	1250	0	57.50	65.00	0	47.50	67.50
	625	0	40.00	55.00	0	35.00	57.50
	312.5	0	35.00	42.50	0	25.00	40.00
	156.25	0	22.50	27.50	0	15.00	32.50
C. citratus	5000	20.00	90.00	92.50	15.00	67.50	82.50
	2500	10.00	80.00	82.50	5.00	55.00	72.50
	1250	2.50	70.00	75.00	2.50	37.50	62.50
	625	0	52.50	62.50	0	27.50	50.00
	312.5	0	35.00	45.00	0	15.00	37.50
	156.25	0	17.50	30.00	0	7.50	25.00
Indoxacarb	20	97.50	100.0	100.0	90.00	100.0	100.0
	10	95.00	100.0	100.0	80.00	100.0	100.0
	5	87.50	100.0	100.0	67.50	100.0	100.0
	1	62.50	100.0	100.0	32.50	97.50	100.0
	0.5	45.00	100.0	100.0	17.50	92.50	100.0

Table 2: Toxicity of tested essential oils and selected insecticides against 2nd instar larvae of *S. littoralis* under laboratory conditions after 3 days of treatment.

Compounds	LC_{50} (µg/ml)	Confidence limit at 95%		LC_{90} (µg/ml)	Confidence limit at 95%		Slope ± SE	Toxicity index
		Lower	Upper		Lower	Upper		
Boswellia carterii	287.68	115.12	516.69	17091.81	3687.67	1.06E+7	0.723±0.230	0.20
Melissa officinalis	457.84	270.83	793.22	2362.41	3278.28	369706.32	0.947±0.255	0.13
Valeriana officinalis	742.98	341.08	1167.59	11995.63	4672.78	2.81E+5	1.061±0.303	0.08
Cymbopogon citratus	505.57	227.91	759.74	4543.64	2285.48	36651.66	1.344±0.369	0.11
Indoxacarb	0.57	0.35	0.98	4.86	2.39	16.72	1.379±0.223	100.0

Table 3: Toxicity of tested essential oils and selected insecticides against 2nd instar larvae of *S. littoralis* under laboratory conditions after 5 days of treatment.

Compounds	LC_{50} (µg/ml)	Confidence limit at 95%		LC_{90} (µg/ml)	Confidence limit at 95%		Slope ± SE	Toxicity index
		Lower	Upper		Lower	Upper		
Boswellia carterii	151.31	29.25	255.70	1683.55	862.29	18767.46	1.225±0.381	0.38
Melissa officinalis	266.03	85.42	422.71	1719.62	1125.40	4568.52	1.581±0.401	0.22
Valeriana officinalis	412.12	112.46	679.26	6198.50	2923.00	68763.43	1.089±0.310	0.14
Cymbopogon citratus	335.19	90.90	540.47	3223.23	1718.61	24721.96	1.304±0.383	0.17
Indoxacarb	0.57	0.35	0.98	4.86	2.39	16.72	1.379±0.223	100.0

Tables 4 and 5 show that all EOs and Indoxacarb have a potential larvicidal effect against the 4th instar after 3 and 5 days of treatment. The median lethal concentrations of the essential oils ranged from 650.32 µg/ml for *M. officinalis* to 2012.95 µg/ml for *C. citratus.* Table 4 shows that *M. officinalis* has the highest larvicidal effect at 10191.94 µg/ml, while *V. officinalis* has the lowest larvicidal effect at 24540.47 µg/ml. The larvicidal effects of all tested EOs were noticeable after 5 days, with LC_{50} and LC_{90} values of 132.94 and 621.83 µg/ml and 3253.26 and 9713.73 µg/ml, respectively. Table 5 shows that *M. officinalis* had the most larvicidal efficacy, with both lethal concentrations followed by *B. carterii*, *V. officinalis*, *and C. citratus*, respectively.

Table 4: Toxicity of tested essential oils and selected insecticides against 4th instar larvae of *S. littoralis* under laboratory conditions after 3 days of treatment.

