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Characterization of Green *Mentha pulegium* (L.) oil Nanotechnology and Adverse Effect on Two Cotton Bollworms, *Pectinophora gossypiella* (Saund.) and *Earias insulana* (Boisd)

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ABSTRACT

In our study, laboratory trials to determine the effectiveness of *Mentha pulegium* leaves essential oil nanoparticles against the pink *Pectinophora gossypiella*, and spiny bollworms (Saunders) Earias *insulana*, too. Gas mass spectrometry was used to study *M. pulegium* spectroscopy (GC/ MS) for the identification of the chemical the makeup of essential oil. Large three active components were identified as D-Carvone (28.46%), Menthol (27.88%) and l-Menthone (15.92%) the oil constituent. The level of LC values, *P. gossypiella* exhibited more sensitivity to *M. pulegium* than *E. insulana* and simply lengthening the exposure period, the death rate increased. A significant percent of larval mortality and malformation were recorded (81.113 and 68.89%) in addition to a remarkable percent of pupal mortality and malformation reached 30.55 and 34.53%, with a significant reduction in adult emergence for *P. gossypiella* and *E. insulana*, respectively. Moreover, some histological deformations at the level of the cuticular layer of treated larvae of both insects as a result of *M. pulegium* treatment were also detected compared to the control check.

INTRODUCTION

The most significant crop is regarded to be cotton (*Gossypium* spp. economic crop in Egypt. Bollworms resulted in Cotton seed yield losses range from 30 to 40 percent (Haque, 1991). *Pectinophora gossypiella* (Saunders), a pink bollworm that belongs to the family Gelechiidae of Lepidoptera, is regarded as one of the most harmful insects that infect cotton areas all over the globe. It causes significant harm to cotton bolls and significant loss in quantity and quality. Spiny bollworm (SBW), the *Earias insulana* (Boisd.) (Lepidoptera: Nolidae) is a serious insect pest infesting many crops and vegetable plants causing considerable damage and leading to a high loss in crops, (Amer ,2015).

In recent times, plant essential oils or extracts have created scientific because of their biological functions. Most of the insecticidal qualities are included in essential oils used as flavouring ingredients against several insect species, (Shah and Mello 2004 and Aflatuni 2005). Flowering plant *Mentha pulegium* a species from the *Lamiaceae* family commonly known as pennyroyal, (Gunby, 1979). one of the powerful plants in pest prevention is *M.*

pulegium, (Regnault *et al.*, 2012), where its According to reports, essential oils are a source of botanical pesticides that can be used to control a variety of insects, (Ghormade *etal.*, 2011). Moreover, it has been claimed to prevent pupation and reduce the expansion of *Peridroma saucia* (Hubner) (Harwood *et al*., 1990).

Regarding the insecticidal activity *M. pulegium* oil is a substitute to chemical insecticides, with adverse effects on non-target organisms, as well as reducing environmental damage, (sarmah *et al*., 2006). Several studies demonstrated that *Mentha* oil can currently use for pest management, (Attia *et al*., 2016 and Hanana *et al*., 2017) with commercial importance, (Lawrence, 2007). However, the main application trouble of essential oils is their instability in their chemical structure in atmospheric conditions due to the destruction of some active components and rapid evaporation, (Anjali *et al*., 2012). Nanotechnology is one of the most challenging technologies in controlling many Insecticides, pesticides, and other substances based on nanomaterials can be used to control insect repellants (Owolade *et al.,* 2008). Traditional integrated pest management techniques in agriculture include inadequate and the adverse consequence is some non-target organisms (Ragaei *et al*., 2014). The creation of nanoparticles devised various processes for inorganic materials, and it contributed to the growth of this little-known location depending on Nano-material biosynthesis (Mohanpuria *et al.,* 2007). Nanoemulsion may be used as an alternative for pest control as insecticidal activity and is less toxic than synthetic pesticides so being eco-friendly, (Massoud *et al.,* 2018).

However, the flavoured oils encapsulation incorporating a Nano-formulation stops destruction and quickly evaporating, improving stability and efficiency of the least application dose and increasing the perseverance (Devi and Maji, 2011) of the active component (Ghotbi *et al*., 2014 and Massoud *et al*., *2018*). The study's objective is to find new materials and evaluate *M. pulegium's* effectiveness as an EO Nano-emulsion a control agent against the two bollworms *p. gossypiella* and *E. insulana* on some biological and histologically two insects.

MATERIALS AND METHODS

The experiment was conducted in a controlled environment at the Plant Protection Research Institute's Bollworms Research Department at 26°C and 70% relative humidity.

