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Efficacy of Nano chitosan and mandarin crust oil on some biological aspects of Cotton Leafworm, *Spodoptera littoralis* (Boisd.)

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Abstract
The effectiveness of mandarin crust oil, (*Citrus reticulate*) and Nano Chitosan was evaluated under laboratory conditions against egg masses, 2_{nd} instars larvae of cotton leaf worm, *Spodoptera littoralis*. The obtained results indicated that the effect of the oil and Nano Chitosan on *S. littoralis* eggs can be summarized as delayed embryonic development and ovicidal activity. According to the no hatchability % of the treated eggs, data showed that at the lowest concentration (10 ppm, 1000ppm), (57%) of the Nano Chitosan, and mandarin crust oil caused a significant reduction in percent non hatchability compared with control (100 %). hatchability On the other hand**,** Nano Chitosan exhibited the highest toxic effect against egg stage followed by mandarin crust oil (*Citrus reticulate*) compared with control. However, the LC_{50} values were 11.5393, and 550.423 ppm, respectively. The treatments showed toxic and morphogenetic effects on *S. littoralis* 2^{nd} instars larvae. Nano Chitosan was the most effective against 2^{nd} instars larvae of *S.littoralis* followed by mandarin crust oil. The LC_{50} values were 6.537, and 2645.48 ppm, respectively. Also, the treatments were shown to cause some morphological changes in treated larvae. On the other hand, all treatments caused malformations in the emerged moths. So that, tested treatments can be applied safely in IPM program for the cotton leafworm, *Spodoptera littoralis* control.

Keywords: Spodoptera littoralis, Nano Chitosan, mandarin crust oil, Malformation

1. Introduction
The Egyptian cotton leaf worm (CLW), *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is one of the most destructive pests of several crops such as cotton, corn, peanut, clover, vegetables and various fruits in Africa, Asia and Europe (Smagghe and Degheele, 1997; [29] El-Aswad *et al*., 2003 [9] and Ragaei and Sabry, 2011) [24]. This pest has a very wide host range of at least 87 plant species over 40 plant families including many vegetable, fruit and ornamental crops. Thus, this pest seemed a destructive pest, not only to cotton, but also to other field crops (Reda *et al*., 2013) [26].It has at least 7 generations a year (Magd Eldin& El-gengaihi, 2000) [17]. The intensive use of broad spectrum insecticides against the cotton leaf worm (CLW) had led to the development of resistance to many of them (Aydin and Gurkan, 2006 and Rizk *et al*., 2010) [27]. In Egypt, many problems have been uncounted as a result of the extensive use of synthetic chemical insecticides. Increasing problems concerning with the application of insecticides including insecticides resistance, residual contamination of human foods, mammalian toxicity and pollution of the environment (Abd El- Wahab, 2003 [2]. Nanotechnology has brought enormous amount of manufactured nanoparticles in the various areas of science, mainly due to its widespread application in a broad range of subjects including engineering, medicine, agriculture, chemistry, biology to applied sciences (that is electronics and materials). In recent years, there has been considerable interest in exploring the potential of nanotechnology in encapsulation at Nano scale (1nm=10-9 m) that is Nano encapsulation. Nano encapsulation is a process of coating or encapsulation of nanoparticles such as an insecticide using biodegradable polymeric matrix material (Bhattacharyya *et al.,* 2010). Nanoparticles have a major challenge due to the extremely small size, high surface energy, and high surface area. And there were reduction of feeding ratio and mean larval weight while larval mortality and antifeedant index increased compared to those of control. For pupal stage, pupal duration and pupal mortality increased while pupal average weight and percent adult emergence decreased compared to those of control. For adult stage, there were reductions in both fecundity and fertility while percent sterility increased compared to those of control (El-Kholy *et al*., 2014) [12]. Chitosan is derived from chitin, a polysaccharide found in exoskeleton of shellfish such as shrimp, lobster or crabs and cell wall of fungi. Chitosan poly (1, 4)-2-amino-2-deoxy-β-Dglucose, is a deacetylation product of chitin, a polysaccharide second by the prevalence in nature after cellulose. It is not only pollution safe, but nourishes the plant and less costly too. Chitosan is preferred due to its, antioxidant, biodegradability, biocompatibility, antimicrobial, and non-toxic properties (Dash *et.al.,* 2011) [8] Generally essential oils are secondary metabolites, volatile, with strong odor and are formed of mixture of several up to dozens of mono- and di-, sesqui-terpenes (Pavela, 2005) [22]. The composition of essential oils varies with every plant species and also on the growth stage of the plant. Different essential oils have been used as repellent, fumigant, larvicidal, ovicidal and adulticidal against different insect orders (Isman, 2000 [16], Mossa, 2016 [19] and Ali *et.al.,* 2017). The aim of this study is to evaluate the biological activity of (mandarin crust oil) and nanoparticles on *S. littoralis*, (Boisd) immature stages (eggs, and, 2nd instars larvae).

