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Toxicological and Histological Evaluation of Insecticides and Essential Oils in Nano Emulsions and Formulated Form Against *Spodoptera littoralis*

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ABSTRACT: Toxicity and histological effects of two essential oils (Peppermint oil and Eugenol) and two insecticides (Chlorpyrifos and Chlorfenapyr) in nano and traditional EC formulations have been tested against 4th instar larvae of *Spodoptera littoralis*. The results revealed that nano emulsion exhibited considerable toxic activities against the laboratory strain of cotton leafworm. Using bioassay test, showed that the nano-emulsion of chlorpyrifos was 10.33 times more than traditional EC emulsion followed by nano chlorfenapyr was 2.77 times more than traditional emulsion. On the other hand, the nano-emulsion of peppermint oil was affected 2.54 times more than traditional EC emulsion while, nano eugenol was 1.55 times more than traditional emulsion in nano and traditional formulations. Histological studies in the midgut of 4th instar larvae treated with LC_{50} concentration of the tested compounds showed great destruction compared with untreated larvae. Our results indicated that the nano formula of essential oils and two tested insecticides were more effective against *S. littoralis* than commercial formulation. nano forms of essential oils (peppermint oil, eugenol), and insecticides (chlorfenapyr, and chlorpyrifos) exhibited histopathological studies in the midgut of *S. littoralis*.

Keywords: Insecticides, Essential oils, Nano-emulsions, traditional formulations. Histological parameters.

1.INTRODUCTION

The Egyptian cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctüidae) is one of the most destructive pests to about 112 host plants from different families. Infestation often leads to significant crop losses and reduced marketability (Ismail *et al*. 2020). The most effective control of *S. littoralis* has been achieved by using chemical pesticides. Extensive use of these pesticides led to the development of resistance and caused many other problems such as environmental pollution and harm to beneficial non-target organisms (Hilliou *et al*. 2021).

Traditional pesticide formulations suffer from multigate problems such as poorly or insoluble in water, so large amounts of organic solvents are required to solubilize them. Most of the organic solvents contaminated the environment (Sanni and Mutta 2014). Currently researchers the use of Nano formulations of insecticides or essential oils similar traditional formulas, but with enhanced features such as more soluble, slower to release, not prematurely degradable, the effective concentration that is expected to be much lower compared to original materials and they could be formulated in water without organic solvents (Bhattacharyya *et al*. 2010).

Therefore, this study aims to evaluate the lethal toxicity of the nano - and commercial formulated form of essential oils; peppermint oil and eugenol and insecticides; chlorpyrifos and chlorfenapyr on 4th instar larvae of *S. littoralis*. In addition, to analyze the morphological damage caused by tested essential oils and insecticides to the target organs of *S. littoralis* larvae: the midgut, epithelial cells.

2.MATERIALS AND METHODS

2.1 Insects

Laboratory strain of the *S. littoralis* was received from Faculty of Agricultural, El-Shatby, Alexandria University, Egypt and was reared on castor bean plant leaves under controlled conditions at 25 ± 1 ºC and 65 ± 5 % R.H for a period 24 hr (16L:8D) without any exposure to insecticides.

2.2. Essential oils (EOs) and insecticides used

peppermint essential oil and eugenol that were obtained from El-Gomhoria Co., for Chemicals supplies in Alexandria, Egypt. The peppermint essential oil main compounds (Eucalyptolss 11.26%, D-menthone 19.7%, Isopropyl-5-methylcyclohexanone 13.26%, Menthol 43.43%, γ-Terpineol 8.07%, Caryophyllene 4.29%) according to Wahba, and Attia (2019). Two commercial insecticides; chlorfenapyr (challenger 24 % EC) and chlorpyrifos (Indophose 50%EC) were obtained from BASF Chemicals Co. Egypt and Indogolf group Sci., of Pesticides-India respectively.

2.3.Preparation of insecticide nano-emulsions

The oil-in-water nano- emulsions were prepared by the procedure previously reported by Sugumar *et al*. (2013) The chlorfenapyr and chlorpyrifos insecticides were used to prepare 1% nano-emulsion. In the final form the emulsion was prepared by adding one portion of the organic phase containing chlorfenapyr or chlorpyrifos insecticides in 95% technical grade dissolved in one portion of Dimethyl sulfoxide (DMSO) and three portions of olive oil in ratios 1:3:1 (v/v/v) this mixture was added in small portion to a conical flask that containing the aqueous phase which consists of the deionized water containing one portion of surfactant (tween 80), all these steps were carried out under magnetic stirring at approximately 800 rpm for 15 min. Then the prepared formulation was subjected to ultrasonic emulsification and subjected to a 75 kHz Sonicator for 30 minutes. Sonicator probe diameter was13 mm dipped into coarse emulsions under cooling conditions. Keeping the emulsion sample beaker in a comparatively bigger beaker containing ice to keep the solution at a constant temperature. Then, the nano-formulated form was characterized to evaluate stability of the new nano-formulation by a dynamic light scattering (DLS) method using Zetasizer Nano ZS (Malvern *Instruments*, *model*: ZEN3600, UK).

2.4. Preparation of essential oils nano-emulsions.

The oil-in-water nanoemulsion was formulated using a modified version of the method described by Sugumar *et al.*, (2014). Eugenol and peppermint oil, surfactant (tween 80) and deionized water. Initially, coarse emulsion was prepared by adding 40ml water to organic phase containing 5% oil and 5% surfactant at ratio 1:1 (v/v) using a magnetic stirrer at 800 rpm for 15m. Then subjected to ultrasonic emulsification using a 75 kHz Sonicator for 30 minutes. Sonicator probe diameter was13 mm dipped into coarse emulsions under cooling condition. Keeping the emulsion sample beaker in a comparatively bigger beaker containing ice to keep solution at constant temperature. Then, the nano-formulated form was characterized to evaluate stability of the new nano-formulation.

2.5. Technique of the tested insect

Insecticides and oils concentrations were applied to the fourth instar larvae of *S. littoralis.* Using the method of feeding treatment was applied as Disc dipping technique.The method of Ishaaya and Klein (1990) was conducted and used to evaluate the efficacy of essential oils, pesticides and nano-emulsions of them against fourth instar larvae of the cotton leafworm *S. littoralis*. Each castor bean leaves disc (2 cm^2) was dipped in each essential oil, pesticides and nono-emulsions for 15s. Discs and held vertically to allow the excess solution to drip off at room temperature (25◦C) to dry. The treated leaves were placed at the bottom of glass jars containing 10 larvae of 4^{th} instar cotton leaf worm three replicates were made for each concentration and three untreated check with distilled water were used. The percentage of mortality was calculated each concentration for recorded after 24 hours of application to insecticides and after 72 hours to oils, corrected for natural mortality according to Abbott equation (Abbott, 1925). Results were illustrated graphically as probit regression lines, and toxicity LC_{50} values as well as the slope were obtained according to Finney, (1971).

