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Essential oils as environmentally friendly insecticides for controlling cotton mealybug, *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae)

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Abstract
Eight plant essential oils were extracted and tested for their efficiency as natural insecticides against the cotton mealybug, *P. solenopsis* (Tinsley). *In-vitro* bioassay, the contact toxicity of the tested oils to adult females and 2nd instar nymphs was determined. Also, repellence activity of the tested oils were tested. The most effective oil against adult female's after 72 hrs of application was *L. nobilis* followed by *Apium graveolens*, *M. longifolia*, *C. articulates*, *M. sativa*, *A. graveolens*, *O. vulgar*, then *F. vulgar*. The 2nd instar nymphs showed more susceptibility for all tested essential. Regarding repellency, *M. longifolia* showed the highest repellency effect but unfortunately with high degradation rate. The most repellent essential oils with satisfactory stability were *L. nobilis*, *F. vulgar* and *C. articulates*. Also, the efficacy of the tested oils were tested against *P. solenopsis* in open field conditions. The most efficient oil at 72hrs after treatment was *L. nobilis* which showed great reduction percentage (89.3%) followed by *Apium graveolens* (88.6%), *M. longifolia* (88.6%), *C. articulates* (87.8%), *M. sativa* (87.7%), *A. graveolens* (86.9%), *O. vulgar* (85.9%) and *F. vulgar* (85.2%), respectively.

Keywords: *Phenacoccus solenopsis*, essential oils, contact toxicity and repellency

1. Introduction
The cotton mealybug, *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae) is a serious invasive polyphagous insect infesting approximately 300 plant species belong to 65 botanical families such as Asteraceae, Amaranthaceae, Cucurbitaceae, Fabaceae, Euphorbiaceae, Malvaceae, Lamiaceae, and Solanaceae [1]. It may attack all aerial parts of the plant preferring the young shoots or branches carrying fruitlets, twigs, and fruiting bodies [2]. Intense infestation of mealybugs cause chlorosis, distortion, defoliation, general weakening, plant stunt and plant death of susceptible plants [3]. Moreover, honeydew egested by the pest stimulates black sooty mold growth which hindering the photosynthesis [4].
The excessive and injudicious use of traditional synthetic insecticides led to many environmental problems which inflict damage on human health and non-target organisms [5, 6], besides development of insect resistance leading to population outbreaks [7-9]. In recent decades, the interest in a more secure and eco-friendly pest-control strategy was increased. Essential oils represent one of the most potential bio-pesticides playing an important role as safe alternatives of traditional pesticides. They may act as repellents [10, 11], antifeedants [12, 13], growth and molting inhibitors [14, 15], fecundity suppressant or toxins [16, 17]. The present study attempts to assess the efficacy of eight essential oils extracted from local Egyptian available plants against the 2nd instar stage-nymphs and adult females. Also, their repellency effects of adult females was estimated.

2. Materials and Methods
2.1 Plant materials and essential oils extraction
The seeds of *Medicago sativa* L. (Fabaceae); fruits of *Foeniculum vulgare* Mill. (Apiaceae); tubers of *Cyperus articulates* L. (Cyperaceae); aerial parts of *Anethum graveolens* L. (Apiaceae) and *Apium graveolens* L. (Apiacea) and Leaves of *Laurus nobilis* L. (Lauraceae), *Mentha longifolia* (L). Huds. (Lamiaceae), *Origanum* vulgar L.

(Lamiaceae) were subjected to hydro-distillation for 4 hours at least in Clevenger-type apparatus in order to extract their volatile eesential oils which were then dried over anhydrous sodium sulfate and kept frozen in dark glass tubes until application. All essential oils were formulated as emulsion in water and 0.3% Tween-80. Four concentrations of each essential oil were tested immediately after preparation.

2.2 Pure culture of cotton mealybug, *P. solenopsis* and Host Plants

A pure culture of *P. solenopsis* was obtained from insecticides-free okra plants, *Abelmoschus Esculentus* at the farm of faculty of Agriculture, Mansoura University. The identification of the tested insect was confirmed at Scale Insect Department, Plant Protection Research Institute, Agric. Res. Center, Giza, Egypt as *P. solenopsis*. The mealybug was reared on okra (3-4 weeks old) planted in small pots (15 cm^3) and preserved under plastic greenhouse conditions of 27±5 ˚C, 70±5 RH and 16:8 hours L:D.

