Academic Journal of Entomology 16 (3): 105-112, 2023
ISSN 1995-8994

DOI: 10.5829/idosi.aje.2023.105.112

Toxicity, Phytochemical Analysis and Biochemical Responses of Some Selected Plant Essential Oils Against Cotton Mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)

Mohamed E. Mostafa, Naglaa M. Youssef and Hanaa M. Raghib

Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

Abstract: Essential oils can potentially be employed as natural pest control products, thus presenting a viable eco-friendly alternative for typical synthetic insecticides. Toxicological tests of ten isolated essential oils against adult females of cotton mealybug, *Phenacoccus solenopsis* after 24h and 72h of treatment using spray method technique revealed that *Nigella sativa*, *Melissa officinalis* and *Cuminum cyminum* were the most potent essential oils at 24h with LC_{50} 52.57, 163.88 and 1311.03 ppm while at 72h were 17.23, 44.52 and 71.79 ppm, respectively. The most potent essential oils were analyzed by gas chromatography/mass spectrometry (GC/MS), several natural product classes were characterized and the main constituents in each essential oil were qualitatively and quantitatively identified. In addition, the effect of these essential oils on biochemical aspects including transaminases enzymes (aspartate aminotransferase AST and alanine aminotransferase ALT), phosphatases enzymes (acid phosphatase ACP and alkaline phosphatase ALP), acelylcholinesterase (AChE) and chitinase revealed that activation in both transaminases and phosphatases enzymes and inhibition in the activity of acelylcholinesterase and chitinase.

Key words: Cotton Mealybug · *Phenacoccus solenopsis* · Essential oils · Chemical composition · Biochemical responses

INTRODUCTION

Phenacoccus solenopsis (Pseudococcidae; Hemiptera) is one of the major sucking pests attaching commonly cotton, so it well-known as cotton mealybug. This invasive and polyphagous insect infests fruit crops, vegetable crops, ornamentals and different weed plants. *P. solenopsis* colonized and propagated on healthy young plants and at heavily infestations, crinkling and curling of leaves were clearly observed resulting in growth malformation. Honeydew production produced at heavily infestations and associated with sooty mold growth which resulted in a reduction in the photosynthetic efficiency of the plant [1-4].

Synthetic chemical insecticides provide an efficient control of pests in agricultural systems. Several negative impacts have been raised on the application of these insecticides targeting environment, human health, reducing non-target beneficial organisms, including predators, parasitoids and pollinators. Meanwhile, new insights are directed towards development of highly selective and biodegradable natural insecticides which are available resources, safe, ecofriendly and compatible with the use of beneficial organisms [3, 5, 6].

Searching for bioactive phytochemicals, plant derivatives, of a potential pesticidal activity is vast and reflects the renewed interest in the field of plant-based pesticides. Essential oils- producing plants can potentially be employed as natural pest control products against a wide range of specific agricultural pests using fumigant, contact and repel applications [7].

Essential oils are complex natural heterogeneous mixtures composed of various low molecular weight terpenoids, as well as non-terpenoid volatile compounds [8]. Several studies addressed the insecticidal activity of essential oils or their chemical constituents against mealybugs suggested a neurotoxic action from its observed symptoms [3, 9, 12-14].

This study aimed to investigate the toxic effect of some selected ecofriendly green essential oils against

Corresponding Author: Mohamed E. Mostafa, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

cotton mealybug, *Phenacoccus solenopsis* in addition to identify their chemical constituents and evaluate its biochemical effects.

MATERIALS AND METHODS

Plant Materials: *Origanum majorana* L. (Lamiaceae) (Aerial parts), *Nigella sativa L.* (Ranunculaceae) (Seeds), *Piper nigrum* L. (Piperaceae) (Seeds), *Cuminum cyminum L.* (Apiaceae) (Seeds), *Boswellia Carterii* Birdw. (Burseraceae) (Resin), *Melissa officinalis L.* (Lamiaceae) (Aerial parts), *Valeriana officinalis* L. (Caprifoliaceae) (Roots) were bought from the herbal markets of Mansoura (Egypt) while *Cymbopogon citratus* (DC.) Stapf (Poaceae) (Aerial parts), *Ocimum basilicum* L. (Lamiaceae) (Leaves), *Coriandrum sativum* L. (Apiaceae) (Aerial parts) were collected from Mansoura University farm.

Essential Oils Isolation: Hydrodistillation of the ten essential oils using a Clevenger type apparatus were performed for 8 h and stored in the dark at -20°C, until analysis according to the European Pharmacopoeia [15].

GC-MS Analysis of Essential Oils: GC/MS analyses were performed 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. The column used was DB-5. The temperature of the oven was programmed from 50°C for 3 min., at isothermal, then heating by 7°C/ min. to 250°C and isothermally for 10 min., at 250°C. Ionization energy was set at 70eV. (Mansoura University, Egypt).