Materials and Methods
Insect sources
To have a culture of the cotton leaf worm, *Spodoptera*

littoralis (Boisd.), freshly eggs masses were obtained from a susceptible laboratory strain maintained in the Integrated Pest Management (IPM) laboratory; Cotton leaf worm Department, Plant Protection Research Institute, Dokki, Egypt. All stages of *S. littoralis* were reared and tested at 27±2° C and 65± 5 % R.H (EL-Defrawi *et al.*, 1964). Larvae were fed on fresh caster bean (*Ricinus communis* L) leaves until pupation. The full grown larvae were collected and placed in clean Jars with moist saw dust placed at the base to provide the pupation site. Adults were fed on 10% sugar solution offered in a piece of cotton tissue soaked in this solution., fresh green leaves of tafla, *Nerium oleander* (L.) were provided for egg laying.

Treatments

Preparation Nano-Chitosan:

Preparation Nano-Chitosan using the bottom-up approach, the ionic gelation method for the preparation of nanoparticles of hydrophobic polymers. The preparative methods were extremely mild and involved a mixture of two aqueous phases at room temperature. One phase of solvent contained the chitosan, while other phase contained polyanion sodium tripolyphosphate (TPP). Chitosan (CS)-g-poly (acrylic acid) (PAA) nanoparticles. It is bought from Naqaa Company. Plant materials and isolation of essential oil Mandarin crust oil is an oil expressed from the peel of the mandarin orange, (*Citrus reticulate*) To have Peel oil of mandarin from host plant, (*Citrus reticulate*) as well as fresh peels fruits of mandarin (*Citrus reticulate* L) were washed and dried at room temperature. Essential oil (volatile oil) of mandarin (*Citrus reticulate* L) was tracted by steam distillation apparatus in the laboratory at Plant Protection Institute, Mansoura, Egypt. The oil was separated dried over anhydrous sodium sulfate and stored in dark glass bottles at 4° C in refrigerator until used (Mohammed and Hany, 2013).The Chemical formula was: Crude oil was dissolved to give 100000 ppm stock solution of Essential oil. The stock solutions were prepared fresh by prior to doing the experiments, Tween-80 emulsifier was added at a concentration of 0.01% to the dilution before preparing the tested concentrations (1000, 2500, 5000, and 10000 ppm) for bioassays was used.

Preparing the stock solution of the tested materials

Convenient stock concentrations of each material were prepared on basis of the tested material weight and the volume of the distilled water (w/v). Four diluted concentrations for each material were used to draw the LC-P lines. Three replicates were used for each concentration.

Laboratory bioassay

1. Influence of tested oil and Nano-Chitosan on egg stage:

To study the effect of the treatments on *S. littoralis* egg stage, healthy tafla leaves with homogenous egg masses (100 eggs/ mass/ leaf) were collected from the laboratory colony. Leaves with egg masses were sprayed with different concentrations (1000, 2500, 5000, and 10000, ppm) of the tested oil and (5,10,20,40 ppm) of Nano-Chitosan on the tested Treatments. Leaves sprayed with distilled water served as control. After spraying, the leaves were air - dried and placed at the bottom of glass Petri dishes. Three replicates were used for each concentration, Microscopic examination was made after 3&4 days. In each treatment the number of hatcheing egg was calculated.

2. Influence of tested oils and Nano-Chitosan on 2nd instars larvae

The leaf-dipping technique, similar to that described by Tabashink *et al*. (1990) [31] was used to determine the toxicity of treatments against the 2nd instar larvae, Fresh castor bean leaves homogenous in size were dipped for approximately 10 seconds into each of different concentrations (1000, 2500,5000, and 10000, ppm) of the tested oil and (5,10,20,40 ppm) of Nano-Chitosan treatments. Leaves dipped in distilled water served as control. After dipping, the leaves were air - dried and placed at the bottom of glass Petri dishes. Ten 2nd instars larvae /replicate were allowed to feed on treated leaves.three replicates were done for each treatment. Larvae were allowed to feed on treated leaves for only 24 h, then, live individual were supplied with untreated castor bean leaves. The larvae were observed and examined daily for 7 successive days after treatment. The toxicity were recorded after one, three, five and seven days post treatment and LC_{50} values were calculated.

The mortality percentage was estimated and corrected according to the Abbott's formula (1925). LC_{50} values were determined using probit analysis statistical method of Finney (1971) [13].

