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Efficacy of Some Pyrethroid Nanoemulsions against cotton Leafworm *Spodoptera littoralis* (Boisd.): Toxicity, Biochemical and Molecular Docking Studies

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Abstract

Although pesticides are favourable for pest controlling because more than 30–40% of the food production is lost due to pests, the extensive use of pesticides has harmful effects on the environment and health. Nanotechnology techniques are recently used in developing agrochemical formulations. Nanoemulsions as new approaches have attracted great attention in delivering many poorly soluble in water active compounds. In this study, the most widely used pyrethroid insecticides (cypermethrin, deltamethrin, and lambda-cyhalothrin) were formulated as nanoemulsions by high-energy ultrasonication. The droplet sizes of prepared nanoemulsions have been investigated by Dynamic Light Scattering (DLS) and Transmission Electron Microscope (TEM), characterization result reveals the size of particles in the range 24.42-84.99 nm. Formulated nanoemulsions in comparison with the active ingredient (a.i) and emulsifier concentrate (EC) were evaluated against 4th instar larvae of *S. littoralis*. The *in vivo* activity levels of targeted enzymes, acetylcholinesterase (AChE), and adenosine triphosphate (ATPase) were studied in two bioassay methods (leaf dipping and topical application). Our finding showed that cypermethrin nanoemulsion has the highest activity with LC_{50} = 19.92 mg/l and LD_{50}= 2.11 ng/larva. Furthermore, the developed nano-formulation showed adequate toxicity levels on AChE and ATPase compared to the active ingredient and EC formulations. The protein-ligand docking was also studied, and the docking poses showed that the insecticides had an excellent binding affinity to the active site of the target enzymes. On this basis, these results suggest that oil in water (O/W) nanoemulsions would be beneficial and can be applied for delivery insecticide formulations.

Key words: Nanoemulsion, Pyrethroid insecticides, *Spodoptera littoralis*, Biochemical studies, Docking.

1. Introduction

The cotton leafworm *S. littoralis* (Boisd) is a major polyphagous pest in Egypt (1). It is active all year round without hibernation period and attacking more than 60 different cultivated and wild plants, mainly cotton, clover, maize, wheat, rice, barley, and vegetables (2-4). Pesticides play a significant role in providing reliable agricultural products cheaply to consumers, improving the yield quality and quantity, and ensuring high profits to farmers (5). Nearly 2 million tons of active pesticide ingredients are utilized annually worldwide. However, by the year 2020, global pesticide usage has been estimated to increase to 3.5 million tons (6).

Synthetic pyrethroids (the fourth group of insecticides) have a toxic effect against many pests. The global usage of pyrethroids has been estimated at 4.67 billion dollars in 2015 and is expected to touch 6.45 billion dollars by 2021 (7). The overuse of conventional pesticides led to many environmental problems such as air contamination (8), surface and groundwater contamination (9), the development of insect resistance to many registered pesticides (10), and health hazards (11, 12). To avoid these problems, scientists and researchers have been showing interest in developing new effective, environmentally friendly pesticides (13-15). Nanotechnology is science, engineering, and technology conducted at the nanoscale, about 1 to 100 nanometers. It can be used across all the other science fields such as chemistry, biology, physics, materials science, engineering (16), and pest management through successful employed formulations of nanomaterial's-based pesticides (17-19). Oil-in-water emulsions (O/W) are now receiving

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great attention due to the need to reduce or eliminate organic solvents for safer handling.

Moreover, it can have significant advantages over emulsifiable concentrates (EC) formulations in cost and safety, manufacture, transportation, and use. However, they require careful selection of emulsifiers to prevent flocculation, creaming, and coalescence of the oil droplets (20). Nanoemulsions are emulsions with droplet sizes in the range of 20–200 nm. Several potential advantages over microemulsions and conventional emulsions include less surfactant required (21), better aggregation stability, less gravitational separation, low viscosity and optical transparency. All these make them desirable systems for many industrial applications (22, 23). Nanoemulsions are suitable for low water-soluble compounds such as pesticides. It can be easy to formulate, handle, and obtained at a low cost (24).