Compounds	LC_{50} (µg/ml)	Confidence limit at 95%		LC_{90} (µg/ml)	Confidence limit at 95%		Slope ± SE	Toxicity index
		Lower	Upper		Lower	Upper		
Boswellia carterii	1239.01	414.74	2020.02	18122.90	7318.60	563306.51	1.100±0.341	0.18
Melissa officinalis	650.32	250.81	1080.86	10191.94	4270.16	139752.43	1.072±0.292	0.34
Valeriana officinalis	1383.58	768.53	2682.85	24540.47	7944.34	926749.13	1.026±0.285	0.16
Cymbopogon citratus	2012.95	1163.55	3241.23	21301.57	8930.26	328756.57	1.251±0.344	0.11
Indoxacarb	2.19	1.41	3.65	20.58	9.68	91.02	1.318±0.242	100.0

Table 5: Toxicity of tested essential oils and selected insecticides against 4th instar larvae of *S. littoralis* under laboratory conditions after 5 days of treatment.

Compounds	LC_{50} (µg/ml)	Confidence limit at 95%		LC_{90} (µg/ml)	Confidence limit at 95%		Slope ± SE	Toxicity index
		Lower	Upper		Lower	Upper		
Boswellia carterii	306.09	54.98	582.19	5167.50	2551.58	37974.21	1.044±0.289	0.72
Melissa officinalis	132.94	6.26	305.28	3253.26	1653.64	31621.94	0.923±0.288	1.65
Valeriana officinalis	389.71	81.38	703.55	6779.61	3029.67	91185.87	1.033±0.301	0.56
Cymbopogon citratus	621.83	86.38	1154.51	9713.73	4939.06	92185.09	1.074±0.323	0.35
Indoxacarb	2.19	1.41	3.65	20.58	9.68	91.02	1.318±0.242	100.0

Essential oils GC-MS analyses

The chemical composition of the most effective essential oils (*B. carterii* and *M. officinalis*) was analyzed using the GC-MS technique to characterize their bioactive components by matching their mass spectra with the deposited analogous masses in the NIST library. A total of fifteen peaks were characterized for *B. carterii* Fig. 1, accounting for 92.60 % of essential oil. The main identified chemical classes were oxygenated sesquiterpenoids (32.1%), diterpene hydrocarbons (1.79%), oxygenated diterpenes (13.86%), and acetogenins derivatives (46.54%). n-Octyl acetate (37.95%), E-Nerolidol (27.40%), Incensole (9.56%), Isoincensole (4.30%), Acetic acid-10,11-dihydroxy-3,7,11-trimethyl-dodeca-2, 6-dienyl ester (3.79%), and 1-Octanol (2.41%) were the main constituents of *B. carterii* essential oil (Table 6). There were also, 31 peaks as indicators for the presence of 31 compounds of *M. officinalis* Fig. 2, accounting for 88.96% of *M. officinalis*, with five main groups of oxygenated monoterpenoids (3.86%), sesquiterpene hydrocarbons (19.11%), oxygenated sesquiterpenoids (54.54%), oxygenated diterpenes (1.79%) and acetogenin derivatives **(**9.66%). Six key components of the most effective *M. officinalis* essential oil were identified as (-)-Spathulenol (23.94%), Caryophyllene oxide (19.09%), β-Curcumene (10.63%), n-Hexadecanoic acid (5.71%), -Cadinol (3.21%), and Aromadendrene oxide-(2) (2.09%) (Table 6).