Equation: Sun, 1950 (to determine LC_{50} index)

$$\text{Toxicity index for } LC_{50} = \frac{LC_{50} \text{ of the most effective compound}}{LC_{50} \text{ of the least effective compound}} \times 100$$

Gas chromatography–mass spectrometry (GC-MS) analysis

The chemical composition of your samples were performed using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 50 C and then increased by 5°C /min to 230°C hold for 2 min. increased to the final temperature 290°C by 30°C /min and hold for 2 min. The injector and MS transfer line temperatures were kept at 250, 260°C respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min and diluted samples of 1 µl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

Results

1. Laboratory Experiments

Influence of tested oil and Nano-Chitosan on egg stage

The effectiveness of tested oil and Nano-Chitosan was evaluated under laboratory conditions. The obtained results are summarized in Tables (1 and 2) and illustrated in Figure (1). The effect of the treatments on *S. littoralis* eggs can be summarized as delayed embryonic development and ovicidal activity. As shown in Table (1), all treatments significantly inhibited the embryonic development of *S. littoralis* eggs, the proportion of non-hatching eggs was considerably high in treated egg-mass in comparison with untreated one. On the other hand, at the concentration

(10000, 40 ppm), the proportion of non-hatching eggs was (85.75 %) and (84 %) of the tested oil and Nano-Chitosan. respectively. It caused a significant reduction in percent non hatchability compared with control (0%). Also, at 1000 and 10 ppm of tested oil and Nano-Chitosan. The Mean of non-hatching percent eggs was 57 %. Respectively. So, it could be concluded that tested treatments had delayed effect on the embryonic development of *S. littoralis*. Statistical analysis indicated that tested oil and Nano-Chitosan had the highest efficiency against *S. littoralis* eggs, compared with control Data presented in Table (2) and Fig. (1), revealed that Nano-Chitosan had the highest toxicity against egg stage of *S.littoralis* followed by tested oil. The LC_{50} values were 11.5393 and 550.423 ppm, respectively. The toxicity index values were 100 and 2.09 for Nano-Chitosan followed by tested oil, respectively. The slope values indicated that, tested oil had the lowest value was 0.9043 followed by Nano-Chitosan 1.9304, respectively. Also, LC_{90}/ LC_{50} values were 26.138, and 4.612, respectively.

Table 1: Efficacy Nano Chitosan and mandarin crust oil against the cotton leaf worm, *Spodoptera littoralis* Boisd. eggs under laboratory conditions (27±2 ◦C and 65±5% RH.) (L.S.D (5 %) =9.4).

Treatments	Conc. (ppm)	Non hatching proportion after treatments				Mean of Non hatching %
		One day	Three days	Five days	Seven days	
Nano Chitosan	5	14	16	20	22	18
	10	20	40	84	84	57
	20	55	58	68	75	64
	40	82	84	84	86	84
mandarin crust oil	1000	42	50	58	78	57
	2500	69	76	79	79	75,75
	5000	80	81	82	82	81.25
	10000	85	85	86	87	85.75
Control (Distilled water)		0	0	0	0	0

Table 2: Toxic effect of Nano Chitosan and mandarin crust oil against eggs of the cotton leaf worm, Spodopter littoralis Boisd.

Treatments	Conc.	Corrected non hatching%	LC_{50}	LC_{90}	Slope± S.D.	Toxicity indexLC_{50}	LC_{90}/ LC_{50}	R	P
Nano Chitosan	5	18	11.5393	53.2197	1.9304± 0.2	100	4.612	0.9610	0.0147
	10	57							
	20	64							
	40	84							
mandarin crust oil	1000	57	550.423	14386.9816	0.9043± 0.19	2.09	26.138	0.9782	0.6026
	2500	75.75							
	5000	81.25							
	10000	85.75							

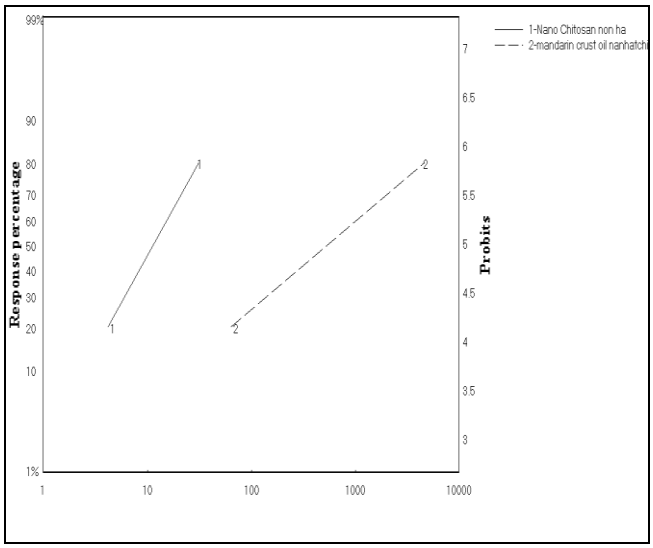

Fig 1: LC-P lines for Nano Chitosan and mandarin crust oil against eggs of cotton leaf worm, *S. litoralis*