Tested Insect: A laboratory strain of *P. solenopsis* was kept under (30±2°C, 65±5%RH) conditions without any pesticides contaminations to obtain a homogenous strain till the time of study. *P. solenopsis* was reared on sprouted potato *Solanum tuberosum* [16].

Bioassay: Ten adult females of *P. solenopsis* were gently moved by the aid of fine brush to a fresh cotton leave and placed in a Petri dish and be ready for the essential oil application. Three replications were prepared for each treatment and the control. Emulsion formulation of the selected essential oils in water containing 0.3% triton X-100 was prepared. After that preparation of a series of five diluted concentrations from each essential oil were performed and immediately tested. Spraying all Petri dishes were carried out using a small hand atomizer with 1 ml aqueous solution of the essential oils. The excess of spraying solutions were removed from the Petri dishes, then confined well with the lids bearing the ventilation holes to prevent vapor accumulation. As the above mentioned procedure the control groups were treated with a spraying solution containing water and 0.3% Triton X-100 only [3].

Mealybug mortality percentage was observed at 24h and 72h following initial treatment, Abotts formula was used for correcting the mortality according to Abbott [17] and data statistically analyzed according to Finney to determine LC_{50}, LC_{90} and slope values [18]. Toxicity index was calculated for each applied essential oil using Sun's equation [19].

Biochemical Tests: *P. solenopsis* was prepared for biochemical testes according to Amin *et al*. [20]. Transaminases enzymes (AST and ALT) were determined based on Ishaaya *et al*. [21] also, phosphatases enzymes (ACP and ALP) were assessed according to Powell and Smith [22], while, acetylcholinesterase (AChE) was determined based on the method derived by Simpson *et al*. [23] and finally, chitinase enzyme according to Bade *et al*. [24] was assigned.

RESULTS AND DISCUSSION

Essential oils can be used as an alternative to traditional pesticides, mimicking their effects on target organisms while avoiding toxicity to non-target species, thus being more selective towards pest targets [25].

The toxic effect of ten plant essential oils against adult females of cotton mealybug, *P. solenopsisi* after 24h and 72h of application were assessed using spray method technique in Table 1. The results revealed that the toxicity effect of each essential oil was significantly increased in a concentration and time dependent manner. The most potent toxic essential oils group against the adult females of *P. solenopsis* after 24 and 72 hours of treatment was found to be the essential oils of *Nigella sativa* ($LC_{50, 90}$ at 24h: 52.57, 1967.99 ppm, and at 72h: 17.23, 357.52 ppm), followed by *Melissa officinalis* ($LC_{50, 90}$ at 24h: 163.88, 1311.03 ppm, and at 72h: 44.52, 402.11 ppm), then *Cuminum cyminum* ($LC_{50, 90}$ at 24h: 393.57, 15159.87 ppm, and at 72h: 71.79, 857.55 ppm), while the moderate toxic group comprises *Coriandrum sativum*, *Origanum majorana*, *Cymbopogon citratus* and *Boswellia Carterii* according to their toxicity index and finally the least toxic group which includes *Ocimum basilicum*, *Piper nigrum* and *Valeriana officinalis,* respectively.

Table 1: Toxicity of essential oils against adult females of *P. solenopsis* after 24 and 72 h of treatment.

	24h				72h			
Essential oils	LC_{50} (ppm) (95% CL)	LC_{90} (ppm) (95% CL)	Slope ± SE	Toxicity index	LC_{50} (ppm) (95% CL)	LC_{90} (ppm) (95% CL)	Slope ± SE	Toxicity index
Origanum majorana	570.41 (283.11-4177.92)	61092.10 (6551.41-1.4xE8)	0.631±0.18	9.22	212.61 (107.38-451.52)	8060.66 (1913.21-1.4xE6)	0.812±0.24	8.10
Nigella sativa	52.57 (11.21-95.81)	1967.99 (609.35-186424.96)	0.815±0.25	100.0	17.23 (1.27-38.51)	357.52 (173.56-3180.87)	0.973±0.29	100.00
Cymbopogon citratus	626.78 (340.17-5697.85)	26354.34 (3749.14-1.3xE8)	0.789±0.25	8.39	104.43 (36.21-175.22)	3570.95 (1063.81-351465.72)	0.836±0.26	16.50
Ocimum basilicum	1461.48 (666.67-18520.98)	23654.09 (4193.90-9553114.90)	1.060±0.30	3.60	527.61 (278.53-4295.20)	12917.78 (879.19-336381.13)	0.923±0.29	3.27
Piper nigrum	2451.37 (938.55-103909.16)	34508.79 (5027.51-8.7xE7)	1.116±0.35	2.14	1125.63 (512.08-14155.48)	15398.36 (2914.98-5.5xE6)	1.128±0.33	1.53
Coriandrum sativum	552.05 (300.79-2312.44)	27358.72 (4688.06-4628730.23)	0.756±0.19	9.52	369.36 (181.52-2683.63)	56324.19 (5233.74-1.1xE9)	0.587±0.19	4.67
Cuminum cyminum L.	393.57 (217.82-1458.83)	15159.87 (2833.84-1.07xE7)	0.808±0.25	13.36	71.79 (31.77-115.19)	857.55 (422.86-4781.44)	1.190±0.28	24.00
Boswellia carterii	633.41 (343.10-4605.37)	20122.39 (3333.98-2.1xE7)	0.853±0.26	8.30	410.75 (219.28-2106.06)	25334.68 (3628.89-5.4xE7)	0.716±0.22	4.19
Melissa officinalis	163.88 (103.21-249.24)	1311.03 (623.17-10803.65)	1.419±0.36	32.08	44.52 (6.60-79.02)	402.11 (236.21-2175.01)	1.341±0.41	38.70
Valeriana officinalis	2826.04 (957.53-160395.67)	96630.31 (9185.10-1.01xE9)	0.836±0.26	1.86	669.60 (303.17-10869.48)	13829.96 (2169.26-1.8xE7)	0.975±0.30	2.57