2.6 Histopathological studies:

The effect of each of the four tested compounds at LC_{50} values was studied on the cellular structure of 4^{th} instar larvae surviving treatment. The specimens from *S. littoralis* larvae samples were collected and fixed in 10% buffered neutral formalin solution. Paraffin sections of 5 microns thickness were prepared and stained with haematoxylin and eosin according to Bancroft *et al*. (1990) and examined microscopically. The show in cross section was mid gut microscopically (200 µm). All sections of *S. littoralis* larvae were done at faculty of Medicine Alexandria University.

2.7. Statistical analysis

The data were calculated as mean ±SE and analyzed statistically. Statistical significant differences between individual means were determined by one-way analysis of variance (ANOVA) using Costat software. Mean values were analyzed with Tukey's test at the 0.05 level or less.

3.RESULTS

3.1.Toxicity of nano and traditional formulation of chlorpyrifos and chlorfenapyr on fourth instar larvae of *Spodoptera littoralis.*

The toxicity of chlorpyrifos and chlorfenapyr, formulations for nano and traditional formulate tested against the fourth instar larvae of *S. littoralis* are given in Table (1) and Figure (1). It was shown that nano chlorpyrifos was 10.33 times more toxic than traditional formula while nano chlorfenapyr was 2.77 times more toxic than

traditional formula after 24hr post-treatment. While LC_{50} values were 0.0031% and 0.0061 % for both traditional formulations of chlorpyrifos and chlorfenapyr, respectively.
The data show a very narrow effect on confidence limits in statical analysis Also, the sharpener of slope. Comparing the slope values of the toxicity lines of these insecticides data in Table (1) show that commercial chlorpyrifos had a high slope value of toxicity line with a 1.92 slope value.

Table 1. Toxicity of nano emulsion and traditional formulations of chlorpyrifos and chlorfenapyr on fourth instar larvae of *Spodoptera littoralis*.

Treatment	Application form	LC_{50}% (95% FL)	Confidence limits		Slop ± SE	X2	P-value
			Lower	Upper			
chlorpyrifos	Nano formula	0.0003	0.0002	0.0003	1.63±0.16	2.09	0.84
	Traditional EC formula	0.0031	0.0027	0.0035	1.92±0.17	1.18	0.95
chlorfenapyr	Nano formula	0.0022	0.0018	0.0026	1.41±0.16	1.75	0.88
	Traditional EC formula	0.0061	0.0048	0.0084	1.08±0.16	1.28	0.94

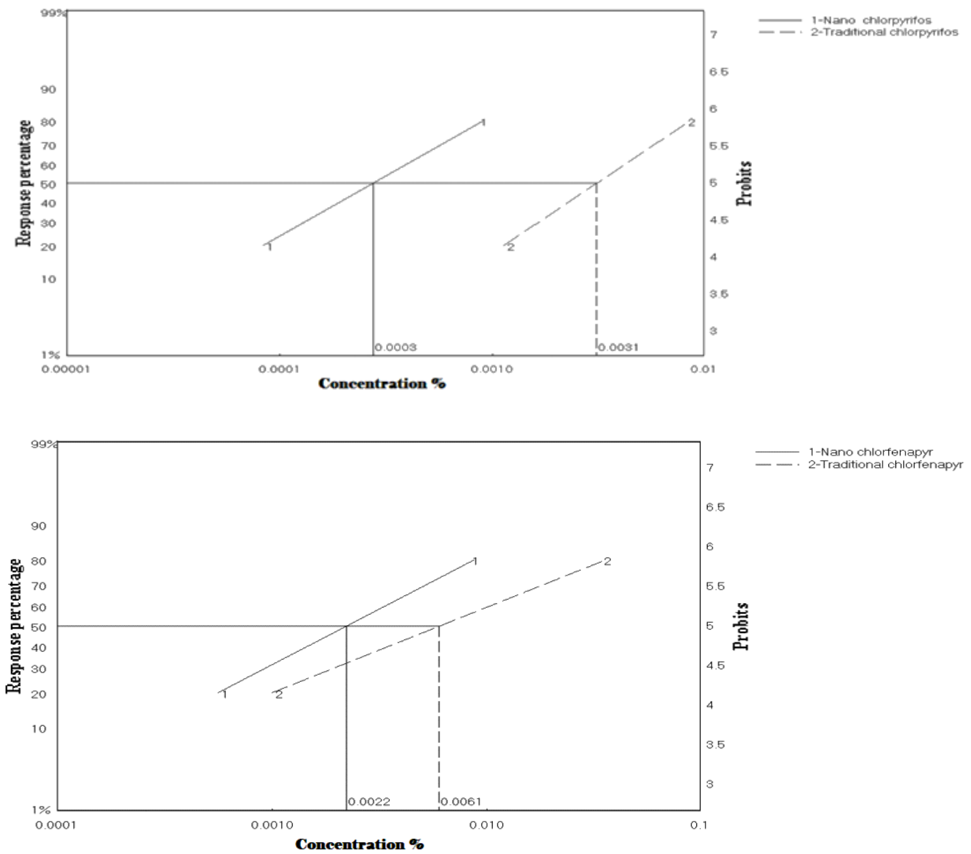


Fig. (1): Toxicity of nano emulsion and traditional formulations of chlorpyrifos and chlorfenapyr on fourth instar larvae of *Spodoptera littoralis* treated with feeding methods.

3.2Toxicity of nano and traditional formulate emulsions of peppermint oil and eugenol on fourth instar larvae of *Spodoptera littoralis*.
The toxicity of nano and traditional peppermint oil and eugenol formulations of nano and traditional formulate tested against the fourth instar larvae of *S. littoralis* are given in Table (2) and Figure (2). Traditional peppermint oil and eugenol formulations shows insecticidal toxicity. LC_{50} values were 3.76% and 3.82% for traditional peppermint oil and traditional eugenol, respectively. The LC_{50} values were 1.48%, 2.46% for nano peppermint oil and nano eugenol respectively. While, after 72hr post-treatment. In

general, two nano forms have highly significant effect than the traditional forms. Also, our data indicated that the nano- peppermint oil showed more effective 2.54 folds than raditional peppermint oil, while nano- eugenol was most effective 1.55 folds than traditional eugenol. The data show a very narrow effect on confidence limits in statical analysis which mean the very sharp data calculate the effect of eugenol or peppermint oil nano or traditional formulation which give high confidence limit for the results obtained. Data in Tables (2) show that peppermint oil and eugenol had very narrow slop values ranging between 1.68 to 1.67.