Many different methods and procedures have been developed for nanoemulsions (low-energy methods, high-energy methods, and combined methods). (25). Recently, nanoemulsions have been extensively studied as an important delivery system against many pests as larvicide (26), insecticide (23, 27), repellent activity (28), acaricide (29, 30), fungicide (31, 32), bactericide (33) and antiparasitic activities (34).

This research aims to develop stable oil in water nanoemulsion containing insecticides (cypermethrin, deltamethrin, and lambda-cyhalothrin) by high-energy method (ultrasonication). Moreover, evaluate the insecticidal activity of these emulsions compared to the commercial and active ingredient of insecticides against the fourth instar larvae of cotton leafworm *S. littoralis* using two bioassay methods leaf dip and topical application. Study the *in vivo* effect of different insecticide formulations on biochemical parameters AChE and ATPase. We are also using MOE (Molecular Operating Environment) software to identify the correct docking poses of ligands in the binding pocket of protein to predict the affinity between the insecticides and the targeted enzymes**.**

2. Experimental

2.1. Insecticides, chemicals, and reagents

2.1.1. Insecticides

Cypermethrin $C_{22}H_{19}Cl_2NO_3$ (90% and 25% EC Sparkill®), deltamethrin $C_{22}H_{19}Br_2NO_3$ (98% and 5% EC Nu-Tox®) and lambda-cyhalothrin $C_{23}H_{19}ClF_3NO_3$ (96% and 10% EC Lambada®) were obtained from Kafr El Zayat Pesticides and Chemicals Co. (Kafr El-Zayat, Gharbia, Egypt).

2.1.2. Chemicals

Acetylthiocholine iodide (ATChI) ($CH_3COSCH_2CH_2N(CH_3)_3I$), adenosine triphosphate (ATP) ($C_{10}H_{16}N_5O_{13}P_3$), bovine serum albumin (BSA), 5,5-dithio bis (2-nitrobenzoic) acid (DTNB), butanol ($C_4H_{10}O$), Foline-Ciocalteu phenol, hydroxymethyl aminomethane (Tris-base) ($C_4H_{11}NO_3$), non-ionic surfactant Tween 80, dimethyl sulfoxide (DMSO) (C_2H_6OS), Ammonium molybdate $(NH_4)_6Mo_7O_{24}$, sodium-potassium tartrate $KNaC_4H_4O_6{\cdot}4H_2O$, ferrous sulfate $FeSO_4$, tricholoroacetic acid (TCA) $C_2HCl_3O_2$, toluene (C_7H_8), sodium phosphate monobasic anhydrous ($NaH_2PO_4.H_2O$) and sodium phosphate dibasic anhydrous ($Na_2HPO_4.H_2O$), were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other commercially presented solvents and reagents such as sodium hydroxide (NaOH), hydrochloric acid (HCl), sulfuric acid (H_2so_4), copper sulfate ($CuSO_4$), and acetone (C_3H_6O) were purchased from El-Gomhoria for pharmaceutical and chemicals Co, (Adeb Ishak St, Manshia, Alexandria, Egypt) and were used without further purification.

2.1.3. Buffers

Phosphate buffer (pH 7.4): Aliquot of 802 mL of 0.1M Na_2HPO_4 and 198 mL of 0.1M Na_2PO_4 to prepare 1 liter of the buffer. Phosphate buffer (pH 8**):** Aliquot of 940 mL of 0.1M Na_2HPO_4 and 60 mL of 0.1M Na_2PO_4 to prepare 1 liter of the buffer. Alkaline copper reagent: 48 mL of 2% (w/v) sodium carbonate in 0.1 N NaOH were added to 1 mL of 1% (w/v) sodium–potassium tartrate and1 mL of 0.5% (w/v) copper sulfate. Tris-HCl buffer (pH 7.4): 0.12 M tris were added to 0.32m sucrose and 0.001m EDTA and adjust pH to 7.4 with the suitable volume of concentrated HCl**.** Colour reagent: 5 g ferrous sulfate in 10 mL ammonium molybdate solution prepared in10 N sulfuric acid.