Our findings was in agreement with Al-Harbi *et al*. [26], who recorded a significant mortality percentage 96.4% of *N. sativa* essential oil against *Sitophilus oryzae* at concentration 6 mg/cm^2 after 48h and 70.3% at 24h of exposure time [26]. The efficacy of *M. officinalis* essential oil as a safe plant-based insecticide was previously assessed against *Tribolium castaneum* Herbst and showed a potent toxic effect in fumigant (LC_{50}=0.071 µL/mL air) and contact bioassays (100% mortality at 0.157 µL/cm^2) and 100% repellency at concentration ≤0.028 µL/cm^2 [27].

Laboratory experiments established the effectiveness of *C. cyminum* oil at a concentration of 2.5% against *Dactylopius opuntiae* with 100% nymph mortality after 3 h post treatment [28]. Also, a significant mortality rate (58.3–53.3%) against the 2nd and 5th instar larvae of *Cydalima perspectalis* was obtained with *C. cyminum* essential oil [29]. The fumigant and contact toxicity of *C. cyminum* essential oil and its main constituent cuminaldehyde were tested against adults of *Sitophilus zeamais*, after 7 days of exposure and the LC_{50} values were (229.4 and 484.8 mg L^{-1} air) for fumigant assay and LD_{50} values were (120.4 and 96.5 µg per adult) [30].

Essential oils are characterized by a strong odour with its intensity dependent on the chemical composition and concentration of the different components. The composition of these oils comprises a great number of constituents, with a variable number of polar and non-polar molecules in different concentrations.

The major compounds within the essential oil seem to reflect quite well the biological properties of the extraction source [31].

The most promising active essential oils, *N. sativa, M. officinalis* and *C. cyminum*, were analyzed by GC/MS technique to identify their bioactive components by comparing their mass spectra with those authenticated in NIST library. From *N. sativa* (NEO) 18 bioactive compounds were identified, representing 97.94% from the analyzed oil sample, a series of bioactive acetogenines (95.00%) was characterized with the major components being n-hexadecanoic acid (21.02%), linoleic acid ethyl ester (20.18%), (Z,Z)-9,12-octadecadienoic acid (15.34%) and hexadecanoic acid ethyl ester (13.44%) (Table 2).

From *M. officinalis* (MEO), 38 bioactive compounds were identified, representing 98.19% from the analyzed oil sample, two main groups was characterized monoterpenes (11.63%) and sesqueiterpene (85.76) with the highest abundant compounds being (-)-caryophyllene oxide (23.83%), (-)-spathulenol (19.94%) and α-curcumene (14.16%) (Table 2).

Seventeen bioactive compounds were identified from *C. cyminum* (CEO), representing 99.53% from the analyzed oil sample, with the major components being cuminaldehyde (39.73%), p-cymene (31.06%) and β-pinene

Table 2: GC/MS analysis data of the promising essential oils.