Influence of tested oil and Nano-Chitosan on 2nd instar larvae of *S.littoralis*

The obtained data are summarized and illustrated in Table (3 and 4) and Figure (2). The effect of tested oil and Nano-Chitosan on 2nd instars larvae *S. littoralis* can be summarized as follows 1) toxic effect, and 2) morphogenetic effect. The effect of the tested oil and Nano-Chitosan on *S.littoralis* larvae recorded relatively higher mortality percentage in comparison with untreated one (Table, 3). At higher concentration 10000, 40 ppm caused 86.66% mortality of treated *S.littoralis* larvae tested by oil and Nano-Chitosan respectively. As shown in Table (4), Nano-Chitosan exhibited the highest toxicity against 2nd instar larvae of *S.littoralis* followed by tested oil. The LC_{50} values were 6.5374, 2645.482 ppm, respectively. The toxicity index values were 100 and 0.247, respectively. The slope values indicated that Nano-Chitosan had the lowest value was 1.4364± 0.3821followed by tested oil 1.7151± 0.350, respectively. Also, LC_{90}/ LC_{50} values were 7.802and 5.587, respectively.

Table 3: Corrected mortality % of 2nd instar larvae of the cotton leaf worm, *Spodoptera littoralis* treated with Nano Chitosan and mandarin crust oil under laboratory conditions 27±2 ◦C and 65±5% RH.(L.S.D. == 15.4)

No.	Treatments	Conc. (ppm)	Mortality after treatments %				Total Mortality %
			One day	Three days	Five days	Seven days	
1	Nano Chitosan	5	16.67	------	6.67	20	43.34
		10	36.67	------	3.33	20	60

		20	40	6.67	16.67	13.33	76.67
		40	43.33	6.67	20	16.67	86.66
2	mandarin crust oil	1000	13.33	6.66	3.33	3.33	26.66
		2500	36.67	3.33	3.33	------	43.33
		5000	46.67	10	3.33	6.67	66.67
		10000	66.66	6.67	3.33	10	86.66
3	Control (Distilled water	----	0	0	0	0	0

Table 4: Toxic effect of Nano Chitosan and mandarin crust oil against 2nd instar larvae of the cotton leaf worm, Spodoptera littoralis.

Treatments	Conc.	Corrected mortality%	LC_{50}	LC_{90}	Slope± S.D.	Toxicity indexLC_{50}	LC_{50}/ LC_{90}	R	P
Nano Chitosan	5	43.34	6.5374	51.0079	1.4364± 0.3821	100	7.802	0.9992	0.989
	10	60							
	20	76.67							
	40	86.67							
mandarin crust oil	1000	26.66	2645.482	147823492	1.7151± 0.350	0.247	5.587	0.9877	0.712
	2500	43.33							
	5000	66.67							
	10000	86.66							

R: Regression P: Propability

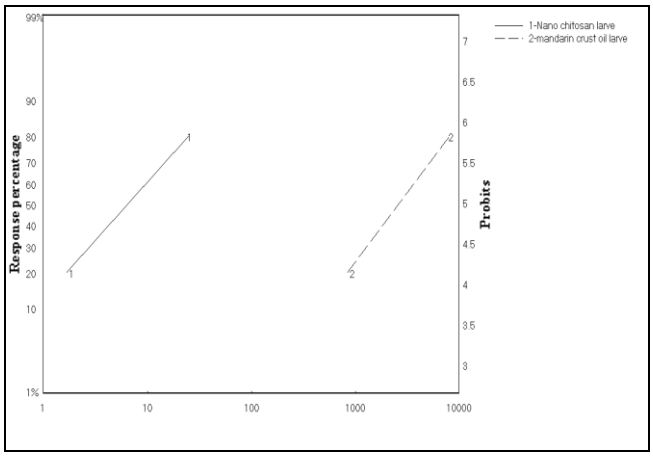

Fig 2: LC-P lines Nano Chitosan and mandarin crust oil against 2nd instar larvae of cotton leaf worm, S. litoralis

1- Biological aspects of treated larvae with Nano-Chitosan:

When the larvae treated with Nano-Chitosan, concentration (5, 10, 20, 40 ppm), the results in Table (5) showed that, Nano-Chitosan increased the 3th larval duration by increasing the concentration to 2.3±0.29, 2.6±0.29, 3.16±0.76 and 3.5±0.5, respectively, compared with 2.3±0.29 for control. There was a significant difference between the treated larvae with (10, 20.40 ppm) and untreated larvae, the L.S.D. (0.84). Similar results were obtained in larval duration for 4th & 5th & 6th. Instar and Pre-pupal duration. there was a significant difference between the treated larvae with different concentration (20,40 ppm) and the untreated larvae, the L.S.D.(0.52), (0.62), (0.62) and.(0.33) for 4th & 5th & 6th and Pre-pupa, respectively