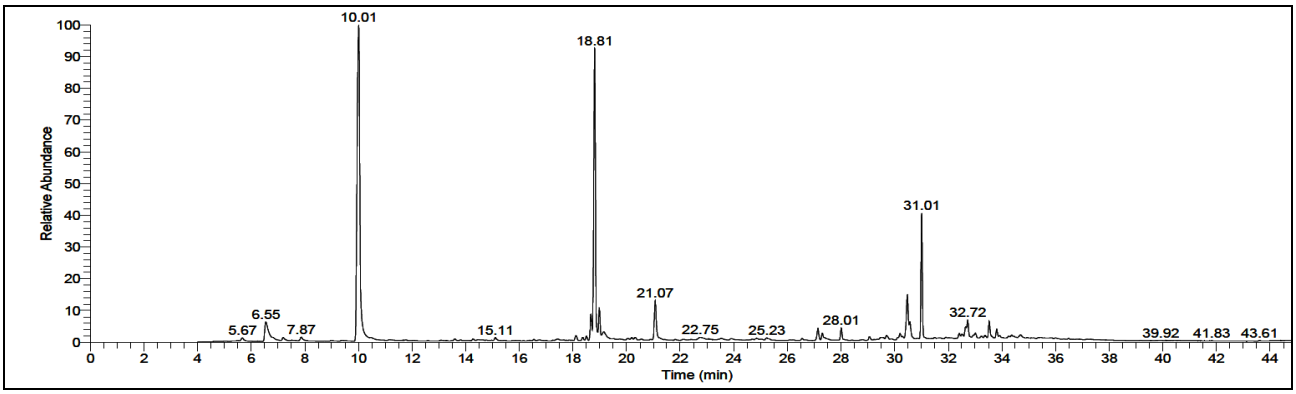

Fig 1: GC chromatogram of the identified constituents of *B. carterii* essential oil

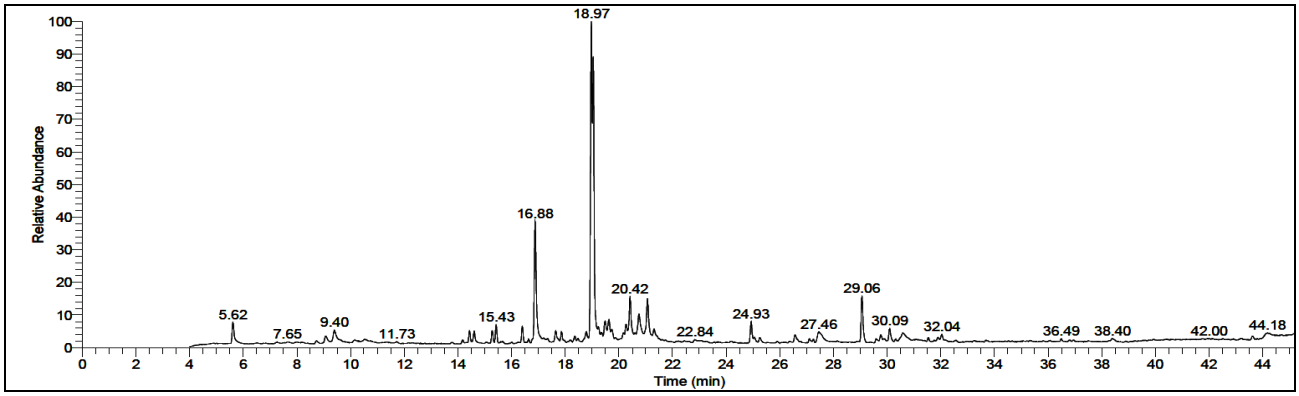

Fig 2: GC chromatogram of the identified constituents of *M. officinalis* essential oil

Table 6: GC-MS chemical profiling of *B. carterii* and *M. officinalis* essential oils.