2.3 *In-vitro* bioassay

2.3.1 The insect pest bioassay for contact toxicity

Ten adult females/or newly molted second instar nymphs of *P. solenopsis* were randomly selected and carefully transferred to a plastic Petri dish (9cm in diameter) containing an okra leaf using camel hair brush to be considered as a replicate. Then, they were sprayed with 1 ml of the aqueous solution of the tested essential oil containing 0.3% Tween 80. The excess of solution was get rid of immediately after spraying, then, the lid of Petri-dish, bearing the ventilation holes was sealed. Each treatment was repeated thrice in addition to control (sprayed only with water containing 0.3% Tween 80) [18]. Mortality percentages of adult females/or the second instar nymphs were determined after 24h and 72h of treatment.

2.3.2 Bioassay for repellency effect

Sixteen okra seedlings with three leaves which were planted in pairs in pots (60 cm Length, 20 cm Depth and 16 cm Width) at 40cm distance of each other. One plant of pair was sprayed with the tested essential oil at LC_{90} of adult females and the other was sprayed with water containing 0.3% Tween 80 (control). 50 adult females of *P. solenopsis* were released into the center of the distance between each pair of seedlings directly and/or after 24hrs and 48hrs after treatment. The repellence effect of each essential oil was tested after different elapsed time post treatment by counting numbers of adult females settled on both pair of seedlings. The experiment was replicated thrice under the same conditions of 27±3 ºC, 70±5 RH and 16: 8 L: D photoperiods.

2.4 Field bioassay

Essential oils were tested against *P. solenopsis* on okra in open field conditions. The field was divided into equal blocks. Each four blocks were sprayed with an essential oil (four replicates) with *invitro*-LC_{90} of adult females. In addition, there were four replicates sprayed with water containing 0.3% Tween 80 to serve as controls. The field blocks were completely arranged randomly. Thirty leaves from okra plants in each replicate were examined for counting *P. solenopsis* individuals. The efficacy of the tested oils was recorded daily till the 3rd day after application.

2.5 Statistical analysis

For contact toxicity, the average of mortality percentages of *P. solenopsis* adult females/ or 2nd instar stage-nymphs were determined and corrected using Abbott's formula (1925) [19]. Then, they were statistically analyzed according to Finney (1971) [20] to estimate LC_{50}, LC_{90} and slope values. Also, Toxicity index was computed for different essential oils by comparing them with the most effective one using Sun's equation [21].

Repellence index (RI) [22-24] was calculated according to the equation: RI=2T / (T+C), where T is the number of insects on treated plants and C is the number of insects on the control plant. When RI values <1, it indicated repellence effect of thetested essential oil; RI>1, it indicated attractive effect of the tested essential oil. RIs values were statistically analyzed by Two-Way ANOVA using Co-Stat Software (2004) [25].

For the field experiment, the efficiency of the tested essential oils were determined according to Henderson-Tilton (1955) [26].

3. Results and Discussions

3.1 *In vitro* bioassay

3.1.1 Bioassay for contact toxicity

In vitro bioassay clarify that all the tested oils had a significant contact toxic effect on both adult females and 2nd instar nymphs of *P. solenopsis*. Some essential oils showed a peak level of efficiency at 24hrs after treatment unlike others which revealed the highest mortality percentage with increasing the time elapsed after treatment. At 24hrs after treatment, it was found that *M. longifolia* was the most efficient oil against adult females followed by *L. nobilis*, *Apium graveolens*, *M. sativa*, *C. articulates*, *A. graveolens*, *O. vulgar*, then *F. vulgar*. Our results about the high contact toxicity of *Mentha longifolia* essential oil against *P. solenopsis* were agreed with those recorded by Roozdar *et al*. (2020) [27]. But, after 72hrs of application, *L. nobilis* essential oil was the most efficient followed by *Apium graveolens*, *M. longifolia, C. articulates*, *M. sativa*, *A. graveolens*, *O. vulgar*, then *F. vulgar*. The 2nd instar nymphs were more susceptible for all tested essential oils at the same pace. LC_{50}, LC_{90} values and toxicity index for mortality values after 24hrs and 72hrs of tested essential oils against adult females and 2nd instar nymphs are given in table 1 & 2, respectively.

The insecticidal activity of the essential oils was varied depending up on their chemical constituents which the majority are terpenoids. These compounds showed neurotoxic activity by inhibition of acetylcholinesterase (AChE) via blocking receptors of octopamine, non-mammalian target [28]. Also, they conflict the ɣ-aminobutyric acid (GABA) gate chloride channels in insects [29].