While the pupal duration averaged 4.3±0.29, 4.8±0.29, 5.16±0.76, and 5.8±0.76, days for concentration (5, 10, 20, 40 ppm) respectively, compared with 4.25±0.25 days for control. Also, There were high significant difference between the treated larvae with concentration (10,20,40 ppm) and the untreated larvae but there was a significant difference between the treated larvae with (5ppm)and untreated, the L.S.D.(1.02)

2- Biological aspects of treated larvae with mandarin crust oil:

When the larvae treated with mandarin crust oil concentration 1000, 2500, 5000, 10000 ppm of the tested oil, the results represented in Table (6) indicated that, the tested oil increased the 3th larval duration by increasing the concentration to 2.5±0.5, 3.3±0.29, 4.3±0.29 and 4.5±0. 5 compared with 2.3± 0.29 for control. There were high significant difference between the treated larvae with concentration (2500, 5000, 10000 ppm), the L.S.D. (0.70).

Similar results were obtained in larval duration for 4th & 5th & 6th. Instar & Pre-pupal duration and pupal duration, there were high significant difference between the treated larvae with all of concentration of the tested oil and the untreated larvae, the L.S.D. (0.62), (0.62), (0.62), (0.57) and (0.84), respectively.

Table 5: Effect of Nano Chitosan on the biological aspects of 3rd instar larvae of the cotton leaf worm, *Spodoptera littoralis*.

Developmental stages	Control	Duration of different stages treated with Nano Chitosan				LSD
		5ppm	10ppm	20ppm	40ppm	
3rd instar	2.3±0. 29	2.3 ± 0.29	2.6± 0.29	3.16 ± 076	3.5± 0.5	0.84
4th instar	2.3±0.29	2.1± 0.29	2.3± 0.25	2.6± 0.29	2.5± 0.5	0.52
5th instar	1.3±0. 29	1.16 ±0.29	1.3± 0.29	1.5± 0.5	1.8 ± 028	0.62
6th instar	1.3±0.29	1.3± 0.29	1.3± 0.29	1.5± 0.5	1.8 ± 0.28	0.62
Pre-pupa	0.5±0	0.5± 0	0.5± 0	0.6± 0.29	1.3±0.29	0.33
Pupa	4±0.5	4.3± 0.29	4.8± 0.29	5.16± 0.76	5.8±0.76	1.02

Table 6: Effect of mandarin crust oil on the biological aspects of 3rd instar larvae of the cotton leaf worm, Spodoptera littoralis

Developmental stages	Control	Duration of different stages treated with mandarin crust oil				LSD
		1000ppm	2500ppm	5000ppm	10000ppm	
3rd instar	2.3±0. 29	2.5 ± 0.5	3.3 ±0.29	4.3 ± 0.29	4.5± 0.5	0.70
4th instar	2.3±0.29	2.3 ± 0.29	3.3 ± 029	4.16 ± 0.29	4.5± 0.5	0.62

5th instar	1.3±0. 29	2.3 ± 0.29	2.6±0.29	3.16 ± 0.29	3.5±10.5	0.62
6th instar	1.3±0.29	2.16± 0.29	2.6 ±0.29	3.16 ± 029	3.5±0.5	0.62
Pre-pupa	0.5±0	0.6 ± 0.29	0.8 ±0.29	1± 0.5	1.3±0.29	o.57
Pupa	4±0.5	4.5± 0.5	4.8 ±0.29	5± 0.5	5.5±0.5	o.84

Influence and Malformation of on 2nd instar larvae of *S.littoralis* due to treatment with tested oil and Nano-Chitosan

The obtained data are summarized and illustrated in Table (7) and Figure (3, 4, 5, and 6). The effect tested oil and Nano-Chitosan on *S. littoralis* 2nd instars larvae can be summarized in Table (7) represents the morphogenetic effects of tested oil and Nano-Chitosan on 2nd instar larvae of *S.littoralis*. The larval mortality among the thirty larvae tested in the present study ranged between 86.66% for mandarin crust oil and Nano-Chitosan in the high concentration 10000, 40 ppm respectively, and (63.33,43.34%) in the low concentration (1000, 5 ppm) for mandarin crust oil and Nano-Chitosan respectively, pointing out two possibilities *viz*., toxicity to mandarin crust oil and Nano-Chitosan and malnutrition of larvae were reduced in size and lethargic in nature when compared to those in the control. Fig. (3, 4) Successful pupation of the treated larvae was observed in the present study ranged between 13.33 % for mandarin crust oil and Nano-Chitosan in the high concentration 10000, 40 ppm respectively. The formation of deformed pupae indicates defects in the moulting process. Fig (6) Furthermore, adult moths which emerged showed some malformations in the wing. Fig (5).Treated 2nd instar larvae of cotton leaf worm, *S. littoralis* with each of tested oil and Nano-Chitosan caused malformation in treated larvae as shown in Fig. (3.4).However, the tested oil and Nano-Chitosan had been shown to cause some morphological changes in treated larvae. The tested oil and Nano-Chitosan caused malformation in the emerged moths as shown in Fig (5, 6).