Table 2. Toxicity of nano and traditional formulation emulsions of on peppermint oil and eugenol fourth instar larvae of *Spodoptera littoralis.*

Treatment	Application form	LC_{50}% (95% FL)	Confidence limits		Slop ± SE	X2	P-value
			Lower	Upper			
Peppermint oil	**Nano formula**	1.48	1.19	1.72	1.68±0.22	0.78	0.99
	Traditional EC formula	3.76	3.27	4.60	1.65±0.23	1.02	0.98
Eugenol	**Nano formula**	2.46	2.17	2.70	1.67±0.22	1.11	0.98
	Traditional EC formula	3.82	3.20	4.73	1.60±0.23	2.67	0.85

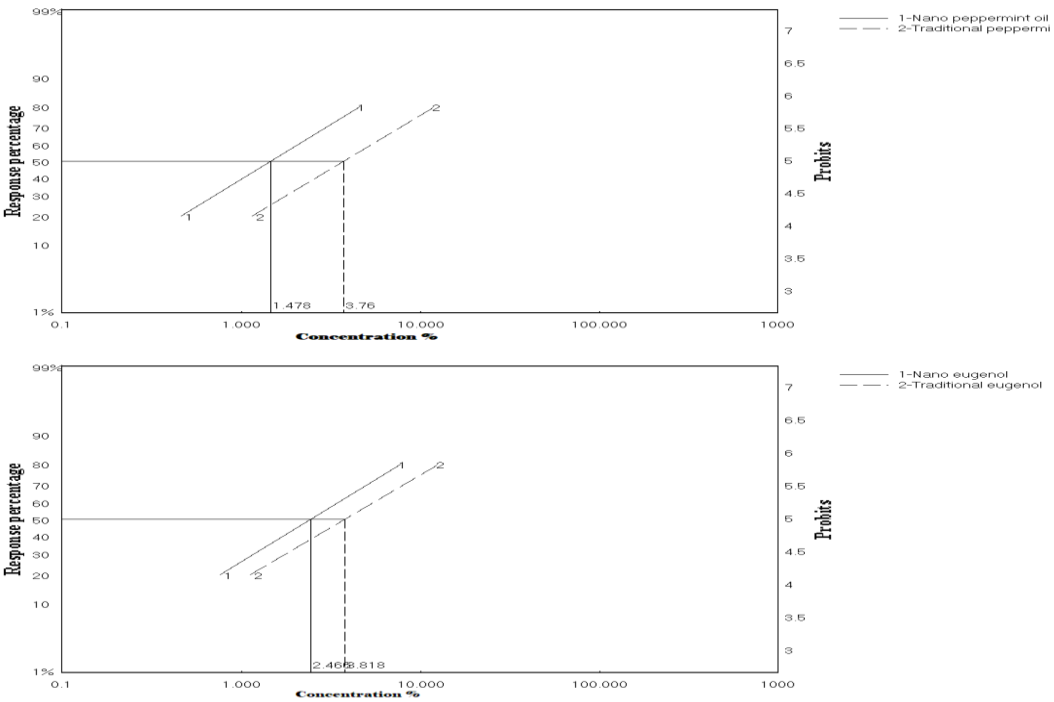


Fig. (2): Toxicity of nano and traditional formulation emulsions of on peppermint oil and eugenol fourth instar larvae of *Spodoptera littoralis* treated with feeding methods.

1.1. Effect of physical charactrisation of nano-emulsion

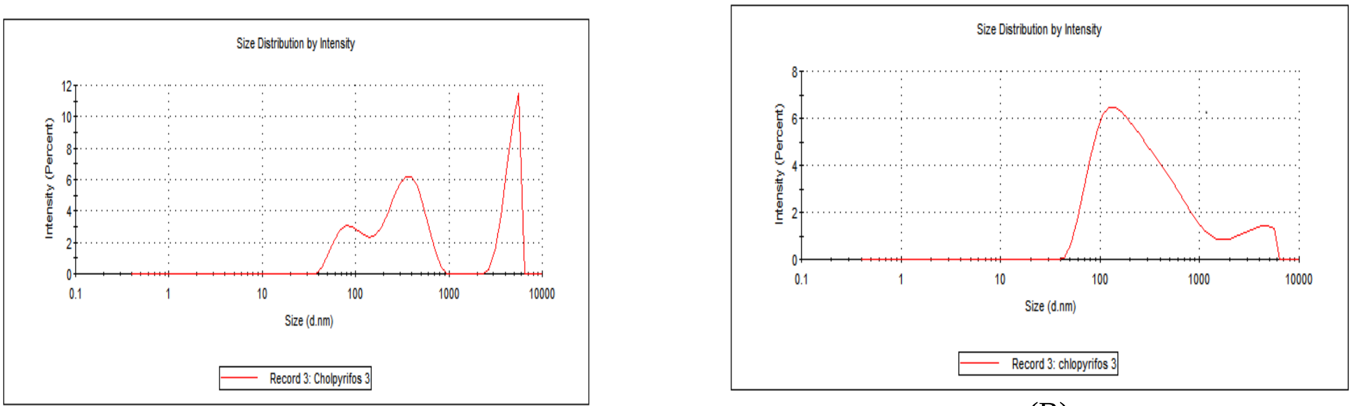
The data show in Table (3) that formulations droplet size decrease from 401.2 to 177.6 nm after sonication for chlorpyrifos traditional and nano formulation Fig (3). However, the droplet size was 1453 to 90.23 nm after sonication for chlorfenapyr traditional and nano formulation Fig (4) . However, the droplet size was 543.7 to 188.6 nm after sonication for eugenol conventional and nano formulation Fig (5). Also, formulations droplet size decrease from 599.8 to 66.16 nm after sonication for peppermint oil traditional and nano formulation Fig (6).The droplet size was 177.6 to 90.23 nm for chlorpyrifos and chlorfenapyr nano formulation respectively. Also, Fig (7) show the appearance of emulsions prepared of nano-emulsion (O/W) of eugenol, peppermint oil essential oil, chlorpyrifos, chlorfenapyr before Sonication and After Sonication. This indicates that when the droplet size decreased in comparison between nano and Traditional formulation, the toxicity increased 2.27 folds of chlorpyrifos and 16.10 fold of chlorfenapyr.

the droplet size varied from 188.6 to 66.16 nm (Table 3) for eugenol and peppermint oil, respectively. The droplet size decreased in comparison between nano and traditional coarse, the toxicity increased 9.07 folds of peppermint oil and 2.88 fold of eugenol Which indicates that the toxicity of both eugenol and peppermint oil increase with decreeing droplet diameter.

Table (3) show that PDI (poly dispersity index) value decrease from 0.885 to 0.460 nm and 0.603 to 0.398 nm after sonication for chlorpyrifos and chlorfenapyr respectively, and also, it was decrease from 0.616 to 0.366 nm and 0.976 to 0.206 nm after sonication for eugenol and peppermint oil respectively.