2.2. Preparation of insecticide nanoemulsions

Insecticide nanoemulsions were prepared by the procedure reported by Badawy and co-authors (35). All nanoemulsions were prepared in two phases, organic phase, and aqueous phase. The organic phase consists of 10 ml of insecticides (cypermethrin, deltamethrin, and lambda-cyhalothrin) dissolving in 10 ml toluene as solvent and 1 ml butanol as co-solvent. The aqueous phase was consisting of 9 ml of surfactant (tween 80) and 70 ml water. The emulsions were prepared by dropwise of organic phase on the aqueous phase with stirring at 4000 rpm for 30 min. Then the nanoemulsions formed by ultrasonic, the process was carried out at 15 min, 50 % of sonicator power (20 kHz) and pulses 7cycle/sec**.** The difference of temperature from the initial coarse emulsion to the final emulsion was not more than 25°C**.**

2.3. Insecticide nanoemulsions characterization

Droplet size distribution and polydispersity index (PDI) of nanoemulsion were determined using Zetasizer Nano ZS (Malvern Instruments, UK) at room temperature (36). Transmission Electron Microscopy (TEM) was carried out to visualize the shape and morphology of formulated nanoemulsions. The TEM samples were observed with JEOL JSM-1200EX II (Peabody, MA, USA). The formulations' viscosity was measured without further dilution using a Rotary Myr VR 3000 digital viscometer with L4 spindle at 200 rpm at 25°C. For thermodynamic characterization, nanoemulsions were exposed to extreme storage conditions (centrifugation, heating-cooling cycle, and freezing-thaw cycle) to predict the samples' ability to tolerate over a certain period (37).

2.4. Insect and bioassay methods used

The culture of cotton leafworm *S. littoralis* was obtained from a laboratory strain maintained for several generations without any insecticidal or microbial pressure in the cotton leafworm research department, Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza. The insects were reared on castor leaves (*Ricinus communis*), under laboratory conditions at 25 ± 2 °C and 60 ± 5 % R.H according to Eldefrawi, Toppozada (38). Two different methods of bioassay were used (leaf dip and topical application).

In the leaf dip method, castor leaves were dipped in insecticide solution for ten seconds and allowed to dry for 45 min at room temperature. The treated leaves were exposed to the larvae in tri replicate. Each insecticide's active ingredient was dissolved in DMSO with 0.01% tween 80 and diluted to desired concentrations by water. However, EC and nanoemulsions were diluted to desired concentrations with water (39). According to Metcalf (40), the topical application method was used. One microliter of an acetone solution containing insecticide concentration was applied to the thoracic terga of the larva using the micro applicator. Freshly molted 4th instar larvae of *S. littoralis* were used, mortality was determined 24 h after treatment.

2.5. Biochemical studies

The sub-lethal doses and concentrations equivalent to the LC_{50} and LD_{50} values of different insecticides in different formulations were used to determine the effects on some biochemical responses. Live insects from each treatment were selected. The larvae's definite weight was homogenized in 3 mL of phosphate buffer (pH 7.4) with a tissue-tearor on ice. The homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant was used in protein determination and as the crude enzyme extract. Lowry method (41) was used to determine protein content; the sample of protein content was determined by comparing to the standard curve of BSA. AChE activity (in vivo) in head capsule of *S. littoralis* larvae were assayed using a procedure of Ellman (42). The absorbance was measured at 412 nm using Unico 1200 spectrophotometer. ATPase activity was determined colorimetrically according to the method of Koch (43), absorbance was measured at 740 nm by using a Unico 1200 spectrophotometer. The enzyme activity was represented as δ OD_{740} min^{-1}.mg $protein^{-1}$.