Insect Cultures:

The two susceptible Laboratory strains of pink and spiny bollworms, *p. gossypiella* and *E. insulana*, The PBW was kept on a modified artificial diet described by Rashad and Ammar, (1985), while the SBWs were maintained on an artificial diet described by Amer, (2015). Both of two insects were raised for several generations without the use of any insecticides in a laboratory environment of 26°C and 70°RH.

Plant Composition:

Mentha leaves were bought at a neighborhood market. Leaves were dried in the shaded area of the laboratory, and 120 grams of dried leaves were subjected to hydrodistillation for two hours to obtain essential oil extraction. The surfactant (tween 80) was used and ethanol as a co-surfactant from El-Gomhoria Chemicals Company, Cairo, Egypt.

Isolation and Analysis of Essential Oil:

The *Mentha* leaves were weighted and hydro-distilled by employing a Clevenger apparatus that has been modified to fit a six-liter round-bottom flask. The distillate underwent centrifugation at 965.36 g for 10 minutes Featuring a crosspiece that is horizontal (Model R 206 T FANEM BL); oil was collected by To get rid of any moisture, pipette pasteur into a vial and keep it in a drying pistol. Following that, The bottle was frozen until used in

laboratory testing. Five grams of the chopped plant was extracted using a Dean-Stark extractor and 80 mL of cyclohexane. The amount of moisture was measured three times.

The oil was analysed qualitatively and quantitative using a Shimadzu Under the following circumstances, a GC-2010 gas chromatograph fitted with a GC-MS-QP 2010 Plus mass spectrometer: The lower and upper temperatures were maintained for 3 and 10 minutes, respectively, on the temperature-programmed gas chromatography, which was set at 35°C and 250°C with a 5°C/min gradient. Gas chromatography was employed to determine the retention indices, and two fused silica capillary columns (30 m 0.25 mm) were used. The flow rate of the carrier gas (helium) is 1 mL/min. 1 l of injection in split mode was used (split ratio 1:100). Using the information from the GC/MS internal library, the components of essential oils can be identified based on their mass spectra. According to the elution of their retention index, the compounds were found.

Nanoemulsion Preparation:

Preparation of Leaves of *M.pulegium* L EO The oil-in-water nanoemulsion experiment was carried out in the lab. of the National Research Center. Tween 80 and the essential oil of *M. pulegium* were used to create a nanoemulsion, co-surfactant ethanol and distilled water. Ten mL of *M. pulegium* oil, 3mL Tween 80-, and 3-mL ethanol were added to water 84 mL under gentle stirring until a uniform mixture was created, to reach the final 100 mL of the mixture were created and swirled with a magnetic stirrer for 15 minutes. Finally, 15 minutes of ultrasonic emulsification at a 20 kHz Sonicator with a 750 W power output. Oil and 10% *M. pulegium* oil Nano-emulsion was exposed to ATR-FTIR by using The combined platinum and diamond ATR Bruker Vertex 80 (Germany) uses a diamond disc as an internal reflector with a resolution of 4 cm-1 and a refractive index of 2.4. and 10% oil Nano-emulsion was exposed to UV-Vis and measured by using JascoV-630 from range190-1000nm.

***M. pulegium* Nanoemulsion Morphology**:

The morphology and form of Nano-emulsion oil were determined by EM unit's transmission electron microscope (TEM), National Research Centre, Dokki, Giza. One drop of the emulsion was laid on a grid of copper. JOEL JEM was used to acquire TEM micrographs -1230 plus utilising a tungsten source and operating at 120kv.

Stability:

The stability of the Nano-emulsion was tested for a month at 25 ºC. Also, the Nano-emulsion was spun at 10,000 rpm for 30 minutes according to (Ghosh *et al*., 2013). No observed creaming, phase separation or cracking was noticed which confirmed the Stability of the formulated Nano-emulsion.

Toxicity Bioassay:

To evaluate the toxicity of *M. pulegium* Nano-emulsion oil against *P. gossypiella* and *E. insulana* neoate larva, serially diluted concentrations of 20, 10, 5 and 2.5% were prepared. Each conc. sprayed on the surface of a modified diet, described above for each insect. A method of spraying 1 mL of tested concentration on five grams of the diet poured at petri dishes (9 cm) was applied. 30 newly hatched larvae of three replicates, each with three replicates the two tested insects were exposed to diet treatment for 3 days. The untreated was sprayed only with water. All treatments were kept under the same conditions. After 48 and 72 h., the dead number larvae mortality percentages were calculated. Analysis was estimated LDP line analysis software's LC_{25}, LC_{50}, LC_{90}, and slope values in accordance with (Finney, 1971)**.**

Biological Study:

The latent effect when tested *M. pulegium* relating to some biological characteristics of PBW and SPW-treated newly hatched larvae was studied. LC_{50s} (calculated after 48 hours) of the tested compound for both insects were spread on the artificial diet surface, allowing

larvae to feed on fifty larvae divided into three duplicates for each treatment used. Another group of larvae treated only with water was used as a control check. All treated and untreated larvae of both tested insects were kept under the previously mentioned conditions and inspected daily until pupation. The biological parameters such as mortality, malformation, duration for larvae and pupae, percent Pupation and adult emergency were registered and estimated.