Table 7: Malformation of 2nd instar larvae of *Spodoptera lituralis* due to treatment with some materials.

Parameters	mandarin crust oil				control	Nano Chitosan			
	10000ppm	5000ppm	2500ppm	1000ppm		40ppm	20ppm	10ppm	5ppm
Number of larvae tested	30	30	30	30	30	30	30	30	30
Number of dead larvae	26	25	22	19	--------	26	23	18	13
Mortality (%)	86.67	83.33	73.33	63.33	---------	86.67	76.67	60	43.34
Pupation (%)	13.33	16.66	26.66	36.66	100	13.33	23.33	40	56.66
Number of deformed pupae	2	2	2	4	------	3	3	3	7
Deformed pupae (%)	6.66	6.66	6.66	13.33	--------	10	10	10	23.33
Number of emerged adults	2	3	6	7	30	1	4	9	10
Emerged adults (%)	6.66	10	20	23.33	100	3.33	13.33	30	33.33
Number of Deformed adults	2	2	3	2	-------	1	2	5	3
Deformed adults (%)	6.66	6.66	10	6.66	--------	3.33	6.66	16.66	10

(a) (b) (c)

Fig 3: Malformation of *S. littoralis* larvae treated with mandarin crust oil (a), Nano Chitosan (b) and control (c).

(a) (b) (c)

Fig 4: Malformation of *S. littoralis* larvae treated with mandarin crust oil (a), Nano Chitosan (b) and control (c).

(a) (b) (c)

Fig 5: Malformation of cotton leaf worm adults due to treatment with mandarin crust oil (a), Nano Chitosan (b) and control (c

(a) (b) (c)

Fig 6: Malformation of cotton leaf worm adults due to treatment with mandarin crust oil (a), Nano Chitosan (b) and control (c