2.6. Docking of tested insecticides into enzymes

The modeled protein structure, AChE (PDB:2ACE), ATPase (PDB:3A3Y), CaE (PDB:1CI8), GST (PDB: 1PN9), in their PDB formats were downloaded from the protein data bank (PDB) and imported on to the MOE. The protein chemistry of the missing hydrogen was corrected after which the heteroatoms and the crystallographic water molecules were removed from the protein (44). Insecticides (cypermethrin, deltamethrin, and lambda-cyhalothrin) were constructed using the Chem Draw professional 15 Builder module. The ligands were minimized before initiating the docking using CHARM m 99 force field, 3D structures generated, removal of duplicates was done, and bonds were added to it. After all the default parameters were set and obtain the minimum energy structures, the ligands were allowed to be flexible, then manually positioned within the enzyme model's catalytic site cavity. The protein-ligand docking was performed using the Molecular Operating Environment (MOE) 2014.13, Chemical Computing Group Inc, Montreal, Quebec, Canada, using an induced fit protocol, which considers the receptor as fixed and the ligand as flexible (45). The binding energy analyzed by a full-force field, the affinity between the ligand and the protein was evaluated with scoring functions, which calculated free binding interaction energies based on molecular force field terms. RMSD and scoring functions were computed, the best ligand interaction was analyzed and assessed at the end of the dock results.

2.7. Statistical analysis

Statistical analysis was done using the statistical package SPSS software version 25 (SPSS, Chicago, IL, USA) (46). The log dose-response curves allowed the determination of LC_{50} and LD_{50} for the bioassays according to probit analysis (47). The 95% confidence limit and standard error for the range of LC_{50} and LD_{50} values for the compound for assays on mortality were determined. Abbott formula used to get correction mortality (48). Statistical significance data was determined with one-way analysis of variance (ANOVA) by comparing means using the SNK method at the probability of ≤0.05 (49).

3. Results and Discussion

3.1. Preparation and characterization of insecticide nanoemulsions

The Physicochemical characterization results, TEM and DLS micrograph of nanoemulsions are presented in Table 1 and Figure 1. The droplet size and polydispersity index (PDI) of prepared emulsions confirm the formation of nanoemulsions. The droplet size of insecticide nanoemulsions obtained by the ultrasonic method was 24.42-84.99 nm, PDI values varied from 0.121 to 0.377.

Table 1. Physicochemical characterization of insecticide nanoemulsions

Insecticides	Droplet diameter (nm)	Polydispersity index (PDI)	Viscosity ± SD (mPa.s)
Cypermethrin	84.99	0.121	2.1±0.12
Deltamethrin	24.42	0.377	27.23±0.21
Lambda-cyhalothrin	79.05	0.162	2.5±0.06

PDI value reflects the distribution of particle size in the formulations and ranges from zero to one. PDI value lower than 0.2 reflects the homogeneity, and PDI of >0.3 indicates the system's heterogeneity (50). These results proved that all these liquid formulations were successful in their preparation in the nanometric size range. Other studies have reported matching results on successful preparation O/W nanoemulsions by the ultrasonic method with pesticidal effects (26, 35, 51).

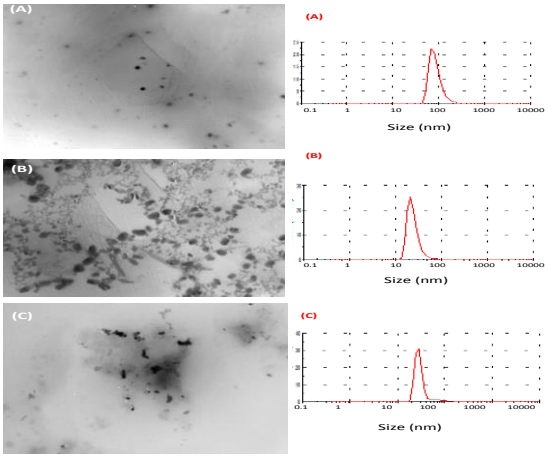

Figure 1. Left: Transmission electron microscopy (TEM) photographs of cypermethrin (A), deltamethrin (B), and lambda-cyhalothrin (C). Right: Droplet size distribution of cypermethrin (A), deltamethrin (B), and lambda-cyhalothrin (C) nanoemulsions by a dynamic light scattering (DLS).