Table 1: Toxicity of plant essential oils against adult females of *P. solenopsis* after 24hrs and 72hrs of treatment under laboratory conditions of 27± 2 °C., 70 ± 5% RH, and 16:8hs L:D

Essential oils	adult females													
	24hrs							72hrs						
	LC50 (ppm) and confidence limits at 95%		LC90 (ppm) and confidence limits at 95%		Slope ± SE	X^2	Toxicity index	LC50 (ppm) and confidence limits at 95%		LC90 (ppm) and confidence limits at 95%		Slope ± SE	X^2	Toxicity index
A. graveolens	188.21		2461.33		1.148 ± 0.358	0.09	20.78	103.605		1260.48		1.18 ± 0.376	0.134	14.58
	76.07	301.08	984.31	86448.59				19.81	174.81	611.11	20014.54			
Apium graveolens	39.59		395.87		1.282 ± 0.384	0.133	98.81	14.62		102.64		1.51 ± 0.368	0.359	92.90
	24.08	166.27	115.4	38892.86				9.81	22.13	50.81	658.94			
C. articulates	48.095		461.403		1.305 ± 0.367	0.174	81.34	29.43		311.495		1.251 ± 0.36	0.338	51.339
	31.72	108.7	165.93	13689.38				17.85	50.11	123.18	7174.49			
F. vulgar	1619.17		22245.46		1.126 ± 0.355	0.369	2.42	606.19		5183.63		1.375 ± 0.38	2.862	2.492
	947.14	3217.19	7315.38	2013558.36				214.44	936.47	2832.15	115962			
L. nobilis	39.221		291.56		1.47 ± 0.369	1.569	99.74	15.11		92.97		1.624 ± 0.39	0.181	100
	27.02	67.68	129.65	2804.04				8.05	21.48	55.74	323.88			
M. longifolia	39.12		377.175		1.302 ± 0.363	0.093	100	26.24		176.16		1.5 ± 0.37	0.869	57.58
	25.71	75.48	144.53	8521.26				17.34	38.38	91.45	947.59			
M. sativa	46.73		481.62		1.265 ± 0.364	0.19	83.72	34.53		319.75		1.326 ± 0.36	0.239	43.761
	30.49	107.85	167.33	18511.33				22.53	60.71	130.25	5361.03			
O. vulgar	576.75		5751.563		1.283 ± 0.371	0.047	6.783	308.89		3967.82		1.156 ± 0.36	0.132	4.891
	371.85	1605.16	1895.65	275373.27				180.76	577.58	1380.2	232542.84			

Table 2: Toxicity of plant essential oils against 2nd instar nymphs of *P. solenopsis* after 24hrs and 72hrs of treatment under laboratory conditions of 27± 2 °C., 70 ± 5% RH, and 16:8hs L:D

Essential oils	2nd instar nymphs													
	24hrs							72hrs						
	LC50 (ppm) and confidence limits at 95%		LC90 (ppm) and confidence limits at 95%		Slope ± SE	X^2	Toxicity index	LC50 (ppm) and confidence limits at 95%		LC90 (ppm) and confidence limits at 95%		Slope ± SE	X^2	Toxicity index
A. graveolens	145.05		895.17		1.622 ± 0.389	0.221	17.962	85.085		559.54		1.567 ± 0.392	0.368	12.168
	75.14	207.21	539.58	3088.21				27.45	135.59	367.43	1447.58			
Apium graveolens	34.25		389.3		1.214 ± 0.373	0.143	76.06	10.98		74.53		1.541 ± 0.371	0.581	94.29
	21.04	135.39	110.48	52942.26				6.81	15.75	39.997	369.08			
C. articulates	39.662		429.735		1.239 ± 0.361	0.216	65.688	23.273		193.62		1.393 ± 0.364	0.459	44.485
	25.54	82.00	152.86	16035.08				13.794	34.92	92.88	1622.19			
F. vulgar	1355.06		12714.74		1.318 ± 0.361	0.592	1.923	549.20		1721.6		2.583 ± 0.663	0.07	1.885
	824.61	2161.38	5504.92	173921.94				310.54	723.66	1241.75	3860.74			
L. nobilis	28.77		280.71		1.296 ± 0.371	0.752	90.547	10.53		63.11		1.633 ± 0.376	0.765	100
	18.62	78.43	93.89	12127.49				6.503	14.53	35.96	246.1			
M. longifolia	26.05		259.87		1.28 ± 0.36	0.013	100	21.64		119.12		1.73 ± 0.38	1.32	47.853
	15.3	41.71	109.996	4220.33				14.27	29.88	70.37	398.26			
M. sativa	36.61		272.96		1.47 ± 0.368	0.113	71.164	23.67		162.85		1.53 ± 0.369	0.47	43.743
	25.1	61.16	123.53	2471.32				15.03	34.27	85.47	866.92			
O. vulgar	351.659		4174.57		1.193 ± 0.358	0.063	7.409	229.329		2384.533		1.26 ± 0.36	0.09	4.514
	218.29	694.49	1460.64	199347.58				123.79	359.51	1021.91	39782.64			