No	Component Name	RT	MEO Area %	CEO Area %	NEO Area %	MF	M.wt
	Acetogenines						
1	1-Octen-3-ol (**1**)	4.31	0.80			$C_8H_{16}O$	128
2	Tetradecanoic acid (**2**)	22.20			0.39	$C_{14}H_{28}O_2$	228
3	Tetradecanoic acid, ethyl ester (**3**)	22.92			0.52	$C_{16}H_{32}O_2$	256
4	Hexadecanoic acid, methyl ester (**4**)	25.54			1.97	$C_{17}H_{34}O_2$	270
5	n-Hexadecanoic acid (**5**)	26.29			21.02	$C_{16}H_{32}O_2$	256
6	Hexadecanoic acid, ethyl ester (**6**)	26.87			13.44	$C_{18}H_{36}O_2$	284
7	(Z,Z)-9,12-Octadecadienoic acid, methyl ester (**7**)	28.53			2.65	$C_{19}H_{34}O_2$	294
8	(E)-9-Octadecenoic acid, methyl ester (**8**)	28.71			2.13	$C_{19}H_{36}O_2$	296
9	(Z,Z)-9,12-Octadecadienoic acid (**9**)	29.24			15.34	$C_{18}H_{32}O_2$	280
10	Oleic Acid (**10**)	29.40			7.88	$C_{18}H_{34}O_2$	282
11	Linoleic acid ethyl ester (**11**)	29.76			20.18	$C_{20}H_{36}O_2$	308
12	Ethyl Oleate (**12**)	29.93			8.79	$C_{20}H_{38}O_2$	310
13	Octadecanoic acid, ethyl ester (**13**)	30.48			0.69	$C_{20}H_{40}O_2$	312
	Total		0.80		95.00		
	Monoterpene hydrocarbon						
14	β-Pinene (**14**)	4.31		15.06		$C_{10}H_{16}$	136
15	β-Myrcene (**15**)	4.52		0.50		$C_{10}H_{16}$	136
16	m-Cymene (**16**)	5.05	0.21	2.65		$C_{10}H_{14}$	134
17	p-Cymene (**17**)	5.06		31.06		$C_{10}H_{14}$	134
18	o-Cymene (**18**)	5.05			0.88	$C_{10}H_{14}$	134
19	γ-Terpinene (**19**)	5.81		0.25	0.20	$C_{10}H_{16}$	136
	Total		0.21	49.52	1.08		
	Oxygenated monoterpenes						
20	Cineole (**20**)	5.21	5.97			$C_{10}H_{18}O$	154
21	Linalool (**21**)	6.59	0.30			$C_{10}H_{18}O$	154
22	(E)-Pinocarveol (**22**)	7.39		0.60		$C_{10}H_{16}O$	152
23	Pinocarvone (**23**)	7.75		0.35		$C_{10}H_{14}O$	150
24	E,E-2,6-Dimethyl-3,5,7-octatriene-2-ol (**24**)	8.03		0.41		$C_{10}H_{16}O$	152
25	Terpinen-4-ol (**25**)	8.33	1.04		0.47	$C_{10}H_{18}O$	154
26	p-Menth-3-en-7-al (**26**)	8.53		2.55		$C_{10}H_{16}O$	152
27	(-)-*β*-Fenchol (**27**)	8.62	1.60			$C_{10}H_{18}O$	154
28	*trans*-Carveol (**28**)	9.27	0.87			$C_{10}H_{16}O$	152
29	Cumaldehyde (**29**)	9.57		39.73		$C_{10}H_{12}O$	148
30	Carvone (**30**)	9.68	0.69			$C_{10}H_{14}O$	150
31	2-Isopropenyl-5-methyl-4-hexenal (**31**)	10.44	0.36			$C_{10}H_{16}O$	152
32	Terpinen-7-al (**32**)	10.63		0.87		$C_{10}H_{14}O$	150
33	1,4-p-Menthadien-7-al (**33**)	10.80		0.28		$C_{10}H_{14}O$	150
34	1-(6-Methyl-7-oxabicyclo[4.1.0]hept-1-yl)ethanone (**34**)	11.05		1.02		$C_9H_{14}O_2$	154
35	Carvacrol (**35**)	11.33			0.35	$C_{10}H_{14}O$	150
36	2-Hydroxy-3-(3-methyl-2-butenyl)-3-cyclopenten-1-one (**36**)	12.27		0.35		$C_{10}H_{14}O_2$	166
37	(6E)-3,7-Dimethyl-6-nonenal (**37**)	12.96		0.50		$C_{11}H_{20}O$	168
38	β-(E)-Damascenone (**38**)	13.30	0.34			$C_{13}H_{18}O$	190
39	(R)-lavandulyl acetate (**39**)	13.41	0.25			$C_{12}H_{20}O_2$	196
40	Cuminic acid (**40**)	14.51		3.04		$C_{10}H_{12}O_2$	164
41	2-Caren-4-ol (**41**)	31.56		0.31		$C_{10}H_{16}O$	152
	Total	11.42	50.01	0.82			
	Total Monoterpene	11.63	99.53	1.9			
	Sesqueiterpene hydrocarbons						
42	α-Copaene (**42**)	13.56	1.32			$C_{15}H_{24}$	204
43	(-)-α-Bourbonene (**43**)	13.72	1.05			$C_{15}H_{24}$	204
44	Longifolene (**44**)	14.17			0.78	$C_{15}H_{24}$	204
45	Cedr-8-ene (**45**)	14.36	1.35			$C_{15}H_{24}$	204
46	Caryophyllene (**46**)	14.51	1.68			$C_{15}H_{24}$	204
47	Aromandendrene (**47**)	15.47	1.46			$C_{15}H_{24}$	204
48	α-Longipinene (**48**)	15.71	0.33			$C_{15}H_{24}$	204
49	γ-Muurolene (**49**)	15.85	0.23			$C_{15}H_{24}$	204
50	α-Curcumene (**50**)	15.94	14.16			$C_{15}H_{22}$	202
51	trans-Calamenene (**51**)	16.77	0.26			$C_{15}H_{22}$	202
52	(+)-δ-Cadinene (**52**)	16.92	1.25			$C_{15}H_{24}$	204
53	Cadala-1(10),3,8-triene (**53**)	17.18	0.51			$C_{15}H_{22}$	202
	Total	23.6		0.78			