The results of thermodynamic characterization studies (centrifugation, heating-cooling cycle, and freezing-thaw cycle) on prepared nanoemulsions showed that all formulations were found to be stable in their homogenous state. Generally, the nano size and large surface area of the droplets increase the nanoemulsions' kinetic stability by their continuous Brownian motion (52). The viscosity values were 2.1, 27.23, and 2.5 mPa.s for cypermethrin, deltamethrin, and lambda-cyhalothrin, respectively.

3.2. Bioassay against *S. littoralis* by leaf dipping and topical application techniques

The toxicity against *S. littoralis* after 24 h from the beginning of feeding on castor leaves treated with insecticides by leaf-dip and others treated by the topical application are presented in Tables 2 and 3, respectively. The results show that all tested insecticide formulations exhibited significant toxicity with LC_{50} ranging from 19.92 to 436.48 mg/L by leaf dip method and LD_{50} ranging from 2.11 to 77.21 ng/larvae by topical application method after 24 h of treatment. In Table 2, cypermethrin nanoemulsion showed higher toxicity than the tested insecticides caused the highest toxicity (LC_{50} = 19.92 mg/L) followed by deltamethrin and Lambda-cyhalothrin (LC_{50} = 47.10 and 306.87mg/ L, respectively) by leaf dipping method. Besides, it can be noted that the active ingredient showed lower toxicity than EC insecticide formulations with LC_{50} in the range of 49.26-436.48 mg/L by leaf dipping method. Comparing the obtained results with those reported by several authors (53-55) who studied the activity of cypermethrin and deltamethrin insecticides against cotton leafworm and found similar results with LC_{50} = 5.84 and 8.21 mg/L, respectively. However, our results were in disagreement with other authors (56) who found that the LC_{50} of lambda- cyhalothrin was ranged from 10.77 to 152.54 mg/L. The results of tested insecticides against *S. littoralis* by topical application method against 4th instar larvae of *S. littoralis* are presented in Table 3. Compared to the active ingredients and EC, the LD50 of nanoemulsion ranged between (2.11 to 30.89), all nanoemulsions exhibited high toxicity. The assay results suggested that cypermethrin was the most toxic insecticide among all tested insecticides with different formulations, followed by deltamethrin and Lambda-cyhalothrin. The LD_{50} of other insecticides ranged from 2 to 78 ng/larva. Several research groups evaluated the toxicity of these insecticides by topical application against *S. littoralis*, and they found that the LD_{50} was ranged from 1.3 to 42 ng/larva (57,58).

Table 2. Toxicity of active ingredients, EC and nanoemulsion of insecticides against 4th instar larvae of *S. littoralis* by leaf dip method after 24 h of treatment

Insecticides	LC_{50}[a] (mg/L)	95 % Confidence limits		Slope[b] ± SE	Intercept[c]± SE	(χ2)[d]
		Lower	Upper			
Active ingredients						
Cypermethrin	22.42	4.63	45.90	1.86±0.16	-2.51±0.25	20.64
Deltamethrin	74.09	68.52	80.34	5.27±0.43	-9.86±0.79	2.64
Lambda-cyhalothrin	436.48	207.43	885.96	1.90±0.14	5.02±0.73	15.81
EC formulations						
Sparkill® (25% EC)	20.84	5.31	39.86	1.95±0.17	-2.57±0.26	18.76
Nu-Tox® (5% EC)	49.26	31.48	75.75	2.68±0.21	-4.54±0.36	12.23
Lambada® (10% EC)	367.57	115.27	996.81	2.00±0.14	-5.15±0.38	27.57
Nanoemulsion formulations						
Cypermethrin	19.92	8.95	32.18	2.02±0.17	-2.62±0.26	11.40
Deltamethrin	47.10	31.53	68.76	2.76±0.21	-4.62±0.36	10.45
Lambda-cyhalothrin	306.87	52.32	1185.18	1.87±0.13	-4.66±0.35	36.43

[a] Median lethal concentration (concentration which caused 50% mortality of the tested larvae). [b] Slope of the concentration - mortality regression line ± standard error. [c] Intercept of the regression line ± S.E. [d] Chi-square value. EC: Emulsifiable concentrate.