No.	Compound name	Rt	Mol. Wt.	Mol. formula	BCEO Area %	MOEO Area %
Oxygenated Monoterpenes						
1	Eucalyptol	5.62	154	$C_{10}H_{18}O$		1.83
2	trans-4-Thujanol	9.08	154	$C_{10}H_{18}O$		0.86
3	α-Terpineol	9.40	154	$C_{10}H_{18}O$		1.17
Total OA					------	3.86
Sesquiterpene Hydrocarbons						
4	γ-Elemene	14.18	204	$C_{15}H_{24}$		0.35
5	α-Copaene	14.43	204	$C_{15}H_{24}$		0.94
6	(-)-β-Bourbonene	14.61	204	$C_{15}H_{24}$		1.06
7	Cedrene	15.28	204	$C_{15}H_{24}$		1.02
8	β-Caryophyllene	15.53	204	$C_{15}H_{24}$		1.49
9	Aromandendrene	16.40	204	$C_{15}H_{24}$		1.40
10	β-Curcumene	16.64	204	$C_{15}H_{24}$		0.31
11	α-Curcumene	16.88	202	$C_{15}H_{22}$		10.63
12	7-epi-α-Cadinene	17.65	204	$C_{15}H_{24}$		1.12
13	δ-Cadinene	17.86	204	$C_{15}H_{24}$		0.79
Total SH					------	19.11
Oxygenated Sesquiterpenes						
14	α-Bisabolol oxide	18.12	238	$C_{15}H_{26}O_2$	0.51	
15	Isocaryophyllene oxide	18.36	220	$C_{15}H_{24}O$		0.57
16	(2E,6E)-9-(3,3-Dimethyl-2-oxiranyl)-2,7-dimethyl-2,6-nonadien-1-ol	18.50	238	$C_{15}H_{26}O_2$	0.40	
17	E-Nerolidol	18.82	222	$C_{15}H_{26}O$	27.40	0.89
18	(-)-Spathulenol	18.97	220	$C_{15}H_{24}O$		23.94
19	Caryophyllene oxide	19.05	220	$C_{15}H_{24}O$		19.09
20	7-epi-cis-sesquisabinene hydrate	19.35	220	$C_{15}H_{24}O$		0.26
21	Aromadendrene oxide-(2)	19.63	220	$C_{15}H_{24}O$		2.09
22	Ledene oxide-(II)	19.74	220	$C_{15}H_{24}O$		0.87
23	11,11-Dimethyl-4,8-dimethylenebicyclo [7.2.0] undecan-3-ol	20.27	220	$C_{15}H_{24}O$		0.68
22	τ-Cadinol	20.42	222	$C_{15}H_{26}O$		3.21
24	Acetic acid-10,11-dihydroxy-3,7,11-trimethyl-dodeca-2,6-dienyl ester	21.07	298	$C_{17}H_{30}O_4$	3.79	
25	(1R,7S)-Germacra-4(15),5,10(14)-trien-1β-ol	21.31	220	$C_{15}H_{24}O$		0.94
26	Hexahydrofarnesyl acetone	24.93	268	$C_{18}H_{36}O$		2.00
Total OS					**32.1**	54.54
Diterpenes hydrocarbons						
27	Neocembrene	27.14	272	$C_{20}H_{32}$	1.13	
28	Cembrene	27.30	272	$C_{20}H_{32}$	0.66	
Total DH					**1.79**	------
Oxygenated diterpenes						
28	Isoincensole	30.47	306	$C_{20}H_{34}O_2$	4.30	
30	Incensole	31.01	306	$C_{20}H_{34}O_2$	9.56	
31	Phytol	30.09	254	$C_{20}H_{40}O$		1.79
Total OD					**13.86**	1.79
Acetogenins derivatives						
32	1-Octanol	6.55	130	$C_8H_{18}O$	2.41	
33	3-Methyl-6-hepten-1-ol	7.86	128	$C_8H_{16}O$	0.42	
34	n-Octyl acetate	10.02	172	$C_{10}H_{20}O_2$	37.95	
35	1-[2-Methyl-2-(4-methyl-3-pentenyl) cyclopropyl] ethanol	18.67	182	$C_{12}H_{22}O$	1.60	
36	Hexadecanoic acid, methyl ester	26.57	270	$C_{17}H_{34}O_2$		0.79
37	n-Hexadecanoic acid	27.45	256	$C_{16}H_{32}O_2$		5.71
38	cis-5,8,11,14,17-Eicosapentaenoic acid	29.70	302	$C_{20}H_{30}O_2$	0.99	
39	Oleic acid	29.78	254	$C_{18}H_{34}O_2$		1.12
40	Methyl hydroxylinolenate	30.57	308	$C_{19}H_{32}O_3$	1.19	
41	(3Z,13Z)-2-Methyl-3,13-octadecadien-1-ol	30.58	236	$C_{19}H_{36}O_2$		1.05
42	Arachidonic acid methyl ester	32.72	318	$C_{21}H_{34}O_2$	1.98	
43	Butyl 6,9,12-hexadecatrienoate	44.12	306	$C_{20}H_{34}O_2$		0.99
Total A					46.54	9.66
Total					92.60	88.96