For histopathological examination, the tested compound at its LC_{50} was applied to neonate larvae of *P. gossypiella* as well as *E. insulana*. After seven days, ten active larvae from each treatment and control were kept in a 10% formaldehyde solution in the refrigerator until the microscopic inspection. The morphological alterations of cuticle structure of each sample were analysed by microscopic inspection and vs the comparison check.

Histopathological Studies:

The result of *M. pulegium* on the histological structures of PBW and SPW-treated larvae was studied compared to untreated. Both PBW and SPW-treated larvae were placed in 10% formal saline for a day. In tap water samples were washed Then, to dehydrate, methyl, ethyl, and 100% ethyl alcohol were diluted successively. Specimens were cleaned in xylene and submerged in paraffin for 24 hours at 56 degrees in a hot air oven. Sled microtomes were used to make paraffin beeswax tissue blocks for sectioning at a thickness of 4 microns. For examination using a light electric microscope, the tissue sections were placed on glass slides, deparaffinized, and stained with hematoxylin and eosin (Banchroft *et al.*, 1996).

Analytical Statistics:

Data were acquired, and one-way analysis of variance was used to analyse them (ANOVA) ($P < 0.05$ %) Snedecor ,(1971) and Duncan's multiple range test of means state that according to Duncan, (1955) was used.

RESULTS AND DISCUSSION

Characterisation of Nano-emulsions:

M. pulegium EO Nano-emulsion is turbid and milky white in colour while the turbidity decreases and the emulsion became optically clear after sonication. McClements, (2002).

UV-VIS and FTIR Spectroscopic Analysis:

UV-VIS Analysis:

UV-Vis is depended on visible proton and irradiation of ultraviolet on the sample and measures the absorption rate of matter at different wavelengths. In the UV-VIS spectral range in the region between 200 to 400 nm, the appearance of one or more peaks is an unmistakable sign that unsaturated groups and heteroatoms such N, S, and O according to Njokua *et al.*, (2013). The spectrum for *M. pulegium* EO Nano-emulsion displays two peaks at wavelengths of 196 nm and 241 nm, the obtained data is given as follows in Fig. 1 and Table 1, (1).

Table (1): UV-VIS peak values of *M. pulegium* Nano-emulsion.

No.	Wavelength (nm)	Absorbance
1	196	1.7
2	241	1.47

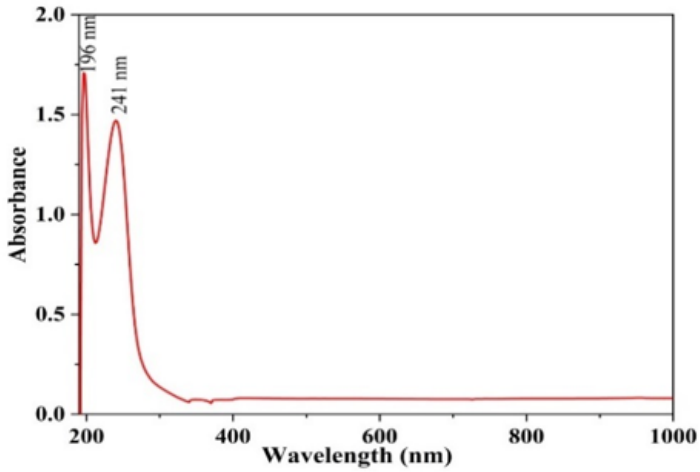

Fig. 1: UV-VIS spectra of *M. pulegium* Nano-emulsion.

FTIR Analysis *M. pulegium* L. Functional Groups:

The FTIR of the *M. pulegium* L.oil and its emulsion are presented in Figures (2&3) respectively, and Table (2).

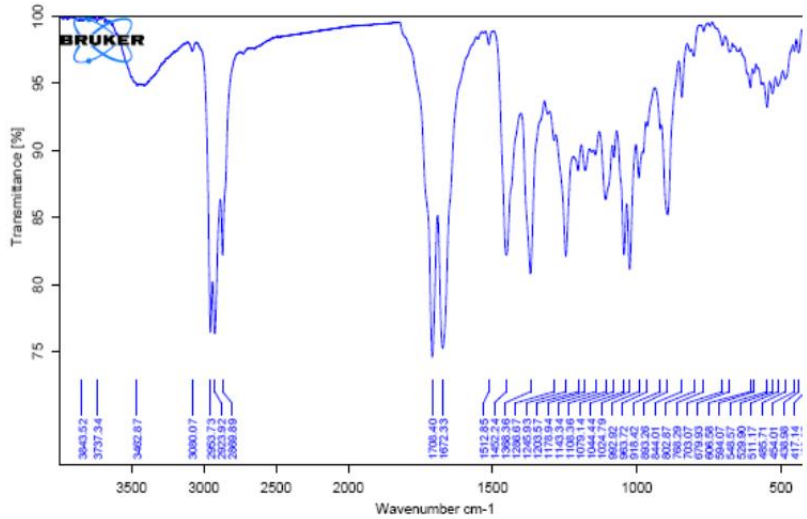

Fig. 2: FTIR spectrum of *M. pulegium* oil.