It can be noted that the application of nanoemulsion formulations improving the delivery of insecticide. The bioassay data indicated that the nanometric O/W nanoformulations' possible use could be the starting point for developing new formulations for pest control. In this direction, there is worldwide interest in the development of nanoemulsion for agrochemical applications. Different reports support the efficacy of nanoemulsion against different pests, including stored product insect (59), mosquitoes (26), plant fungi (32), cotton bollworms (60), cattle tick (30), bacteria (33), and spider mite (29). Generally, nanoemulsions are a valid option for potent, safer, and eco-friendly tools in the agro-system. Future research on toxicity on non-target organisms, field experiments, and develop the technical sides needed.

3.3. Biochemical effects of pesticides on *S. littoralis*

3.3.1. Effects on (AChE)

AChE is a serine hydrolase found at neuromuscular junctions. In chemical synapses of the cholinergic synapses, the biological role of AChE is termination of impulse transmission by rapid hydrolysis of the neurotransmitter acetylcholine (ACh) to acetate and choline. AChE has a remarkably high specific catalytic activity, especially for a serine hydrolase (61,62).

The effect of insecticides on AChE of *S. littoralis* treated by leaf dipping method recorded in Table 4, the result showed that nanoemulsion had a high inhibitory effect on the enzyme activity of larvae treated. However, they are still lower than organophosphate insecticides due to the AChE enzyme not being the target enzyme for pyrethroid. EC and a.i insecticides showed the lowest inhibitory effects.

The effect of insecticides by topical application method is shown in Table 5. The effect of different insecticides on AChE revealed that significant differences were observed among treatments. Deltamethrin showed the highest effect at the different formulation followed by cypermethrin and lambda-cyhalothrin. The specific activities of treated AChE with deltamethrin are 0.386, 0.183 and 0.175 OD/ mg protein/min for a.i, EC and nanoemulsion, respectively. Little research has been studied on the effect of pyrethroids on AChE. For example, treatment with fenitrothion (0.1, 0.2, 0.4, 0.8, and 1 nmol/bee) led to a decrease in AChE activities in honeybees (63).

Table 3. Toxicity of active ingredients, EC and nanoemulsion of insecticides against 4th instar larvae of *S. littoralis* by topical application method after 24 h of treatment

Insecticides	LD_{50}[a] (ng/larvae)	95 % Confidence limits		Slope[b] ± SE	Intercept[c] ± SE	(χ2)[d]
		Lower	Upper			
Active ingredients						
Cypermethrin	2.63	0.025	4.75	2.13±0.23	5.51±0.54	5.37
Deltamethrin	10.53	8.59	12.64	1.66±0.19	3.28±0.37	2.23
Lambda-cyhalothrin	77.21	57.41	110.02	1.02±0.13	1.14±0.18	0.56
EC formulations						
Sparkill® (25% EC)	2.27	1.83	2.69	2.71±0.29	7.15±0.69	2.90
Nu-Tox® (5% EC)	8.37	6.57	10.19	1.58±0.19	3.28±0.37	3.56
Lambada® (10% EC)	56.72	40.87	81.32	0.91±0.13	1.13±0.18	0.64
Nanoemulsion formulations						
Cypermethrin	2.11	1.72	2.47	3.03±0.33	8.10±0.83	1.42
Deltamethrin	7.41	5.95	8.87	1.85±0.20	3.93±0.40	1.40
Lambda-cyhalothrin	30.89	22.53	40.93	1.07±0.13	1.61±0.19	1.31

[a] Median lethal dose (dose which caused 50% mortality of the tested larvae). [b] Slope of the concentration - mortality regression line ± standard error. [c] Intercept of the regression line ± S.E. [d] Chi-square value. EC: Emulsifiable concentrate.