Biochemical indicators of larvae

The effect of LC_{50} of *B. carterii* and *M. officinalis* was examined on the activity of biochemical parameter alkaline phosphatases and acid phosphatases **(**Table 7). The activity of ALP showed a non-significant reduction of 2.79% and 1.16% in larvae treated with both selected BCEO and MOEO, respectively. On the other hand, Indoxicarb insecticide showed a significant reduction compared to the control. In the case of ACP, there was a significant activation for BCEO reaching 135% compared with the control, while a mild reduction was achieved by MOEOs and Indoxcarb insecticides reaching 2.39% and 7.95%, respectively.

The detoxification enzymes, GST and AChE, demonstrated significant differences compared to the control group. As shown in Table 8, a remarkable inhibition in the activity of GST was observed in Indoxcarb (64.40 %), followed by BCEO (47.58%) and MOEO (30.67%) in comparison to the control. Also, a significant inhibition in AChE activity was recorded in MOEO (57.28%), followed by a mild activation in BCEO (4.85%) and Indoxcarb (21.05%) compared to the control.

The results in Table 9 represent the effectiveness of EOS treatments on the level of transaminase activity in *S. littoralis*. All essential oil treatments, as well as Indoxcarb, significantly inhibited GPT activity, with the highest inhibition value recorded in MOEO (45.92%), followed by BCEO (44.95%) and Indoxcarb (34.18%) compared to the control. Also, a remarkable activation was noticed for GOT activity, and Indoxcarb recorded the highest activation (66.23%), followed by BCEO (13.09%) and MOEO (10.21%) in comparison to the control.

Table 7: Effect of LC_{50} of the most potent essential oils on the activity of phosphatases (ALP, ACP) of *S. littoralis*.

Compounds	ALP (U/L) ±SE	Change %	ACP (U/L) ±SE	Change %
Control	75.20±0.23^{a}	---	15.47±0.61^{b}	---
B. carterii	77.30±1.76^{a}	2.79	36.50±0.74^{a}	135.94
M. officinalis	74.33±0.43^{a}	-1.16	15.10±0.51^{b}	-2.39
Indoxacarb	40.70±1.00^{b}	-45.88	14.27±0.20^{b}	-7.95
LSD $_{0.05}$	3.397		1.798	

LSD: Least Significant Difference at $P < .05$

Table 8: Effect of LC50 of the most potent essential oils on the activity of AChE and GST of *S. littoralis*.

Compounds	GST activity (mmol sub. Conjugated/ min/mg protein) ±SE	Change %	AChE activity (ug AchBr /min/gm b wt.) ±SE	Change %
Control	433.28±1.72^{a}	---	164.46±0.54^{c}	---
B. carterii	227.14±1.11^{c}	-47.58	172.44±0.56^{b}	4.85
M. officinalis	300.40±1.70^{b}	-30.67	70.25±0.80^{d}	-57.28
Indoxacarb	154.25±1.21^{d}	-64.40	199.08±0.57^{a}	21.05
LSD $_{0.05}$	4.766		2.040	

LSD: Least Significant Difference at $P < .05$

Table 9: Effect of LC_{50} of the most potent essential oils on the activity of transminases of *S. littoralis.*

Compounds	GPT (ALT) (U/L) ±SE	Change %	GOT(AST) (U/L) ±SE	Change %
Control	18.40±0.26^{a}	---	3.82±0.20^{b}	---
B. carterii	10.13±0.27^{c}	-44.95	4.32±0.11^{b}	13.09
M. officinalis	9.95±0.27^{c}	-45.92	4.21±0.08^{b}	10.21
Indoxacarb	12.11±0.16^{b}	-34.18	6.35±0.25^{a}	66.23
LSD $_{0.05}$	0.798		0.560	