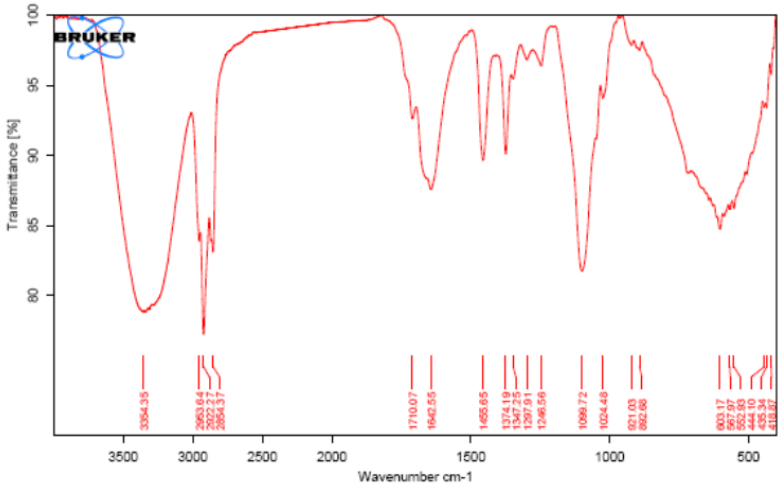

Fig. 3: FTIR spectrum of *M. pulegium* Nano-emulsion.

The FTIR result demonstrated allocated to the strong band at 3354.35 cm1 vibration that the OH group of water molecules, the peaks range from 2854.37-2953.64 cm-1 that vibration is assigned to methanol and menthone according to prakash and yunus, (2013).

Table 2: IR spectra's, refractive indices.

	IR spectra (ATR, cm-1)
Essential oil	3462.87`-3080.07-2953.73-2869.89-1708.40-1672.33-1512.85-1452.24-1286.67-1245.93-1079.14-1044.44-1024.79-918.42-893.26-768.29-679.93-594.07-548.57-529.90-485.71-454.01-483.98-417.14
Nano-emulsion	3354.35-2953.64-2854.37-2922.27-1710.07-1642.55-1455.65-1374.19-1297.91-1246.56-1099.72-1024.48-921.03—892.68-603.17-567.97-552.93-444.10-435.34-418.87

The peaks at 2922.27cm-1 corresponded to the CH2 asymmetric stretch and the CH3 symmetric stretch the bands in the spectra range 1642.55-1710.07cm-1are most probably related to groupings of pulegone and piperitone c=o,1455.65cm-1 to CH2 scissoring,1374.19 cm-1 to CH-CH3present in iso menthone and pulegone according to AL-Shareefi *et al.*, (2019). Finally, we observe in the same spectral area, both have identical conspicuous bands. *Mentha pulegium* L.oil and its Nano-emulsion.

Nano-emulsion Surface Morphology and Particle Size Distribution:

TEM images **(**Fig. 4**)** indicated the Nano-capsules structure of Nano-emulsion. Particles were spherical in shape and sized around 41-137nm in diameters.t The Nano-capsules of both EOs were found to be spherical according to Transmission Electron Microscopy (TEM) studies. The droplet size of a good Nano-emulsion is between 20-200 nm, (Sugumar *et al.*, 2014 and Ostertag *et al.*, 2012) this is in accordance with our findings.