Table 2: Continue

No	Component Name	RT	MEO Area %	CEO Area %	NEO Area %	MF	M.wt
	Oxygenated sesqueiterpene						
54	Ledene oxide-(II) (**54**)	17.45	0.19			$C_{15}H_{24}O$	220
55	Isoaromadendrene epoxide (**55**)	17.72	0.32			$C_{15}H_{24}O$	220
56	Nerolidol (**56**)	17.80	0.75			$C_{15}H_{26}O$	222
57	(-)-Spathulenol (**57**)	17.93	19.94			$C_{15}H_{24}O$	220
58	(-)-Caryophyllene oxide (**58**)	17.38	23.83			$C_{15}H_{24}O$	220
59	Cedr-8(15)-en-9-ol (**59**)	18.20	0.51			$C_{15}H_{24}O$	220
60	α-acorenol (**60**)	18.31	0.20			$C_{15}H_{26}O$	222
61	Alloaromadendrene oxide-(2) (**61**)	18.43	1.50			$C_{15}H_{24}O$	220
62	(1R,7S)-Germacra-4(15),5,10(14)-trien-1β-ol (**62**)	18.69	4.35			$C_{15}H_{24}O$	220
63	Cubenol (**63**)	19.14	0.87			$C_{15}H_{26}O$	222
64	11,11-Dimethyl-4,8-dimethylenebicyclo[7.2.0]undecan-3-ol (**64**)	19.20	1.72			$C_{15}H_{24}O$	220
65	τ-Cadinol (**65**)	19.37	4.35			$C_{15}H_{26}O$	222
66	Cubebol (**66**)	19.46	0.27			$C_{15}H_{26}O$	222
67	Verrucarol (**67**)	23.03	0.31			$C_{15}H_{22}O_4$	266
68	Hexahydrofarnesyl acetone (**68**)	23.93	1.94			$C_{18}H_{36}O$	268
69	Phytol (**69**)	29.03	1.11			$C_{20}H_{40}O$	296
	Total		62.16				
	Total sesqueiterpene		85.76		0.78		
	Aromatic compounds						
70	5-Methyl-2-phenyl-2-hexenal (**70**)	15.63			0.26	$C_{13}H_{16}O$	188
	Total				0.26		

Table 3: Activity levels of some biochemical parameters in adult females of *P. solenopsis* after application the LC_{50} of the promising essential oils

Essential oil	LC_{50} ppm	AST (mU/mg protein) Mean±SE	Change %	ALT (mU/mg protein) Mean±SE	Change %	ALP (µg phenol/ min/g.b.wt) Mean±SE	Change %	ACP (µg phenol/min/ g.b.wt) Mean±SE	Change %	AchE (ug AchBr/min/gm body weight) Mean±SE	Change %	Chitinase (µg NAGA/min /g body weight) Mean±SE	Change %
Control		0.79^{c}±0.001		1.95^{d}±0.084		0.32^{d}±0.007		1.03^{d}±0.011		451.49^{a}±11.33		35.73^{b}±0.520	
N. sativa	52.57	0.95^{b}±0.012	20.3	6.54^{a}±0.137	235.4	0.47^{a}±0.007	46.9	2.65^{a}±0.011	157.3	281.05^{b}±0.86	-37.8	55.79^{a}±3.562	56.1
C. cyminum	393.57	1.35^{a}±0.058	70.9	5.95^{b}±0.172	205.1	0.41^{b}±0.008	28.1	2.27^{b}±0.004	120.4	242.49^{c}±3.33	-46.3	34.72^{b}±0.984	-2.8
M. officinalis	163.88	1.00^{b}±0.002	26.6	5.07^{c} ±0.132	160.0	0.37^{c} ±0.002	15.6	2.15^{c}±0.007	108.7	298.49^{b}±3.78	-33.9	19.00^{c}±1.666	-46.8
$LSD_{0.05}$		0.10		0.44		0.02		0.03		20.27		6.66	
p		0.000		0.000		0.000		0.0000		0.0000		0.0000	
f		62.94		229.34		99.87		5950.93		217.95		54.42	

LSD=less significant differences, F=F-test, P=P-value; According to Duncan's, letters represent the substantial variations between treatments; the data represent the average and standard errors of three replicates, each containing a 30- adult females.

(15.06%) from the main group monoterpene (99.53%) (Table 2).