LSD: Least Significant Difference at $P < .05$

Histological analysis

The histological texture of *S. littoralis* larvae midgut recorded a significant difference after treatments by the most effective Eos and Indoxacarb than control. The cross-section of normal *S. littoralis* larvae midgut (control) showed normal midgut structures with four epithelial cell types resting on intact basement membrane. The epithelium layers consist of goblet cells, columnar cells, and clusters of regenerative cells with large nuclei and strong basophilic cytoplasm. The peritrophic membrane surrounding the midgut lumen protects these epithelium layers from food particles (Fig. 3a).

The most significant difference observed after Indoxacarb insecticide treatment Fig. 3b was that the basement membrane either detaches, separates, or is destroyed. It also shows vacuolar degeneration, severe degeneration, necrosis of all epithelial types, loss of architecture, and shrinkage of the muscular layer. Notice the rupture of the peritrophic membrane.

BCEO caused a separation of the basement membrane and epithelial lining breaks down in large amounts inside the vacuoles. The apical brush border is also deteriorating and disintegrating. In addition, degeneration and loss of some epithelial cell lining Fig. 3c.

MOEO application also showed separation in the basement membrane, leading to severe vacuolar degeneration of its epithelial lining and Malpighian bodies and degeneration and loss of the epithelial brush border. The severe necrosis and loss of most epithelial cells lining the midgut are shown in Fig.3d.