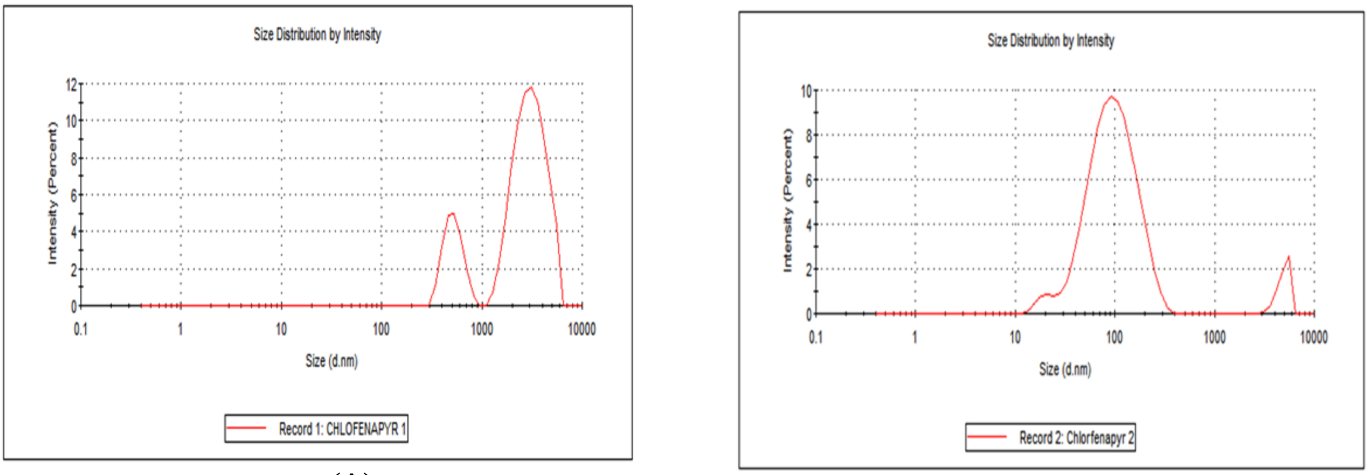
Table (3). Physicochemical properties for different course and nano-emulsions.

Compound	Droplet diameter(nm)		PDI (Polydispersity index) (nm)	
	Conventional formulation	Nano formulation	Conventional formulation	Nano formulation
Chlorpyrifos	401.2	177.6	0.885	0.460
Chlorfenapyr	1453	90.23	0.603	0.398
Eugenol	543.7	188.6	0.616	0.366
Peppermint oil	599.8	66.16	0.976	0.206



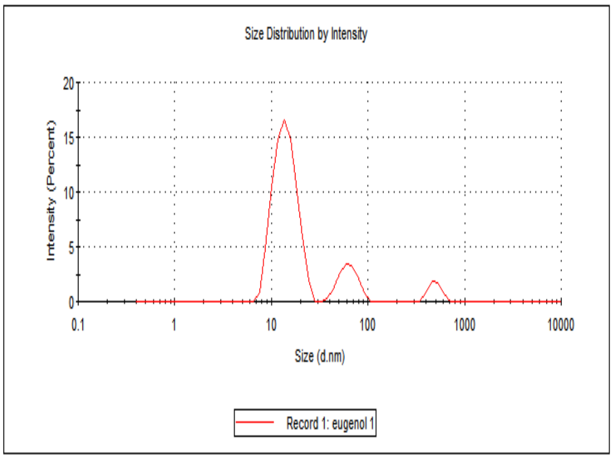
(A) (B)

Fig (3): Droplet size distribution of course (A)and nano-emulsions (B) chlorpyrifos of preparations using a dynamic light scattering.

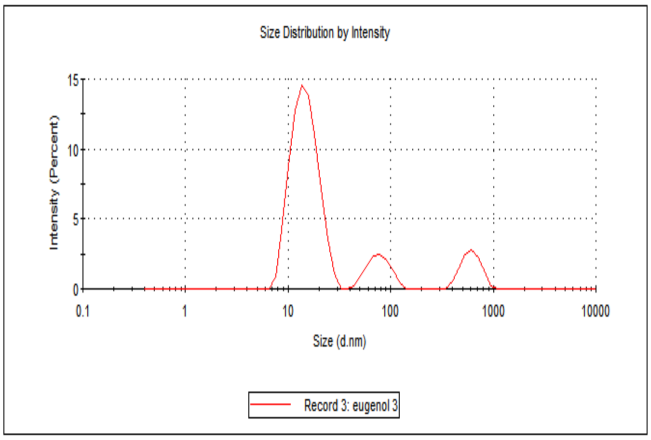


(A) (B)

Fig (4): Droplet size distribution of course (A) and nano-emulsions (B) chlorfenapyr for the preparations usig a dynamic light scattering.

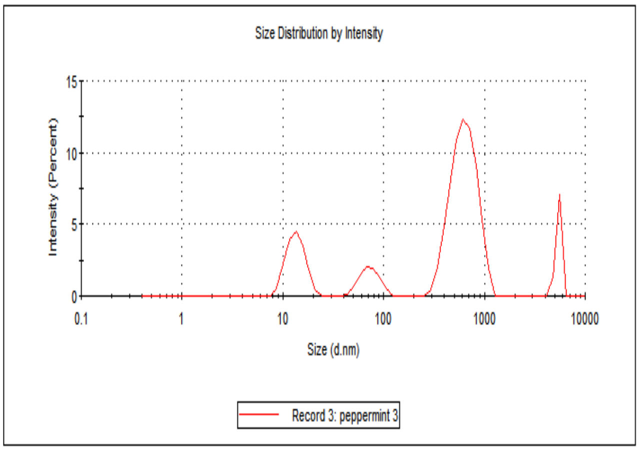


(A)

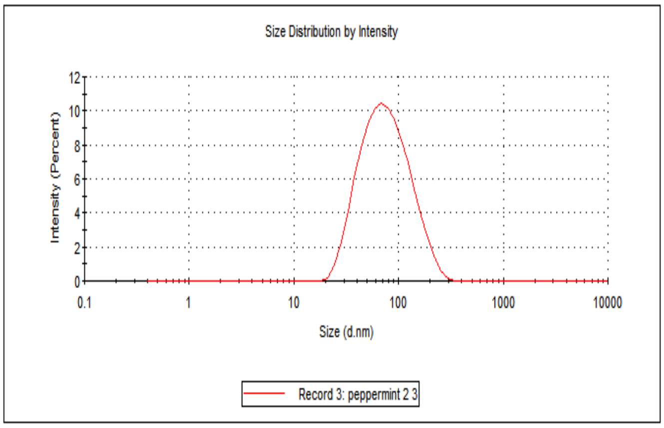


(B)

Fig (5): Droplet size distribution of course (A) and nano-emulsions (B) Eugenol of preparations using a dynamic light scattering.