3.1.2 Bioassay for repellency effect

It was found that all tested essential oils showed repellency effect against *P. solenopsis.* There were high significant differences of the tested oils repellency (F: 115.41; df: 7; $p<0.01$). Data in Fig 1-3 showed that time was an influential factor affecting the repellency of the tested essential oils. All essential oils showed high repellency immediately after treatment. *M. longifolia* showed the highest repellency followed by *L. nobilis*, *F. vulgar*, *Apium graveolens*, *M. sativa*, *C. articulates*, *A. graveolens* then *O. vulgar*.

After 24 hours of application, the repellency effects of the tested oils decreased to some extent and continued declination at 48hrs after treatment. It was clearly observed that time must be taken into consideration. The perfect illustration of that was like *M. longifolia* which showed the highest repellency effect after immediately application but quickly degraded showing high repellency declination at 24 & 48hrs. The most repellent oils with satisfactory stability were *L. nobilis*, *F. vulgar* and *C. articulates.* There were high significant differences among different elapsed times after treatment (F: 1111.81; df: 2; $p<0.01$).

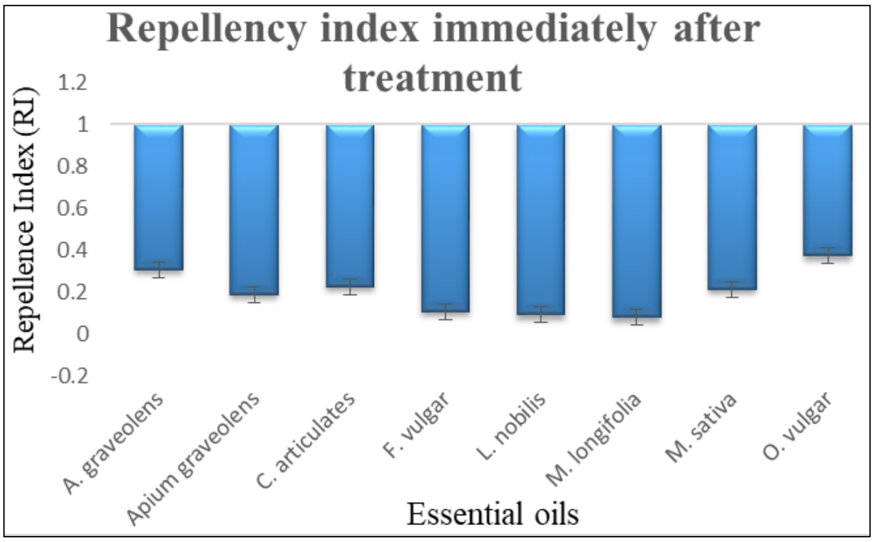

Fig 1: Repellence indices of the tested essential oils directly after treatment

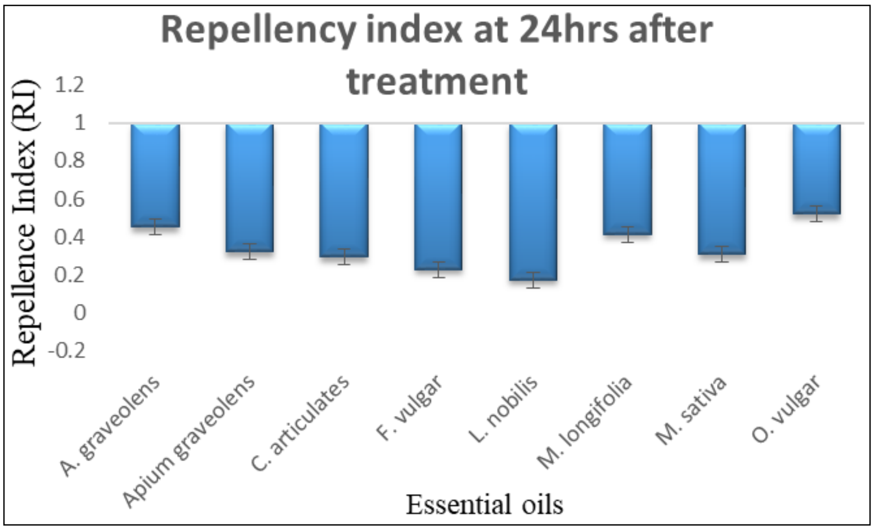

Fig 2: Repellence indices of the tested essential oils at 24hrs after treatment

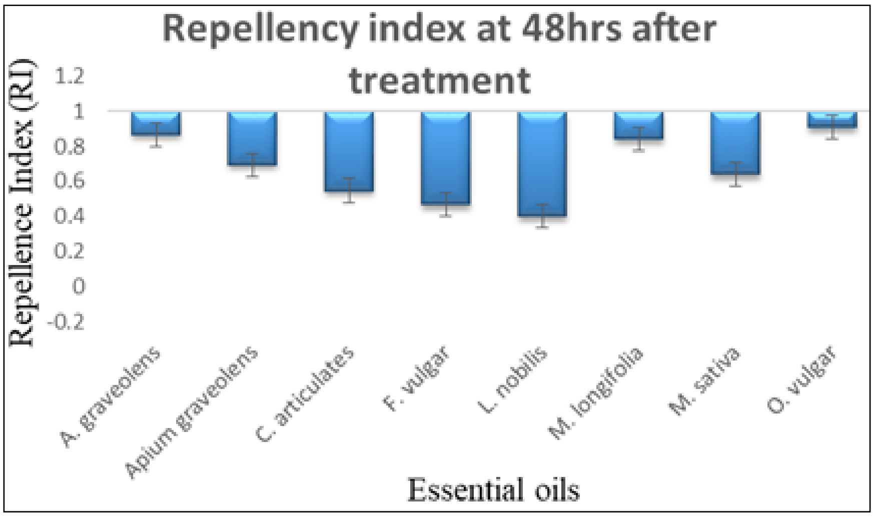

Fig 3: Repellence indices of the tested essential oils at 48hrs after treatment

3.2 Field bioassay

The field experiment demonstrated that essential oils greatly suppressed *P. solenopsis* populations on okra plants. At the 1st day after treatment, *M. longifolia* showed the highest population reduction followed by *L. nobilis, Apium graveolens, M. sativa, C. articulates, A. graveolens, O. vulgar* then *F. vulgar* with mean reduction percentages: 92.4, 91.1, 90.1, 89.9, 89.3, 88.9, 88.6 and 39.6%, respectively as shown in Table 3. At the 2nd and 3rd day after