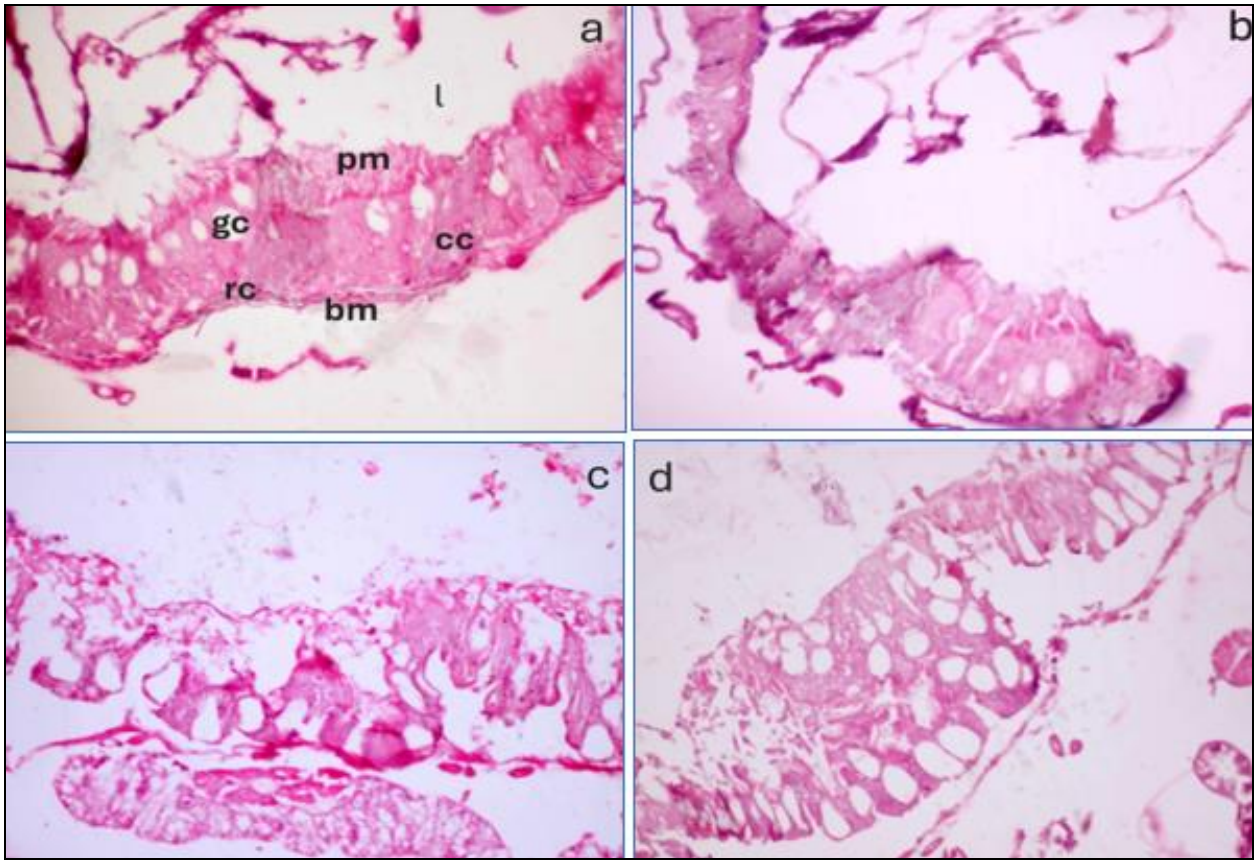

Fig 3: The cross section of the midgut of *S. littoralis* 4th instar larvae (X400): a) normal, b) treated with Indoxacarb insecticide, c) treated with *B. carterii* Eo, and d) treated with *M. officinalis* Eo [Note: bm) basement membrane, gc) goblet cells, cc) columnar cells, rc) regenerative cells, pm) peritrophic membrane, and l) lumen].

Discussions

This study compared the toxicity of four plant-derived essential oils on the Egyptian cotton leafworm. Our results show that *B. carterii* BCEO and *M. officinalis* MOEO could be used as green insecticides to control *S. littoralis* populations. Previous studies highlighted the efficiency of *M. officinalis* essential oil as an antifeedant and fumigant toxicity against *Tribolium castaneum* [20]. In addition, the Meliaceae family has antifeedant, insect growth regulating, and insecticidal properties [21] which closely resemble the obtained results in this study.

B. carterii BCEO toxicity on other pests was documented previously like *Callosobruchus* spp [22, 23], *Trogoderma granarium* [24], *Tetranychus urticae* [25] and spiny bollworm *Earias insulana* [26]. These findings unequivocally demonstrate the superiority of *B. carterii* BCEO and *M. officinalis* MOEO in controlling the Egyptian cotton leafworm.

Similarly, the efficacy of *V. officinalis* essential oil has been reported previously as repellent and toxic activity against red flour beetles and other several pests [27, 28]. Also, *C. citratus* essential oil caused substantial mortality in all larval stages of the fall armyworm *S. littoralis* [29] and against the 3rd instar of *S. littoralis*, CCEO recorded (LC_{50}= 725.2 mg/L) after two days of treatment, which closely resembles to our obtained results [30]. Various secondary metabolites were identified from *C. citratus* essential oils that exhibited insecticidal properties and influenced enzymatic activity and the immune system [31, 32].

The insecticidal activity of any essential oil varied significantly based on its chemical composition. GC-MS analysis of *B. carterii* BCEO identified various terpenes; the most predominant oxygenated sesquiterpene was E-Nerolidol (27.40 %), the well-known pesticide against several pests like *Aedes aegypti*, *T. urticae* Koch, *Rhipicephalus microplus* larvae [33] and *Metopolophium dirhodum* [33]. Similarly, *M. officinalis* afforded several classes; the main oxygenated sesquiterpenes were (-)-spathulenol (23.94%) and caryophyllene oxide (19.09%) which were previously documented as aphicides against *M. dirhodum* and considered as eco-friendly green insecticides [34] also, caryophyllene oxide is a highly efficient *Anopheles gambiae* as mosquito repellent [35].

Biochemical parameters investigation of insect pests are the most important indicators that reflect the insecticidal effect. According to Alfy *et al*. (2020) [2], treating the larvae of *S. littoralis* with chitosan nanoparticles as a natural insecticidal product reduces the activity of the alkaline phosphatase enzyme. *Chrysodeixis chalcites* (Lepidoptera: Noctuidae) larvae showed different activities in ACP, ALP, ALT, and AST levels after feeding on lemon balm *Melissa officinalis* in comparison with corn and the dill herb [36].