These differences between the essential oils are probably due to a mixture of environmental (climate/ nutrition/soil/weather) and genetic factors, which can affect the biosynthesis of the secondary metabolite [28].

The effect of sublethal concentration (LC_{50}) of the promising essential oils on certain biochemical parameters of the adult females of *P. solenopsis* after 72h of treatment was examined to interpret the primary mode of actions of the selected essential oils.

Results represented in Table 3 revealed that the biochemical parameter aspartate aminotransferase (AST) of adult females haemolymph of *P. solenopsis* was significantly activated by all essential oils treatments, where the highest activation value (1.35±0.058 mU/mg protein) observed in *C. cyminum* treatment followed by *M. officinalis* (1.00±0.002 mU/mg protein) and *N. sativa* (0.95±0.012 mU/mg protein) compared to control (0.79±0.001 mU/mg protein). Also, a significant activation in alanine aminotransferase (ALT) activity was noticed, the activation values were 6.54±0.137 (235.4%), 5.95±0.172 (205.1%) and 5.07±0.132 (160.0%) (mU/mg protein) for *N. sativa, C. cyminum and M. officinalis* essential oils, respectively compared with the control 1.95^{d}±0.084 (mU/mg protein).

The level of ALT and AST enzymes are affected by the entry of any toxicants or any infections into the insect body which resulted in physiological challenge [32].

Acid and alkaline phosphatases have been shown to be associated with insect development especially in relation to nutrition and egg maturation. Acid phosphatase has received considerable attention in developmental studies because of its association with histolysis [33].

The activity of acid (ACP) and alkaline (ALP) phosphatase of adult female's haemolymph of *P. solenopsis* (Table 3) was significantly increased after application the LC_{50} of all tested essential oils compared with the untreated one. *N. sativa* showed the highest distinguishable activation in both ACP and ALP by (2.65±0.011 and 0.47±0.007) (157.3 and 46.9%) followed by *C. cyminum* (2.27±0.004 and 0.41±0.008) (120.4 and 28.1%) and *M. officinalis* essential oils (2.15±0.007 and 0.37±0.002) (µg phenol/min/g.b.wt) (108.7 and 15.6%), respectively.

Our findings were in accordance with Zhi-qing *et al*. [34], terpinen-4-ol (**25**), the component identified in both *N. sativa* and *M. officinalis*, increased hydrolyzing metabolism by activating phosphatase. Terpinen-4-ol may participate in hydrolyzing metabolism or stimulate insect to increase metabolizing and drug tolerance. The result was in accordance also with total esterase. That is, terpinen-4-ol increased esterase activity [34].

Acetylcholinesterase enzyme (AChE) hydrolyzes the neurotransmitter acetylcholine to stop neuronal excitement at the postsynaptic membrane [35].

Biochemical investigation of AChE enzyme of adult female's haemolymph of *P. solenopsis* (Table 3) revealed inhibition in the activity of the enzyme after application of all selected essential oils treatments. Data revealed that *C. cyminum* was the highest significantly inhibited AChE enzyme, 242.49±3.33 (ug AchBr/min/gm body weight) (-46.3%), followed by *N. sativa* 281.05±0.86 (-37.8%), and *M. officinalis* essential oils (298.49±3.78) (ug AchBr/min/gm body weight) (-33.9%) compared with the control (451.49^{a}±11.33 ug AchBr/min/gm body weight).

The competitive inhibition of acetylcholinesterase enzyme (AChE) by several essential oils especially monterpenes group have been previously reported [3]. This in accordance with our findings, in which *C. cyminum* showed the highest content of monoterpenes group (99.53 %) and may be in a correlation with the inhibition of AChE enzyme. The anticholinesterase activity of the major volatile constituents of *C. cyminum*, cumaldehyde **(29)** was assessed and a significant inhibitory action was noticed at 214.5 µg mL^{-1} of tested compound against adults of *S. zeamais* [30].

Chitinases are essential in chitin metabolism processes. They enable the regulation of the insect exoskeleton formation, as well as the degradation of chitin. Only *M. officinalis* essential oil showed the highest significant inhibition effect to adult female's haemolymph chitinases of *P. solenopsis* (Table 3) (19.00±1.666) (-46.8%), while a slight inhibition effect was recorded by *C. cyminum* 34.72±0.984 (-2.8%) and an activation effect was observed for *N. sativa* (55.79±3.562) (56.1%) compared with control (35.73±0.520 µgNAGA/ min /g body weight).

Similar results have been reported, after application of *Eucalyptus globulus* essential oil and 1,8-Cineole a significant decrease in chitinFase activity was observed against *Tribolium confusum* likewise, menthol against *Rhyzopertha dominica* [36, 37].