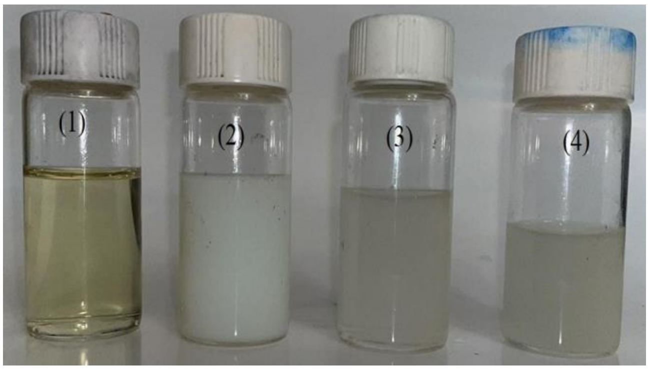
(A)



(B)

Fig (6): Droplet size distribution of course (A) and nano-emulsions (B) peppermint oil of preparations using a dynamic light scattering.

(A)



(B)

Fig (7): The appearance of emulsions prepared of nano-emulsion (O/W) of eugenol (1), peppermint oil (2), chlorpyrifos (3) and chlorfenapyr (4) Before Sonication (A), After Sonication (B).

Histological Studies:

Histopathological effect for the tested insecticides on midgut tissues of the cotton leafworm, *Spodoptera littoralis* 4th instar larvae are shown in Fig (8–12).

1.1. Histological studies on midgut of Spodoptera larvae

The foregut of lepidopteran larvae is composed of an esophagus and an enlarged tisenes, which holds food prior to its passage into the midgut then the hindgut. The transverse sections of treated and check untreated larvae were determinate at a thick of 5µm which were cut in medial larval body where found the midgut. The slides were examined by microscope (100X, 400X). The aim of this study to evaluate the effects of two insecticides (Chlorpyrifos and Chlorfenapyr) and two essential oils (eugenol and peppermint oil) on histological structure and their function on the cell level, comparing with untreated 4th instar larvae of cotton leaf worm midgut section.

Cross sections of untreated 4th instar larvae of *Spodoptera littoralis* which showed that midgut was performed after 24 hrs of feeding on fresh discs of castor bean leaves (Figure 8).

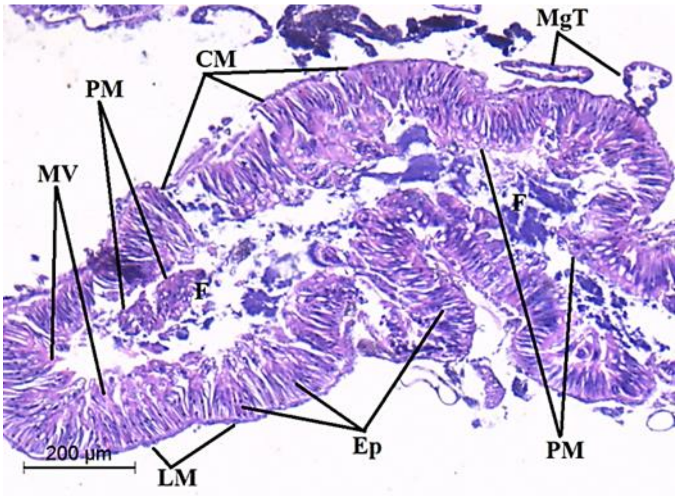


Figure (8): Transverse section of untreated 4th instar larvae cotton leaf worm midgut.

*MgT; Malpighian Tubules, CM; Circular Muscles, PM; Peritrophic Membrane, MV; Microvilli, LM; Longitudinal Muscles, Ep; Epithelium, F; Food.

1.2. Effects of Nano and traditional treatments of Chlorpyrifos on 4th instar larvae of *Spodoptera littoralis* midgut

Traditional treatment of Chlorpyrifos in comparison with the untreated 4th instar larvae of *Spodoptera littoralis* midgut which is illustrated in Fag (9, A), the data showed that undifferentiated epithelial cell, appears on lumen and large vacuoles after treatment by traditional formulation of chlorpyrifos. While after treatment the 4th instar larvae of cotton leaf worm by nano-formulation of chlorpyrifos, the data revealed separated peritrophic membrane, microvilli and many small vacuoles (Fig 9, B). This data are in agreements a with which found before that nano-chlorpyrifos is 10.33 times toxic than traditional chlorpyrifos due to nano more damage inside the cell. Also, data agree with which found before by El-Sabrout *et al* (2013) and Abd-El-Aziz *et al* (2019) that nano treatment were observed large numbers of vacuoles which meaning decrease in epithelial cell which lead to decreasing of insect digestion activities.

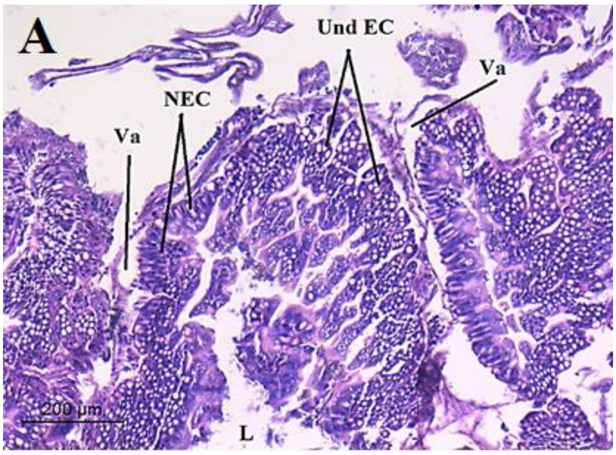


Figure (9, A): Histological section of midgut treated larvae after feeding on fresh discs of castor bean leaves and treated byconventional-treatment of Chlopyrifos.

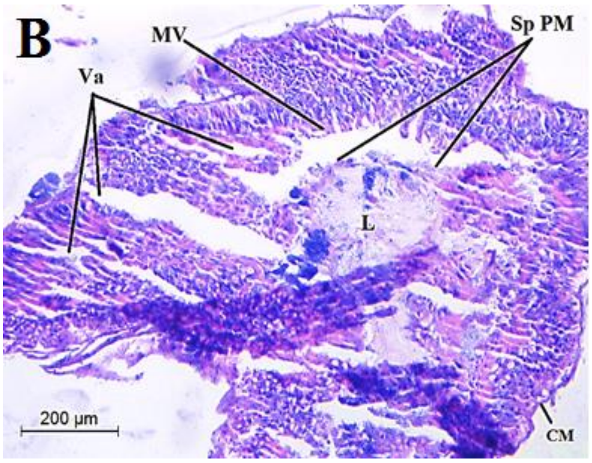


Figure (9, B): Transverse section of midgut of treated larvae after feeding on fresh discs of castor bean leaves and treated by nano-treatment of Chlopyrifos.