The toxicity of essential oils (EOs) arises from their capacity to disrupt or reduce the normal functioning of the insect nervous system [37]. Detoxification enzymes perform a principal role in the problem of insecticide resistance; hence the synthesis of detoxification enzymes is elevated in insects. They can cause target areas to be resistant or destroy foreign substances [38].

The reduction of GST activity level might represent an important contributor that causes insect mortality [39, 40]. The presence of GSTS helps *S. littoralis* larvae to detoxify

insecticides and other xenobiotic chemicals; therefore, the decrease in GST level was a sign of a weakened self-defense and immune system in insects [41].

Essential oils are found to effectively reduce the metabolic process of AChE in insect pests [42]. The presented data was on par with[23] who documented that BCEO significantly affects the antioxidant defense system in *Callosobruchus* spp. and did not affect AChE. The AChE activity was the lowest for the susceptible strain while the maximum for the resistant strain of *Aphis gossypii* [43].

The present results of the histological studies approximately agreed with the study of [16]; they reported that plant extracts of coumarin and azadirachtin caused detachment followed by the destruction of the basement membrane, in addition to the destruction of epithelial cells, and in some cases, the epithelial cells, appeared deformed. The same authors added that the insecticide methomyl caused detachment and destruction in the basement and peritrophic membrane, in addition to the appearance of numerous vacuoles and destruction of epithelial cells which emptied their cytoplasmic contents in the lumen, while protecto caused basement and peritrophic membrane detachment and destruction, vacuolization, and destruction of the epithelial cells. Also [44], reported that the alkaloid extracts of *Ricinus communis* and *Nicotiana glauca* caused severe necrosis, dissolved and ruptured columnar cells, and destroyed the basement membrane of the midgut of *S. littoralis* 4th instar larvae.

According to Abdel-Aal *et al*. (2012) [45], the light microscope examination of *S. littoralis* treated with LC_{50} *Spli*MNPV alone showed vacuolization of the columnar cells, and the peritrophic membrane deteriorated. Also, the same authors found that the larvae treated with the IGR Flufenxuron alone showed exfoliation and vacuolization of the midgut epithelium. The peritrophic membrane was disrupted.

Khalil *et al*. (2021) [46] reported that the midgut of the 3rd instar larvae of *S. littoralis* (Cry1C-tolerant population) showed vacuolization of the epithelium and disruption of the peritrophic membrane. They also said that the lumen was collapsed and that globular bodies and cytoplasmic fragments could be seen pinching off from the tips of the epithelial cells in the lumen next to the damaged peritrophic membrane.

Conclusion

Essential oils 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. Herein, our study's results document the efficiency of *Boswellia Carterii* Birdw.and *Melissa officinalis* L. essential oils to manage *Spodoptera littoralis* larvae as a result of the presence of main components like E-Nerolidol, (-)-Spathulenol, and Caryophyllene oxide that were identified qualitatively and quantitatively in their essential oils by using the GC-MS technique. Further studies were performed to confirm the mode of action, investigating the biochemical parameters we concluded an inhibition in the activity of glutathione S-transferase (GST) and acetylcholinesterase **(**AChE) enzymes. In addition, the pronounced damages in the histological texture of *S. littoralis* larvae midgut. In conclusion, essential oils should be given more consideration for developing eco-friendly and effective insecticides.

Declarations

Consent for publication: Not applicable.

Availability of data and material: The datasets utilized and analyzed during this investigation are available upon reasonable request from the corresponding author.

Ethical Approval

The article doesn't include any studies on the harmful exposure of materials to humans or animals.

Funding

No funding was received from any agency.

Authors' contributions: M.E.M. performed essential oils extraction. Toxicological experiments were carried out by M.E.M., W.Z.A. and N.M.G. M.E.M., W.Z.A., N.M.G. and H. A. handled the conceptualization and performed experimental planning and data analysis. The manuscript was written and reviewed by all authors.

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