REFERENCES

1. Pawar, S.R., H.R. Desai, G.R. Bhanderi, and C.J. Patel, 2017. Biology of the Mealybug, *Phenacoccus solenopsis* Tinsley Infesting Bt Cotton. Int. J. Curr. Microbiol. App. Sci., 6(8): 1287-1297.
2. Noureen, N., M. Hussain, S. Fatima and M. Ghazanfar, 2016. Cotton Mealybug Management: A Review. Journal of Entomology and Zoology Studies, 4(4): 657-663.
3. Mostafa, M.E., N.M. Youssef and A.M. Abaza, 2018. Insecticidal activity and chemical composition of plant essential oils against cotton mealybug, *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae). Journal of Entomology and Zoology Studies, 6(2): 539-543.
4. Abd El-Mageed, A.E.M., N.M. Youssef and M.E. Mostafa, 2018. Efficacy of some Different Insecticides against Cotton Mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) and Its Associated Predators. J. Plant Prot. and Path., Mansoura Univ., 9(6): 351-355.
5. Isman, M.B. and C.M. Machial, 2006. Pesticides based on plant essential oils: from traditional practice to commercialization. *In* M. Rai and M.C. Carpinella (eds.), Naturally Occurring Bioactive Compounds, Elsevier, BV, pp: 29-44.
6. Duke, S.O., H.K. Abbas, T. Amagasa and T. Tanaka, 1996. Phytotoxins of microbial origin with potential for use as herbicides. In: Copping L.G., editor. Crop Protection Agents from Nature: Natural Products and Analogues, Critical Reviews on Applied Chemistry. 35: Society for Chemical Industries; Cambridge, UK., pp: 82-113.
7. Isman, M.B., 2000. Plant essential oils for pest and disease management. Crop protection, 19(8-10): 603-608.
8. Başer, K.H.C. and F. Demirci, 2007. Chemistry of essential oils. In: Flavours and fragrances- chemistry, bioprocessing and sustainability. Berger R.G. (Ed.), Springer, Berlin Heidelberg, pp: 45-83.

9. Mwanauta, R.W., P.A. Ndakidemi, and P.B. Venkataramana, 2023. Biopesticide efficacy of four plant essential oils against papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae). Heliyon, 9(3): 14162.
10. Brahmi, R., K. Abdellaoui, A. Harbi, K. Abbes, R. Rahmouni, S. Tounsi, P. Suma and B. Chermiti, 2022. Toxicity and neurophysiological impacts of three plant-derived essential oils against the vineyard mealybug *Planococcus ficus*. Vitis, 61(1):1 - 10.
11. Ramzi, S., A. Seraji, G.R. Azadi and H.S. Roofigari, 2022. Effects of the extract and the essential oil of *Allium sativum* on tea mealy bug, *Pseudococcus viburni* Sigornet (Hemi- ptera: Pseudococcidae). Biocatalysts and Agricultural Biotechnology, 42: 102359.
12. Arokiyaraj, C., K. Bhattacharyya and S.G.E. Reddy, 2022. Toxicity and synergistic activity of compounds from essential oils and their effect on detoxification enzymes against *Planococcus lilacinus*. Frontiers in Plant Science, 13: 1016737.
13. Kostyukovsky, M., A. Rafaeli, C. Gileadi, N. Demchenko and E.Shaaya, 2002. Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: possible mode of action against insect pests. Pest. Mgt. Sci., 58: 1101-1106.
14. Shaaya E. and A. Rafaeli, 2007. Essential oils as biorational insecticides-potency and mode of action. In: Insecticides design using advanced technologies, Ishaaya I., R. Nauen and A.R. Horowitz (Eds.) Springer Berlin Heidelberg, pp: 249-261.
15. Council of Europe, 2010. European Directorate for the Quality of Medicines, in: European Bioactivity against *B. xylophilus*: nematotoxics from essential oils, essential oils fractions and decoction waters Pharmacopoeia, 7th Edition. Strasbourg, France, pp: 241.
16. Rashid, H., L. Polyak and E. Mosley-Thompson, 2011. Abrupt Climate Change: Mechanisms, Patterns, and Impacts. Journal of Geophysical Monograph Series. American Geophysical Union, Washington D.C. 193:2.
17. Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18(2): 265-267.
18. Finney, D.J., 1982. Probit analysis: a statistical treatment of the sigmoid response curve. Cambridge: Cambridge University Press, 1947. 256.
19. Sun, Y.P., 1950. Toxicity Index-an Improved Method of comparing the relative Toxicity of Insecticides. Journal of Economic Entomology, 43(1): 45-53.
20. Amin, T.R., 1998. Biochemical and physiological studies of some insect growth regulators on the cotton leafworm, *Spodoptera littoralis* (Boisd.). Ph.D. Thesis, Faculty of Science, Cairo Univ. Egypt.
21. Ishaaya, I. and E. Swirski, 1976. Trehalase, invertase and amylase activities in the black scale *Saissetia oleae*, and their relation to host adaptability. J. Insect Physiol., 22: 1025-1029.
22. Powell, M.E.A. and M.J.H. Smith, 1954. The determination of serum acid and alkaline phosphatases activity with 4-amino antipyrine. J. Clin. Pathol., 7: 245- 248.
23. Simpson, D.R. and D.L. Bull and D.A. Lindquist, 1964. A semi microtechnique for the estimation of cholinesterase activity in boll weevils. Annals of the Entomological Society of America, 57(3): 367-371.
24. Bade, M.L. and A. Stinson, 1981. Biochemistry of insect differentiation. A system for studying the mechanism of chitinase activity *in vitro.* Archs Biochem. Biophys, 206: 213-221.
25. Copping, L.G. and S.O. Duke, 2007. Natural products that have been used commercially as crop protection agents. Pest Management Science, 63(6): 524-554.
26. Al-Harbi, N.A., N.M. Al Attar, D.M. Hikal, S.E. Mohamed, A.A.H. Abdel Latef, A.A. Ibrahim and M.A. Abdein. 2021. Evaluation of Insecticidal Effects of Plants Essential Oils Extracted from Basil, Black Seeds and Lavender against *Sitophilus oryzae*. *Plants*, 10(5): 829.
27. Upadhyay, N., V.K. Singh, A.K. Dwivedy, S. Das, A. K. Chaudhari and N. K. Dubey, 2019. Assessment of *Melissa officinalis* L. essential oil as an eco-friendly approach against biodeterioration of wheat flour caused by *Tribolium castaneum* Herbst. Environ. Sci. Pollut. Res., 26: 14036-14049.
28. Naboulsi, I., K. El Fakhouri, H. Annaz, R. Lamzira, C. Ramdani, M. B. G. Thierry, R. Boulamtat, W. Ben Bakrim, I. Mahdi, A. Aboulmouhajir, A. Yasri, M. El Bouhssini, J. L. Ward and M. Sobeh, 2023. Chemical profiling of Artemisia herba-alba, *Cuminum cyminum*, *Cinnamomum camphora*, and *Salvia rosmarinus* essential oils and assessment of their insecticidal potential to control the wild cochineal *Dactylopius opuntiae* (Cockerell). Crop Protection, 17: 106286.