*Und EC; Undifferentiated Epithelial Cell, NEC; Normal Epithelial Cell, Va; Vacuoles, Sp PM; Separated Peritrophic Membrane, MV; Microvilli, CM; Circular Muscles, L; Lumen.

1.3.Effect of Nano and traditional treatments of Chlorfenapyr on 4th instar larva of *Spodoptera littoralis* midgut

Traditional treatment of chlorfenapyr in comparison with the untreated 4th instar larvae of *Spodoptera littoralis* midgut which is illustrated in Fag (10, A), the data showed that undifferentiated epithelial cell after treatment by traditional formulation of chlorfenapyr. While after treatment the 4th instar larvae of cotton leaf worm by nano-formulation of chlorfenapyr, the data revealed incomplete peritrophic membrane, clear separated malpighian tubules and many separated vacuoles (Fig 10, B). the data revealed that nano-treatment of chlorfenapyr showing incomplete peritrophic membrane and smaller size of epithelial cell and undifferentiated epithelial cell which in agree with found before by (Saleh *et al.* (2021).

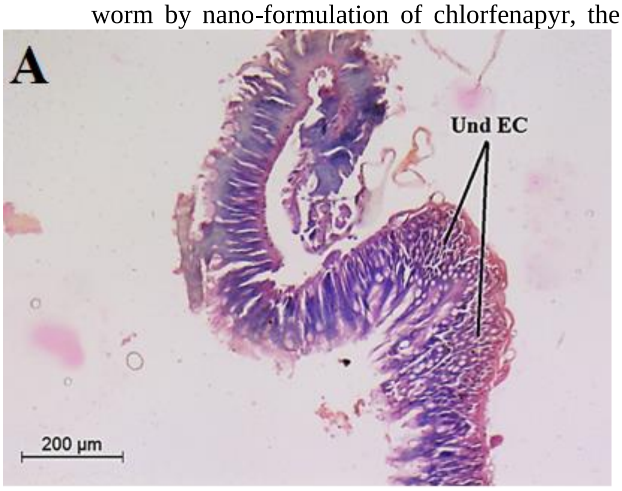


Figure (10, A): Histological section of midgut for the treated larvae after feeding on fresh discs of castor bean and treated by conventional-treatment of Chlorfenapyr.

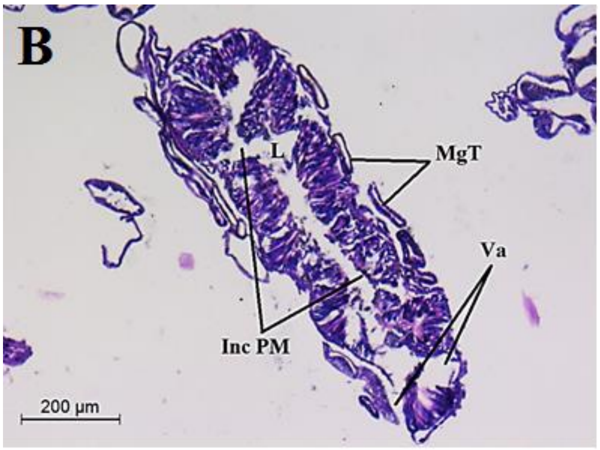


Figure (10, B): Transverse section of midgut of treated larvae after feeding on fresh discs of castor bean leaves and treated by nano-treatment of Chlorfenapyr.

*Und EC; Undifferentiated Epithelial Cell, MgT; Malpighian Tubules, L; Lumen, Inc PM; Incomplete Peritrophic Membrane, Va; Vacuoles.

1.5.Effect of Nano and traditional treatments for eugenol on 4th instar larva of *Spodoptera littoralis* midgut.

Traditional treatment of eugenol in comparison with the untreated 4th instar larvae of *Spodoptera littoralis* midgut which is illustrated in Fag (11, A), the data showed that slight effect on undifferentiated epithelial cell and incomplete peritrophic membrane after treatment by traditional formulation of eugenol. While, after treatment the 4th instar larvae of cotton leaf worm by nano-formulation of eugenol, the data revealed high effect of separated peritrophic membrane, cutting on the Longitudinal Muscles, cutting in Circular Muscles, Microvilli and many vacuoles (Fig 11, B). The data shows that nano- eugenol effect on midgut section by separated peritrophic membrane which agree with which found before by Hanan *et al*., (2020) and clear that junk food which contain dangerous tupes of oils and lipids can does a serious bad effect on cell performance.

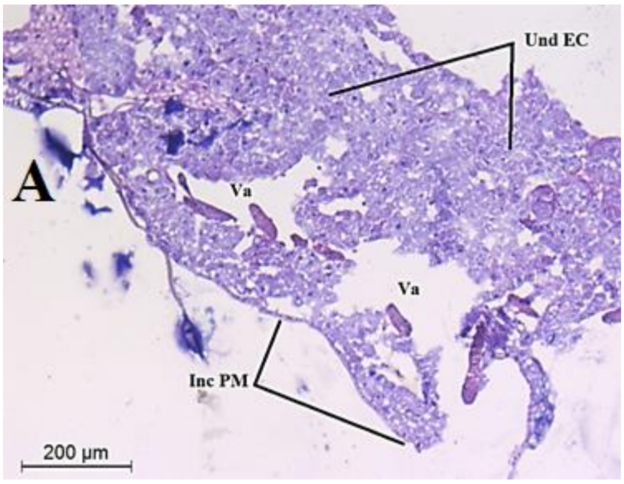


Figure (11, A): Histological section of midgut of treated larvae after feeding on fresh discs of castor bean leaves and treated by conventional-treatment of eugenol.

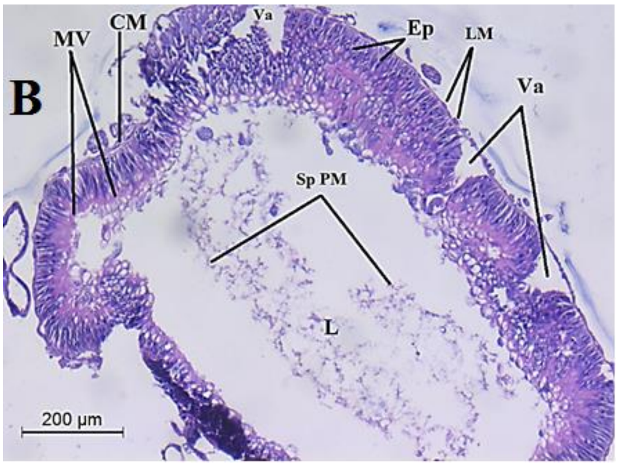


Figure (11, B): Transverse section of midgut of treated larvae after feeding on fresh discs of castor bean and leaves treated by nano-treatment of eugenol.