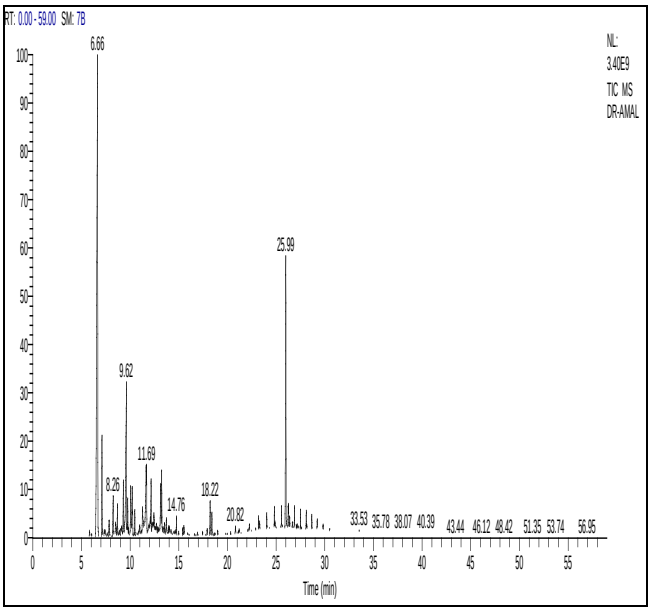

Fig 7: GC/MS analysis of mandarin crust oil, (*Citrus reticulate)*

Chemical analysis

GC/MS analysis detected Forty- Five compounds in mandarin crust oil, (Citrus reticulate). The main defined components are listed in Table (8) and Fig. (7) According to their retention times and their percentage composition. D-Limonene the most abundant compound (32.38%), followed by 1,6-octadien-3-ol,3,7-dimethyl-, propanoate, and L-α-Terpineol, (8.39%), γ-Terpinene (3.40%), Cyclohexanemethanol, 4-hydroxy-α, α, 4-trimethyl- (3.02%), p-Mentha-1(7), 8-dien-2-ol (2.65%), Carveal (2.26%), Vanillin (2.23%), Benzoic acid, 2-(methylamino)-, methyl ester (2.09%), Phenol, 4-methyl-2-(1-methylethyl)- (2.0%), Terpinen-4-ol (1.94%), Methyl anthranilate (1.84%), 1R,4R-p-Mentha-2,8-dien-1-ol (1.61%), (Thymol) (1.34%), Cyclohexanol, 1-methyl-4-(1-methylethenyl)- (β-Terpinol) (1.15%), 2,6,9,11-Dodecatetraenal,2,6,10-trimethyl-, (E,E,E)- (1.07%), decanal (0.97%), Linalool, and cis-p-Mentha-2,8-dien-1-ol (0.82%), Tricyclo [2.2.1. 0(2,6)] heptane-3-methanol, 2,3-dimethyl- (0.81%), -(-)-Carvone) (0.76%), Docosane (0.73%), Pentatriacontane (0.70%), Benzyl Benzoate (0.66%), Heptacosane (0.65%), Benzoic acid, 2-(formylamino)-, methyl ester (0.54 %), 2,6-Octadiene, 2,6-dimethyl-, 6-Octen-1-ol, 3,7-dimethyl-, acetate and (1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo [9.1.0] dodeca-3, 7-diene (0.48 %), S-(+)-5-(1-Hydroxy-1-methylethyl)-2-methyl-2-cyclohexen-1-one (0.45 %), 12-Hydroxydodecanoic acid (0.41 %), Octadecane, 3-ethyl-5-(2-ethylbutyl)- (0.40 %), Bicyclo[2.2.1]heptane, 2,2-dimethyl-5-methylene-, n-Hexadecanoic acid, and Estra-1,3,5(10)-trien-17β-ol, Glycerol 1-palmitate (0.37 %), Phenol, 2-methoxy-4-propyl- (0.36 %), β-Myrcene (0.33 %), p-Cresol, and Retinol (0.31 %), 1H-Cycloprop[e]azulen-4-ol,decahydro-1,1,4,7-tetramethyl-, [1aR-(1aα,4β,4aβ,7α,7aβ,7bα)]- (0.30 %), p-Menth-8-en-1-ol (0.27 %),2-((1R,4R)-4-Hydroxy-4-methylcyclohex-2-enyl) propan-2-yl acetate, and Bergamotol, Z-α-trans-(0.19 %), Citronellal (0.15 %),, Similar results were obtained by Oladipupo *et al*. (2014) who recognized The oils of C. reticulata from Ijanikin had its main compounds as pinocarvone (22.7%), trans-pinocarveol acetate (20.0%), β-thujone (12.8%) while citronellal (38.1%), (Z)-β-ocimene (25.9%), linalool (14.5%) and limonene (12.2%) were the major constituents identified in Ikotun sample.. Also, Behzad Babazadeh-Darjazi.2017 proved that Twenty-six, thirty-five and nineteen peel components were identified in Fortune, Robinson and Osceola respectively including: aldehydes, alcohols, esters, monoterpenes, sesquiterpenes and other components. The major components were limonene, γ-terpinene, (E)-β-ocimene, β-myrcene, sabinene, linalool and α-Pinene. Among the three scions examined, Fortune showed the highest content of aldehydes and Robinson showed the highest content of TSS.

Table 8: Main components of mandarin crust oil, (*Citrus reticulate*) identified by GC/MS

No.	R.T.	Compound Nam	Area %	Molecular Formula	Molecular Weight
1	5.83	β-Myrcene	0.33	C10H16	136
2	6.66	D-Limonene	32.38	C10H16	136
3	7.11	γ-Terpinene	3.40	C10H16	136
4	7.34	2-((1R,4R)-4-Hydroxy-4-methylcyclohex-2-enyl) propan-2-yl acetate	0.19	C12H20O3	212
5	7.42	p-Cresol	0.31	C7H8O	108
6	7.84	Linalool	0.82,	C10H18O	154
7	8.26	1R,4R-p-Mentha-2,8-dien-1-ol	1.61	C10H16O	152
8	8.51	cis-p-Mentha-2,8-dien-1-ol	0.82	C10H16O	152
9	8.69	Cyclohexanol, 1-methyl-4-(1-methylethenyl)- (β-Terpinol)	1.15	C10H18O	154
10	9.05	p-Menth-8-en-1-ol,	0.27	C10H18O	154
11	9.13	Bicyclo[2.2.1]heptane, 2,2-dimethyl-5-methylene-	0.37	C10H18O	154
12	9.30	Terpinen-4-ol	1.94	C10H18O	154
13	9.61	1,6-octadien-3-ol,3,7-dimethyl-, propanoate	8.39	C13H22O2	210
14	9.61	L-α-Terpineol	8.39	C10H18O	154
15	9.76	decanal	0.97	C10H20O	156
16	9.84	Citronellal	0.15	C10H18O	154
17	10.07	Carveal	2.26	C10H16O	152
18	10.21	p-Mentha-1(7),8-dien-2-ol	2.65	C10H16O	152
19	10.48	-(-)-Carvone)	0.76	C10H14O	150
20	11.28	(Thymol)	1.34	C10H14O	150
21	11.64	Cyclohexanemethanol, 4-hydroxy-α,α,4-trimethyl-	3.02	C10H20O2	172
22	11.69	Phenol, 4-methyl-2-(1-methylethyl)-	2.00	C10H14O	150
23	12.15	Methyl anthranilate	1.84	C8H9NO2	151
24	12.26	2,6-Octadiene, 2,6-dimethyl-	0.48	C10H18	138
25	12.26	6-Octen-1-ol, 3,7-dimethyl-, acetate	0.48	C12H22O2	198
26	12.26	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	0.48	C15H24O	220
27	12.69	12-Hydroxydodecanoic acid	0.41	C12H24O3	157
28	13.13	Vanillin	2.23	C8H8O3	152
29	13.25	Benzoic acid, 2-(methylamino)-, methyl ester	2.09	C9H11NO2	165
30	13.55	S-(+)-5-(1-Hydroxy-1-methylethyl)-2-methyl-2-cyclohexen-1-one	0.45	C10H16O2	168
31	13.73	Tricyclo[2.2.1.0(2,6)]heptane-3-methanol, 2,3-dimethyl-	0.81	C10H16O	152
32	14.04	Phenol, 2-methoxy-4-propyl-	0.36	C10H14O2	166
33	15.39	1H-Cycloprop[e]azulen-4-ol,decahydro1,1,4,7-tetramethyl-,[1aR-(1aα,4β,4aβ,7α,7aβ,7bα)]-	0.30	C15H26O	222
34	15.52	Benzoic acid, 2-(formylamino)-, methyl ester	0.54	C9H9NO3	179
35	17.91	Bergamotol, Z-α-trans-	0.19	C15H24O	220
36	18.22	2,6,9,11-Dodecatetraenal,2,6,10-trimethyl-, (E,E,E)-	1.07	C15H22O	218
37	18.40	Benzyl Benzoate	0.66	C14H12O2	212
38	20.82	n-Hexadecanoic acid	0.37	C16H32O2	256
39	20.82	Estra-1,3,5(10)-trien-17β-ol	0.37	C18H24O	256
40	20.82	Glycerol 1-palmitate	0.37	C19H38O4	330
41	23.19	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	0.40	C26H54	346
42	23.31	Retinol	0.31	C20H30O	286
43	24.82	Docosane	0.73	C22H46	324
44	25.55	Heptacosane	0.65	C27H56	380
45	26.88	Pentatriacontane	0.70	C35H72	492