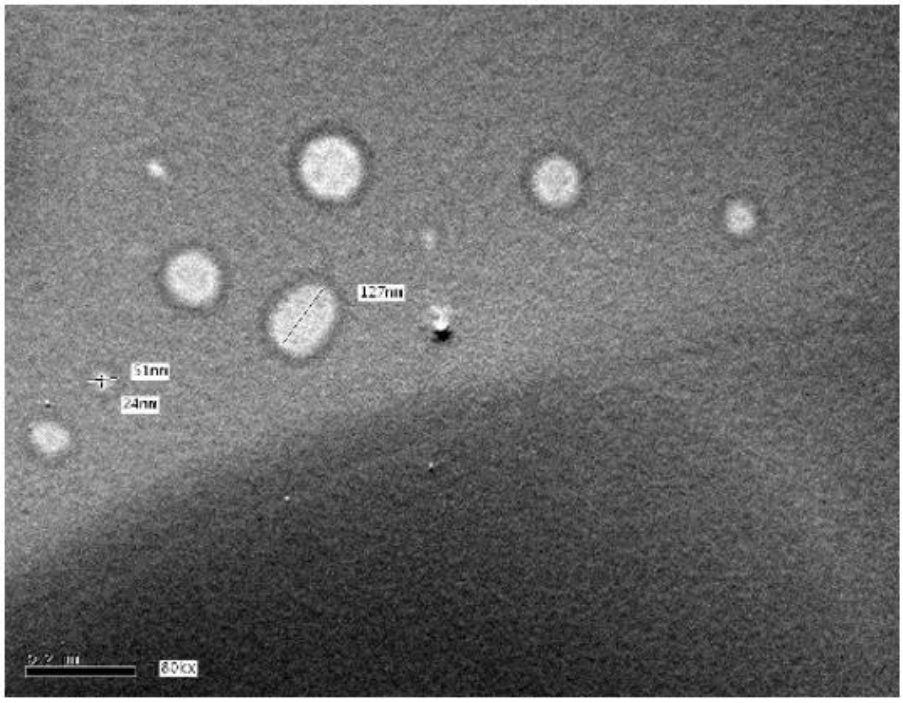

Fig. 4: TEM image of Nano-emulsion essential oils of *Mentha pulegium.*

Chemical Composition of *M. pulegium* L.Oil:

Twenty-six employing these methods, substances were described and identified. GC-mass spectroscopy due to the oil of *M. pulegium* Fig. (5) and Table (3) produced in Egypt. The three main active elements were determined to be D-Carvone (28.46%), Menthol (27.88%), l-Menthone (15.92%) the oil constituent.

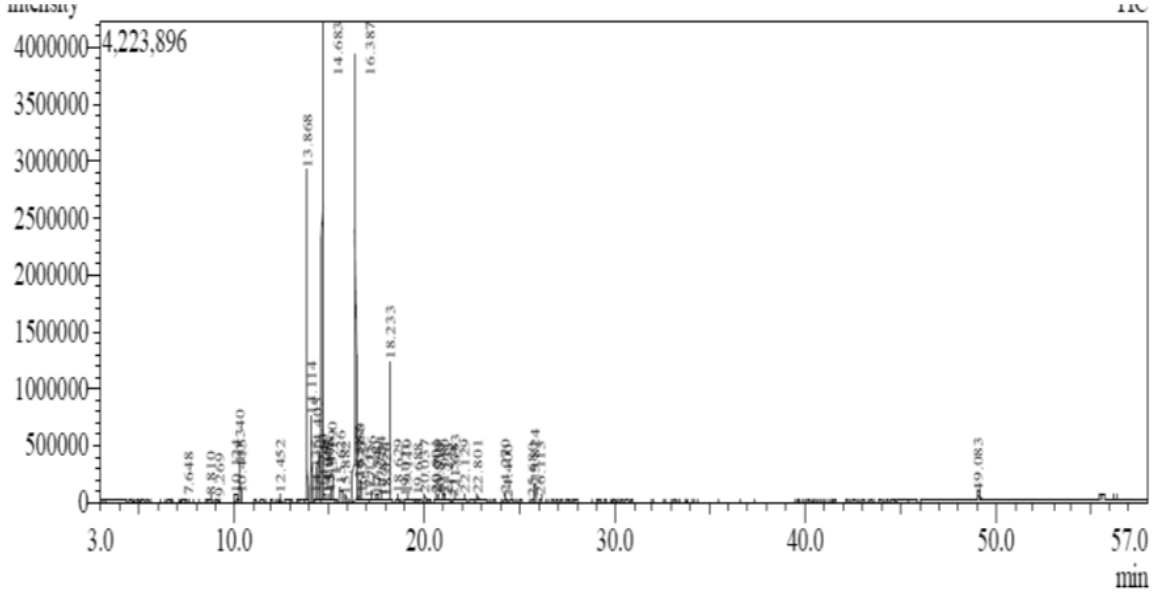

Fig. 5: Gas Chromatography-mass spectroscopy (GC-MS) of the volatile oil of *Mentha pulegium.*

Table 3: The volatile compounds identified from *Mentha pulegium* by using GC-MS.