29. Gokturk, T., N. Chachkhiani-Anasashvili, S. Kordali, G. Dumbadze and A. U. Bozhuyuk, 2021. Insecticidal effects of some essential oils against box tree moth (*Cydalima perspectalis* Walker (Lepidoptera: Crambidae)). Int. J. Trop. Insect. Sci., 41: 313-322.
30. Rosa, J.S.L. Oliveira, R.M.O.F. Sousa, C.B. Escobar and M. Fernandes-Ferreira, 2019. Bioactivity of some Apiaceae essential oils and their constituents against *Sitophilus zeamais* (Coleoptera: Curculionidae). Bulletin of Entomological Research, 110(3): 1-11.
31. Bakkali, F., S. Averbeck, D. Averbeck and M. Idaomar, 2008. Biological effects of essential oils-a review. Food and Chemical Toxicology, 46(2): 446-475.
32. Giboney, P.T., 2005. Mildly elevated liver transaminase levels in the asymptomatic patient. American Family Physician, 71(6): 1105-1110.
33. Salama, Z.A., A.A. Ebied, H.A. Ahmad and T.A. El Sheikh, 2007. Biochemical studies of *Bacillus thuringiensis* and spinosad on the carbohydrates hydrolyzing enzymes and phosphatase enzymes of cotton leaf worm, *Spodoptera littoralis* with special comparison of pyrethroid compound. J. Agric. Sci. Mansoura Univ., 32(7): 5679-5686.
34. Zhi-qing, M., H. Xiu-ling, F. Jun-tao, L. Guang-ze and Z. Xing, 2008. Effects of Terpinen-4-ol on Four Metabolic Enzymes and Polyphenol Oxidase (PPO) in *Mythimna separta* Walke. Agricultural Sciences in China, 7(6): 726-730.
35. Santos, G.K.N., K.A. Dutra, C.S. Lira, B.N. Lima, T.H. Napoleão, P.M.G. Paiva and D.M.A.F. Navarro, 2014. Effects of *Croton rhamnifolioides* essential oil on *Aedes aegypti* oviposition, larval toxicity and trypsin activity. Molecules, 19(10): 16573-16587.
36. Tine, S., F. Tine-Djebbar, A. Debab, A. Mesloub and N. Soltani 2023. Insecticidal efficacy and physiological effects of *Eucalyptus globulus* essential oil and its constituent, 1,8-Cineole against *Tribolium confusum* (Jacquelin du Val, 1868) (Coleoptera, Tenebrionidae). J. Plant. Dis. Prot., 130: 769-780.
37. Tine-Djebbar, F., M. Trad, A.O. Tine, S. Tine and N. Soltani, 2023. Effects of menthol on nutritional physiology and enzyme activities of the lesser grain borer, *Rhyzopertha dominica* (F. 1792) (*Coleoptera*: *Bostrichidae*). J. Plant. Dis. Prot., 130: 509-518.