Discussion

Tested oil and Nano-Chitosan – inhibit embryonic development of *S. littoralis* In the present study, tested oil and Nano-Chitosan inhibit the embryonic development of *S. littoralis* eggs and treated eggs failed to hatch. Also, phenolic compounds significantly inhibited the embryonic development of the lepidopteron species, *Earias vittella* (Fab.) (Rao *et al*, 2005). It is possible that essential oils interact with a component of the membranes of the eggs (Opender and. Dhaliwal, 2001).Essential oils – control metamorphosis in *S. litoralis*. The use of plant essential oils has considered as an important alternative for pest control because of their environmental and mammals safety properties. From results of the present study, could be concluded that the tested oils (Garlic, Peppermint, Eucalyptus and Lavender) possess toxic effect against 2nd and 4th instar larvae of *S. littoralis*. In addition, all the tested oils exhibited antifeedant effects. Because the induction and inhibition of detoxification metabolic system plays an important role in insect's detoxification mechanism, (Suzan and Sara, 2018). The tested oils had significant inhibitory effects on the larval development of *S. littoralis*.A similar response to crude oil extracts has been recorded against *S. littoralis* (Mohammed and Hany, 2013) as well as several lepidopteran species (Abd. El-Aziz and E.zz EL – Din 2007 & Hung *et al*., 2004) [14]. Hung *et al* (2007) [15] demonstrated that the failure of *S. littoralis* larvae to grow was attributed to phenolic compounds in the essential oils. Showed that the particulation of Nano was more effectiveness against treated insects till the minimizing the concentration to the half of it. Nano particles can cover more and large surface area with particles of insecticides. Also, Nano particles can more ability to penetrate enter the target protected plants. These characters make these

products more effective against insect pests attacking the plants. The LC_{50} values revealed that Nano-Chitosan and tested oil was the most toxic to *S. littoralis* larvae. According to (Tahany, R. Abd El-Zaher, 2017) [33] All oils in the Nano – products were very effective compared with that in the traditional form. Moreover, Moreover, the average size of the nanoparticles of the most effective Nano-emulsions (5%) were 55.15, 173.3, 120.3 and 62.14 nm for Flax, Ginger, Garlic and Jojoba plant oil, respectively, It can be conclude that formulated Nano- emulsion with 5% can be used as effective alternative to commercially available formulation on *Spodoptera littoralis* control, and may be more safe after more studies to evaluate their mammalian toxicity. And in the present study tested oil was effective at LC_{50} (2645.482) ppm, on the larval stages. The present investigation obviously indicated that the tested treatments exhibited deleterious effect and malformation on the pupae of *S. littoralis*. Also tested treatments caused reduction in moth performance and malformation of *S. littoralis*. According to (Prakash and Ghosal, 1979). S. Arivoli and Samuel Tennyson (2012).

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