*Und EC; Undifferentiated Epithelial Cell, Inc PM; Incomplete Peritrophic Membrane, separated peritrophic membrane, Va; Vacuoles, CM; Circuler Muscles, MV; Microvilli, LM; Longitudinal Muscles, L; Lumen.

1.6.Effect of Nano and traditional treatments of peppermint oil on4th instar larva of *Spodoptera littoralis* midgut

Traditional treatment of peppermint oil in comparison with the untreated 4th instar larvae of *Spodoptera littoralis* midgut which is illustrated in Fag (12, A), the data showed that cutting in circular muscle, appears of vacuoles and absent peritrophic membrane after treatment by traditional formulation of peppermint oil. While after treatment the 4th instar larvae of cotton leaf worm by nano-formulation of peppermint oil, the data revealed effect on cutting the peritrophic membrane, effect the circular muscles, appears of microvilli, effet on basement membrane and large vacuoles (Fig 12, B). The data shows that the layers of both longitudinal and circular muscles were separated from epithelial layer with nano- peppermint oil treatment which in agree with which found by Abd-El-Aziz *et al* (2019). Other traditional pesticide formulations are relatively poor, which enables pesticide absorption and penetration difficult, severely limiting their biological activity. The size of the droplets can considerably increase their adherence and permeability on the target insects by increasing the biological activity of insecticide and/or decreasing their applied amounts. A nanoemulsion with smaller droplets, high wetting performance, rapid diffusion, and penetration at the target surface is advantageous for increasing pesticide biological activity.

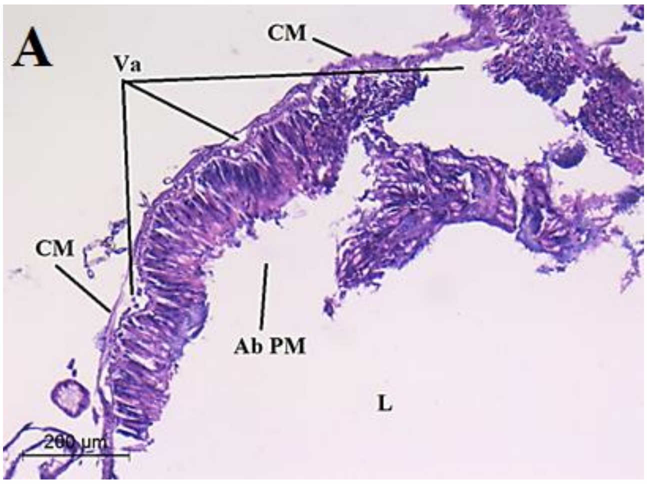


Figure (12, A): Histological section of midgut of treated larvae after feeding on fresh discs of castor bean leaves and treated by conventional treatment of peppermint oil.

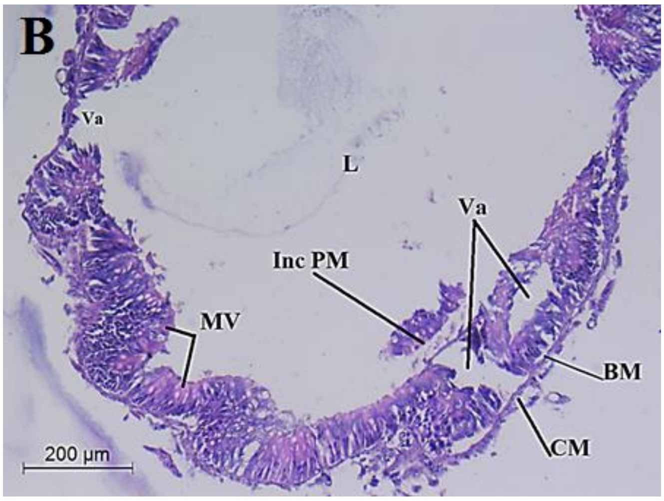


Figure (12, B): Transverse section of midgut of treated larvae after feeding on fresh discs of castor bean leaves and treated by nano-treatment of peppermint oil.

* CM; Circular Muscles, Va; Vacuoles, Ab PM; Absent Peritrophic Membrane, MV; Microvilli, Va; Vacuoles, L; Lumen, Und EC; Undifferentiated Epithelial Cell, CM; Circular Muscles, BM; Basement Membrane.

1.DISCUSSION

The toxicity of chlorfenapyr and chlorpyrifos, formulations of nano and traditional formulate showed that nano chlorpyrifos and chlorfenapyr were more toxic than traditional formula. The toxicity of nano chlorfenapyr was toxic about three fold that of chlorfenapyr because of the low solubility of chlorfenapyr in water (0.12–0.14 mg/L) (U.S. Environmental Protection Agency 2001) but the nanoemulsion formula increases soluble of chlorfenapyr in water. The present findings agreed with those recorded by Ahmed *et al.* (2019); Taktak *et al*. (2021). This result agreement with those (Badawy *et al.*,2021) Median lethal concentration values during a 24 h exposure were 1.27, 2691.12, and 1779.58 mg/L for chlorpyrifos-methyl, diazinon, and malathion nanoemulsions, respectively using a leaf-dip method against the 4th instar larvae of *S. littoralis*. Nano-insecticides have the ability to reduce the hazardous impact of a conventional chemical formulation by reducing pesticide application rates (Nuruzzaman *et al.*, 2016). Chin *et al.*, (2011) revealed that carbofuran a nanosuspension was efficiently against diamondback moth than a micro suspension (commercial formulation). Boehm *et al*., (2003) reported that, the polymer nanoparticles of commercial pesticides-controlled *S. littoralis* efficiently. Guan *et al*., 2010) found, encapsulated nano-imidacloprid was shown to be more effective than commercial imidacloprid against *Martianus dermestoides*.

The data show a very narrow effect on confidence limits in statical analysis which mean the very sharp data calculate the effect of chlorpyrifos or chlorfenapyr insecticides in nano or traditional formulation which give high confidence limit for the results obtained. The sharpener of slope means the high rate of toxicity Comparing the slope values of the toxicity lines of these insecticides. The commercial chlorpyrifos had a high slope value of toxicity. Among the insecticides studied. The high slope indicates that a small increase in insecticide concentration results in significant mortality when compared to the anther formula. The higher the slope, however, the greater the selection pressure on the population. As a result, there will be a higher risk of selecting resistant individuals. as compared to other formula, particularly in cases of continuing to use the same insecticide. But nano formula shows a decrease on the slope from 1.92 to 1.63 which led to a decrease in this risk (Vojoudi *et al*., 2011).