treatment, the population reduction declined gradually as a result of degradation of the essential oils by time. Also, it was clarified that there were great different between the tested essential oils degradation rates which play akey role in changing the efficient arrangement of them at different experimental times. Generally, the most efficient essential oils were *L. nobilis* followed by *Apium graveolens*, *M. longifolia* and *C. articulates*.

Table 3: Reduction % averages of *P. solenopsis* treated with essential oils in open field conditions

Insecticide	Pre-spray	Mean population per plant and percent reduction							
		Days after insecticide treatment						Overall Mean	
		1		2		3			
		Mean No.	% Red.	Mean No.	% Red.	Mean No.	% Red.	Mean No.	% Red.
A. graveolens	362.5^{a}	39.3^{b}	89.3	47.8^{b}	87.2	61.5^{bc}	84.2	49.5b	86.9
Apium graveolens	348.8^{a}	33^{b}	90.8	39.3^{b}	89.1	52.8^{c}	86.0	41.7b	88.6
C. articulates	341^{a}	35.5^{b}	89.9	42.8^{b}	88.0	53.8^{c}	85.5	44.0b	87.8
F. vulgar	355.8^{a}	41.5^{b}	88.6	51.8^{b}	85.9	72.5^{b}	81.1	55.3b	85.2
L. nobilis	363^{a}	33.3^{b}	91.1	37.3^{b}	90.0	51^{c}	86.8	40.5b	89.3
M. longifolia	336.3^{a}	26.5^{b}	92.4	38.8^{b}	89.0	56.5^{c}	84.5	40.6b	88.6
M. sativa	354.8^{a}	36.3^{b}	90.1	44.8^{b}	87.9	57^{c}	85.2	46.0b	87.7
O. vulgar	318.8^{a}	36.5^{b}	88.9	45^{b}	86.4	61^{bc}	82.3	47.5b	85.9
Control	327.8^{a}	336^{a}		341.5^{a}		356.3^{a}		344.6a	
LSD $_{0.05}$	134.9	15.4		14.9		14.7		20.2	

4. Conclusion
The present study spotlighted the importance of the essential oils as ecofriendly insecticides. Our results indicated that *L. nobilis*, *Apium graveolens*, *M. longifolia* and *C. articulates* showed high contact toxicity, repellency effect against *P. solenopsis*. Therefore, they are recommended to be used in wide scales for *P. solenopsis* control. This demonstrates beyond doubt the efficiency of essential oils as bio rational insecticides encouraging further studies for developing their application in wide scales as eco- friendly insecticides.

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