No.	Compounds	RT	Content (%)
1	(1R)-2,6,6- Trimethylbicyclo [3.1.1] hept-2-ene	7.648	0.13
2	6-Undecanol	9.269	0.07
3	α pinene	10.124	0.22
4	Eucalyptol	10.340	1.43
5	Linalyl acetate	12.452	0.23
6	l-Menthone	13.868	15.92
7	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, cis	14.114	3.60
8	2-Furancarboxylic acid, cyclobutyl ester	14.335	0.43
9	Menthyl acetate	14.405	2.22
10	Menthol	14.683	27.88
11	D-Carvone	16.387	28.46
12	Piperitenone oxide	17.236	0.45
13	(-)-Neomenthylacetate	18.233	6.23
14	Mepivacaine	19.688	0.17
15	Glutaric acid, (2-methylcyclohex-1-enyl) methyl tridec-2-yn-1-yl ester	20.709	0.27
16	Isopiperitenone	20.818	0.10
17	(-)-. beta. -Bourbonene	21.080	0.29
18	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	21.233	0.16
19	5-Undecen-4-one	22.129	0.28
20	piperitenone	22.801	0.20
21	(6R)-7a-Hydroxy-3,6-dimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	24.270	0.39
22	1H-Indene, 2,3-dihydro-1,1,5,6-tetramethyl-	24.400	0.10
23	pulegone	25.680	0.13
24	Caryophyllene oxide	25.824	0.80
25	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a. alpha.,4. beta.,4a	26.113	0.17
26	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R-[R*,R*-(E)]]-	49.083	0.54

The activity of *M. pulegium* EO Nano-emulsion against *P. gossypiella* and *E. insulana:*

Results related to *M. pulegium* Nano-emulsion toxicity against bollworms were illustrated in Table (4). Recorded data revealed a toxic effect for *M. pulegium* against both tested insects after 48 h of treatment.

Table (4): Lethal concentration (LC) values of *M. pulegium* EO Nano emulsion against pink bollworm and spiny bollworm treated larvae.

Used comp.	Tested insects	Time (h)	Lethal concentrations (95% Confidence limits)			Slope
			LC_{25} (%)	LC_{50} (%)	LC_{90} (%)	
M. pulegium EO Nano emulsion	*P. gossypiella*	48h	2.7639 (1.270 - 4.060)	6.645 (4.669 - 9.270)	35.184 (20.371 - 121.089)	1.771+/-0.378
		72h	1.5633 (0.550 - 2.501)	3.523 (2.065 - 4.826)	16.4961 (10.989 - 39.037)	1.912+/- 0.413
	E. insulana	48h	1.433	7.898	202.2917	0.910+/- 0.350
		72h	1.5705 (0.188 - 3.003)	5.613 (2.887- 8.913)	63.1397(26.08- 1389.478)	1.220+/- 0.359

P. gossypiella exhibited more sensitivity to *M. pulegium* than *E. insulana* at the level of LC values which may be attributed to the soft outer surface of its body allowing a high opportunity for pesticide absorption compared to the spiny surface of *E. insulana*. The lethal concentrations (LC_{50}) values after 48 h were 6.645 and 7.898% while, decreased to 3.523 and 5.613% after 72h of exposure for *M. pulegium* against *P. gossypiella* and *E. insulana*, respectively, as shown in Table (4). The mortality rate increases by increasing the exposure time and so increasing the internalisation of the harmful chemical by the insect. The small particle of Nano-emulsion size increases the area of emulsion droplets' surfaces and consequently, more opportunity for the particles to adhere and be absorbed by the biological tissues of the insect's body which resulted in their toxic effect, Margulis *et al.*, , 2012, Gonzalez *et al.*, 2014, Nasseri *et al.*, 2016 and Massoud *et al.*, 2018. They added that the location C-C double bond that these two molecules contain enhances the toxicity. Abdelgaleil, (2009) recorded monoterpenes cause inhibitory effects on the activity including *Tribolium castaneum* and *Sitophilus oryzae* (L.) (herbst) by contact toxicities. Also, Abdelgaleil *et al*., (2020) found that carvone can cause significant antifeeding as well as growth inhibitory effects and induce toxicity in *Spodoptera littorallis* larvae. In addition, the essential oil of *M. pulegium* is believed to have insecticidal properties because of menthol, menthone, and menthofuran as major constituents, (Behnam *et al*., 2006. Moreover, not recorded any development of resistance against botanicals, (sarmah *et al*., 2001).

Biological Effects of *M. pulegium* Nano-emulsion against E. insulana and P. gossypiella:

P. gossypiella and *E. insulana* larvae that were previously treated as a neonate with LC_{50} calculated after 48 h. of *M. pulegium* treatment, were inspected to study Biological aspects that differ from the control

Larval Stage:

Information in Table (5) demonstrated the impact of prepared Nano-emulsion against neonate *E. insulana* and *P. gossypiella* larvae till the end of the larval stage. A significant Percent of larval mortality and malformation for both tested insects were recorded at 81.113 and 68.89% *P. gossypiella and E. insulana* as compared to the control, respectively. Moreover, The larval period lasted significantly elongated as a result of

treatment for both insects. The value was 21 days for *E. insulana* and 23 days for *p. gossypiella* compared to 15.26 and 15.90 days for untreated, respectively (Table 5).