Traditional peppermint oil and eugenol formulations showed insecticidal toxicity. Secondary metabolites of EOs are responsible for their insecticidal activities (Ismail *et al*., 2022). major chemical components like (Eucalyptol, D-menthone, , Menthol, γ-Terpineol)found as major components of peppermint oil) are responsible for the insecticidal activity (Adupa and Kagoya, 2018; Fouad *et al.*,2021). Previous studies have shown that the insecticidal efficacy of peppermint oil Heydari *et al*., (2020). Eugenol has been proven effective in controlling a wide range of pests such as *Aedes aegypti* (Adhikari *et al*., 2022), *Musca domestica* (Silva *et al*., 2020), *Callosobruchus maculatus* (González *et al*., 2019). Two nano forms have highly significant effect than the traditional forms. these results agreement with results obtained by (Heydari *et al*.,2020) reported that the synthesized nanoemulsion formulations showed relatively high contact toxicity against cotton aphid. in addiaton, Lucia *et al*., (2020) initiate that nanoemulsions based on thymol-eugenol mixture were highly toxic against *A. aegypti*. Louni *et al*., (2018), reported that the nanoemulsion was more efficient than *Mentha longifolia* essential oil against *E. kuehniella* Zeller. Moreover, nanoemulsion of camphor oil has powerful contact toxicity and influence on mortality rate of *E. kuehniella* fifth-instar larvae is the most effective on mortality than the normal form camphor essential oil with LC_{50} 88.67 ppm and 1699.85 ppm, respectively against 2nd instar larvae of the cotton leafworm (Marouf *et al.*, 2021). The data show a very narrow effect on confidence limits in statical analysis which mean the very sharp data calculate the effect of eugenol or peppermint oil nano or traditional formulation which give high confidence limit for the results obtained. Also, the sharpener of slope means the high rate of toxicity comparing the slope values of the toxicity lines of these oils.

formulations droplet size decrease after sonication for all tested materials Our results are agreement with Ostertag *et al*, (2012) and Massoud *et al*, (2018), found that good nanoemulsion had droplets size between 20-200 nm This indicates that when the droplet size decreased in comparison between nano and Traditional formulation, the toxicity increased. Which indicates that the toxicity of both chlorpyrifos and chlorfenapyr increase with decreeing droplet diameter that agreement with advantage properties such as varied viscosity, permeability, crystallinity, thermal stability, solubility and biodegradability (Badawy, *et al*., 2017).The droplets size of nanoemulsion decreases with an increase in duty cycle; whereas pulsed ultrasound with proper intervals was more functional than continuous ultrasonication (Shahavi *et al.,* 2019). This indicates that when the droplet size decreased in comparison between nano and traditional coarse, the toxicity of peppermint oil and eugenol Which indicates that the toxicity of both eugenol and peppermint oil increase with decreeing droplet diameter, that agreement with found Zhang *et al*., (2014) Comparison of antibacterial activity with pure EOs and emulsification showed that the nanoemulsion was much more effective. This is presumably due to the fact that small fat particles within the nanostructures are able to bring primary oil to the surface of the cell membrane, while pure oil (low water solubility) cannot easily interact with cell membranes.

The stabilization of the nanoemulsions is also dependent on the steric effect of the non-ionic surfactant (Tadros *et al*., 2004). Other typical pesticide formulations are inadequate, which makes it difficult for pesticides to absorb and penetrate, severely limiting their biological activity. The size of the droplets can improve their adherence and permeability to the target insects, increasing pesticide biological activity and/or lowering application rates. The biological activity of insecticides can be improved by using a nanoemulsion with smaller droplets, superior wetting capability, quick diffusion, and penetration to the target surface (Feng *et al.,* 2018). Furthermore, the nano particle size promotes solubility and mobility, resulting in faster penetration of the insect cuticle and increased insecticidal action (Margulis-Goshen and Magdassi, 2013; Dhivya, 2019). The PDI (poly dispersity index) value decrease after sonication, The PDI (poly dispersity index), which ranges from zero to one, is a dimensionless measure of the width of the size distribution computed from the cumulative analysis (Tang *et al*., 2013). The data proved that all these liquid formulations were successful in their preparation in the nanometric size range. All nano-emulsion have low polydispersity indicates that the nano-emulsions will be stable for a long time with high PDI value, might explained the lowest droplet diameter size. Marei, *et al* (2018). Our results according to McClements, (2007) and Tadros *et al*., (2004) show that the attractive forces between the droplets is proportional to the droplet size. When the size of the droplets are in the nano range, the attractive forces between the droplets are weak, thereby, preventing particle aggregation and helps in making the nano-emulsions more stable.

2.CONCLUSION

Overall, our research more knowledge information on the droplet size diameter of pesticides and essential oil nanoemulsions. droplet size is one of the essential properties which could play a major role in the toxicity and the biological activity of nanoemulsions and their effectiveness on the midgut of *spodoptera littoralis* . Nanoemulsion may particularly be an effective delivery system for insecticides and essential oils and their components because of their ability to facilitate the application and increase the effectiveness of their insecticidal effect. Further study on this nanoemulsion is required to assess its potential harmful effects on human health and the environment.

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الملخص العربي

التقييم السمى والنسيجى للمبيدات والزيوت الأساسية فى صورة المستحلب النانوى والتقليدى ضد دودة ورق القطن

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تم اختبار التأثيرات السمية و النسيجية لاثنين من الزيوت الأساسية (زيت النعناع والأوجينول) واثنين من المبيدات الحشرية (Chlorpyrifos و Chlorfenapyr) في تركيبه النانو والتركيبة التقليدية ضد يرقات الطور الرابع من *Spodoptera littoralis*. أظهرت النتائج أن مستحلب النانو له انشطه سامة كبيرة ضد السلالة المختبرية لدودة ورق القطن. باستخدام الاختبارالحيوي ، أظهر أن مستحلب النانو للكلوربيريفوس كان 10.33 مرة أكثر من مستحلب EC التقليدي تبعه النانو كلورفينبير كان 2.77 مرة أكثر من المستحلب التقليدي. من ناحية أخرى ، تأثر مستحلب النانو لزيت النعناع 2.54 مرة أكثر من مستحلب EC التقليدي بينما كان النانو اوجينول1.55مرة أكثر من المستحلب التقليدي في الصيغ النانوية والتقليدية. أظهرت الدراسات النسيجية في المعى المتوسط ليرقات الطور الرابع ان المعاملة بتركيز LC_{50} للمركبات المختبرة تدميرًا كبيرًا مقارنة باليرقات غير المعالجة. تشير نتائجنا إلى أن تركيبة النانو للزيوت الأساسية واثنين من المبيدات الحشرية المختبرة كانت أكثر فعالية ضد *S. littoralis* من التركيبات التجارية. أظهرت أشكال النانو من الزيوت الأساسية (زيت النعناع والأوجينول) والمبيدات الحشرية (الكلورفينابير والكلوربيرفوس) دراسات الأنسجة المرضية في المعي المتوسط من *S. littoralis*.