3.3.2. Effects on ATPase

The *in vivo* effect of pyrethroids on ATPase activity of *S. littoralis* is summarized in Table 5 as LC_{50} and LD_{50} values. LC_{50} of insecticide formulations have significantly inhibited the activity of ATPase.

Values of activity were 0.343, 0.302 and 0.386 OD/ mg protein/min for active ingredients, 0.276, 0.265 and 0.323 OD/ mg protein/min. for EC formulations and 0.260, 0.225 and 0.312 OD/ mg protein/min for nanoemulsion formulations for cypermethrin, deltamethrin and lambda-cyhalothrin, respectively.

In a topical application method, cypermethrin, deltamethrin and lambda-cyhalothrin exhibited reduction of ATPase activity as values were 0.306, 0.293 and 0.358 OD/ mg protein/min, 0.278, 0.253 and 0.326 OD/ mg protein/min, 0.233, 0.218 and 0.301 OD/ mg protein/min and, for active ingredient, EC and nanoemulsion respectively (Table 5). ATPase is identical to the transport system for Na^+ and K^+ across the cell membrane (64). Pyrethroids are known to induce toxic effects by disrupting nerve impulse transmission and modulating the neurotransmitter system (65). Pyrethroids are specific inhibitors to ATPase. ATPases are targets of pyrethroid action and may be used as an in vitro method to compare the toxicities of different pyrethroid compounds (66). Many research showed that a high concentration of pyrethroids has significant inhibition on the insect Na^+ K^+ - ATPase. Some scholars think it is an important target of pyrethroid insecticides against pest insects (67, 68). This study demonstrated that pyrethroids inhibit *in vivo* effects on insect brain Na^+ K^+ - ATPase and inhibitory activities than OP insecticides. These findings are in agreement with other studies in which the exposure to pyrethroid insecticides decreased the activity of Na^+ K^+-ATPase**,** Abbassy *et al* (58) found that inhibitory ratio of ג - cyhalothrin, cypermethrin, deltamethrin and fenvalerate for ATPase in *S. littoralis* larvae were 59.5, 69.8, 69.1 and 68.8%, respectively. Similarly, cyhalothrin has been shown to induce an inhibitory effect against *S. littoralis* Na^+ K^+-ATPase (75.7%:93.6%) (69,70). Compared to conventional and active ingredient insecticides, nanoemulsions with particle size ranging from 24 to 85 nm enhance the effects against AChE, and ATPase, many authors in Pharmaceutical applications, showed that nanoemulsions could cross the barrier and offer additional advantages in drug delivery (71-73).

3.4. Docking of tested insecticides into target enzyme

The selected insecticides were allowed to dock with the homology modeled proteins. For each 30 conformation 3D structures were generated. The dock results were analyzed using MOE. The PDB files of the proteins and ligands files were loaded into the MOE, and then results were read based on ligand binding orientation, binding affinity, and binding-free energies. The docking and interaction energies of AChE and ATPase, models complexed with cypermethrin, deltamethrin, and lambda-cyhalothrin were compared. These data are presented together with energy values in Table 6.

Table 4. *In vivo* biochemical effects of active ingreadient, EC and nanoemulsion of different insecticides at LC_{50} values on AChE and ATPase, activity in fourth instar larvae of *S. littoralis* after 24 h from treatment by leaf dipping method