Table 5: Effects of *M. pulegium* EO Nano-emulsion on treated larvae of *P. gossypiella* and *E. insulana*.

Treatments	*P. gossypiella*		*E. insulana*	
	% Larval mortality and malformation	Larval duration (days)	% Larval mortality and malformation	Larval duration
M. pulegium	81.113[a]	23.00[a]	68.89[a]	21.00[a]
Control	6.67[b]	15.90[b]	8.89[b]	15.26[b]
LSD (0.05)	9.757	4.2168	13.798	3.86
F	448.747	21.854	145.76354	17.045
(P)	0.0000 ***	0.0095 **	0.0003 ***	.0145 *

Pupal Stage:

The effect of *M. pulegium* Nano-emulsion on *Larvae treated with P. gossypiella and E. insulana* extended to include the resulting pupae. A high percent of pupal mortality and malformation was detected as a result of treatment estimated by 30.55% for *P. gossypiella and increased to* 34.53% for *E. insulana* in comparison to control which recorded 3.90 and 2.46%for the two insects respectively. Add to this, significant elongation in pupal duration was recorded at 11.00 and 10.33 days for the two insects, respectively as compared with the control, (Table 6).

Table 6: Effects of *M. pulegium* EO Nano-emulsion on resulting pupae**.**

Treatments	*P. gossypiella*		*E. insulana*	
	% Pupal mortality and malformation	Pupal duration (days)	% Pupal mortality and malformation	Pupal duration (days)
M. pulegium	30.55[a]	11.00[a]	34.53[a]	10.33[a]
Control	3.90[b]	9.6[a]	2.46[b]	7.60[b]
LSD (0.05)	7.974	2.0085	21.932	2.531
F	86.103	3.745	16.489603	8.989
(P)	0.0008 ***	0.1251 ns	0.0153 *	0.0400 *

Adult Stage:

As a latent effect of *M. pulegium* Nano-emulsion treatment, the emerged adults have been affected by both tested insects. Table (7)'s data demonstrated that the rate of adult emergence percentages was significantly reduced due to treatment. The values were 69.45 and 65.47% for *P. gossypiella and E. insulana,* respectively.

Table (7): Effects of *M. pulegium* EO Nano-emulsion on emerged adults.

Treatments	*P. gossypiella*		*E. insulana*	
	% Adult emergence	♀ Sex ratio	% Adult emergence	♀ Sex ratio
M. pulegium	69.45[b]	0.367[b]	65.47[b]	0.63[a]
Control	96.34[a]	0.567[a]	97.54[a]	0.60[a]
LSD (0.05)	7.71	0.131	21.932	0.185
F	93.789	18.00	16.489	0.25
(P)	0.0006 ***	0.0132 *	0.0153 *	0.6433 ns

Compared to 96.34 and 97.54% for control, respectively. On the other hand, the sex to male ratio mer*ged adults* moved to the male side. (0.367♀ Sex ratio**)** in treating *P.*

gossypiella with M. pulegium Nano-emulsion, while to the female side in *E. insulana treatment (*0.63♀ Sex ratio) as well as untreated. Generally, Therefore, *M. pulegium* essential oil can be said to Table (7)'s data demonstrated that Nano-emulsion form was found to exhibit potent insecticidal activity against the two tested bollworms. *E. insulana* was the most sensitive at the level larval stage while *P. gossypiella* showed the highest sensitivity at the pupal and adult stages. Thereby, *M. pulegium* the active ingredients in essential oils have potent larvicidal activity and latent effect efficacy against both PBW and SBW and were created to act as control agents. The ability of essential oils to kill insects their constituents has also been reported against different insect species by many authors. Harwood *et al*., 1990, found that Menthol inhibited pupation and highly reduced growth of the *Peridroma saucia* (Hubner). Brahmi *et al.,* (2016), assessed *Mentha pulegium's* contact toxicity, fumigant toxicity, and repellent impact on adults of *Rhyzopertha dominica.* The LC_{50} of *Mentha longifolia* Nano-emulsion has stronger and contact toxicity effect on the occurrence of *E. kuehniella* deaths 5th instar larva and other various pests, (Louni *et al.,* 2018).

Generally, obtained results indicate the possibility of using *M. pulegium* EO Nano-emulsions as control agents against bollworms which are in keeping with earlier research. Zekri *et al., (*2016). Pointed the potential for *M.pulegium* L the use of a botanical pesticide against dangerous insects Massoud *et al*., (2018) stated that Nano-emulsions are eco-friendly and may serve as alternatives less hazardous than synthetic insecticides for the control of insect pests. Natural products from plants are healthy, reducing environmental pollution and do not have the toxic effect as chemical insecticides so could be used as control programs, (Ramzi *et al.,* 2022).

Histopathological Studies:

The histological examination clears some histopathological alternation of the cuticle layers for both *Treatment of P. gossypiella and E. insulana larvae* as neonates *M. pulegium* Nano-emulsion. The normal cuticle is formed of a well-arranged cellular layer of the Epidermis followed by an inner layer of connective or foundation membrane tissue Endocuticle layer, followed by an outer Exocuticle layer for both *P. gossypiella,* (Fig. 6) as well as *E. insulana,* (Fig. 8**)** larvae.

The treated larvae showed remarkable variations characterized by vacuolization and disorganization in the epidermal layer with mostly irregularly distributed and undistinguishable cuticular layers. The cuticular layers appeared to be compacted and no differentiation between Exocuticle and Endocuticle could be observed with the loose of spins and buds for both *P. gossypiella* and *E. insulana* larvae (Figs. 7&9) compared to those of the control. According to results, *M. pulegium* Nano-emulsion with its essential oils has an impact on the cuticular layers of treated larvae that negatively affect insect development and/or even death. Aziza Sharaby *et al*., (2016) stated the nanoemulsion oil can cause reduction in the rate of chitin deposition and growth of *Agrotis ipsilon* larvae by interfering with activity chitin synthesis in cuticle. When ecdysis occurs, the newly created cuticle loses some of its rigidity and is able to withstand the internal pressure leading to death (Chaibe *et al*., (2007), Al-Dahafer *et al*., (2012) reported that most *A. ipsilon* larvae have died during ecdysis after mint treatment.

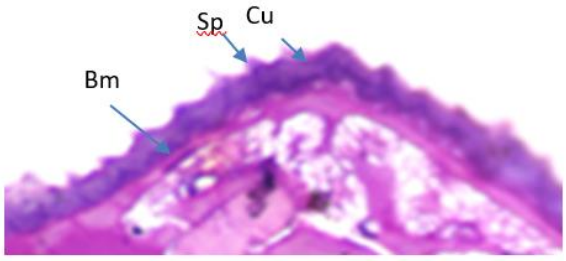

Fig.6: L.S. of the cuticle for untreated *P. gossypiella* larva.

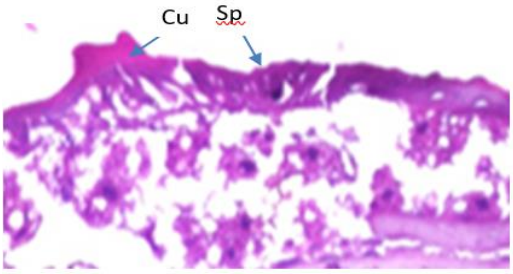

Fig. 7: L.S. of the cuticle for the cuticle of *P. gossypiella* larva treated as neonate with *M. pulegium* Nano-emulsion

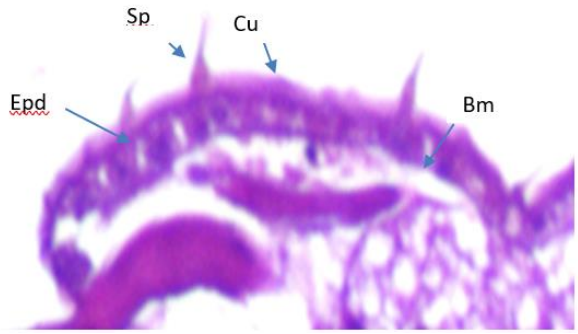

Fig. 8: L.S. of the cuticle for untreated *E. insulana* Larva

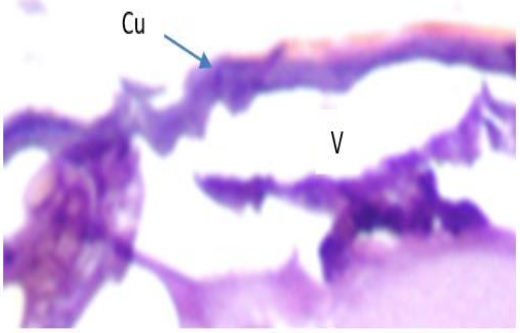

Fig. 9: L.S. of the cuticle for the cuticle of *E. insulana* Larva treated as neonate with *M. pulegium* Nano-emulsion

Note: Cu: Cuticle, **Epd**: epidermis, **V**: Vacuole, **Sp**: Spin, **Bm**: basement membrane.

CONCLUSION

According on the current facts, we may say that, the biosynthesized *M. pulegium* EO Nano-emulsions/nanoparticles were discovered to have strong insecticidal effects on the two tested bollworm pests *P. gossypiella and E. insulana*, and could be developed as a control agent.

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