Treatment	LC_{50} (mg/L)	Enzyme activity (OD.mg^{-1}protien.min^{-1})±SE	
		AChE	ATPase
Active ingredients			
Untreated larvae	-	0.559^{a}±0.02	0.619^{a}±0.03
Cypermethrin	22.42	0.474^{b} ±0.00	0.343^{bc} ±0.04
Deltamethrin	74.09	0.448^{b} ±0.03	0.302^{bcd} ±0.01
Lambda-cyhalothrin	436.48	0.482^{b} ±0.01	0.386^{b}±0.01
EC formulations			
Sparkill® (25% EC)	20.84	0.407^{b} ±0.01	0.276^{cd} ±0.03
Nu-Tox® (5% EC)	49.26	0.403^{b} ±0.00	0.265^{cd} ±0.02
Lambada® (10% EC)	367.57	0.449^{b} ±0.01	0.323^{bcd} ±0.02
Nanoemulsion formulations			
Cypermethrin	19.92	0.389^{b} ±0.05	0.260^{cd} ±0.03
Deltamethrin	47.10	0.388^{b} ±0.00	0.225^{d} ±0.03
Lambda-cyhalothrin	306.87	0.435^{b} ±0.00	0.312^{bcd} ±0.02

Data are means ± SE of three replicates. Values followed by the same letter (s) within a column are not significantly different at P≤0.05, by Student-Newman-Keuls (SNK) Test. AChE: Acetylecoline esterase. ATPase: Adenosinetriphosphate. OD: Optical density. LC_{50}: Median lethal concentration (concentration that caused 50% mortality of the tested larvae). EC: Emulsifiable concentrate.

Table 5. *In vivo* biochemical effects of active ingreadient, EC and nanoemulsion of different insecticides at LD_{50} values on AChE and ATPase activity in fourth instar larvae of *S. littoralis* after 24 h from treatment by topical application method

Treatment	LD_{50} (mg/L)	Enzyme activity (OD.mg^{-1}protien.min^{-1})±SE	
		AChE	ATPase
Active ingredients			
Untreated larvae	-	0. 578^{a}±0.00	0.659^{a}±0.03
Cypermethrin	2.63	0.452^{ab}±0.01	0.306^{bcd} ±0.04
Deltamethrin	10.53	0.386^{bc}±0.00	0.293^{bcd}±0.01
Lambda-cyhalothrin	77.21	0.405^{bc} ±0.00	0.358^{b} ±0.01
EC formulations			
Sparkill® (25% EC)	2.27	0.296^{bcd} ±0.09	0.278^{bcd} ±0.03
Nu-Tox® (5% EC)	8.37	0.183^{d} ±0.01	0.253^{cd} ±0.02
Lambada® (10% EC)	56.72	0.311^{bcd} ±0.03	0.326^{bc} ±0.00
Nanoemulsion formulations			
Cypermethrin	2.11	0.264^{ab}±0.05	0.233^{cd} ±0.02
Deltamethrin	7.41	0.175^{d} ±0.03	0.218^{d} ±0.02
Lambda-cyhalothrin	30.89	0.232^{cd} ±0.07	0.301^{bcd} ±0.04

Data are means ± SE of three replicates. Values followed by the same letter (s) within a column are not significantly different at P≤0.05, by Student-Newman-Keuls (SNK) Test. AChE: Acetylecoline esterase. ATPase: Adenosinetriphosphate. OD: Optical density. LD_{50}: Median lethal dose (dose that caused 50% mortality of the tested larvae). EC: Emulsifiable concentrate.

3.4.1. Docking onto AChE

Docking results of cypermethrin, deltamethrin, and lambda-cyhalothrin binding to AChE are listed in Table 6. The two-dimensional and three-dimensional interaction diagrams are shown in Figure 2. Table 6 showed that the initial docking energies for these compounds in AChE enzyme interaction energies with docking energy were 9.52, 8.83, and 9.25 kcal mol^{-1} for cypermethrin, deltamethrin, and lambda-cyhalothrin, respectively. Six key amino acids (Gly *C*445, Gly *A*445, Asp *C*233, Asp *A*233, Leu *C*444, and Leu *A*444) in the binding pocket interact with cypermethrin via hydrogen bonding and hydrophobic interaction. Gly *C*445 and Gly *A*445 form two hydrogen bonds with chlorine atom in cypermethrin via H- donner interaction (HBD) (2.96 - 2.96Å). The benzene ring of cypermethrin formed two H–pi (3.76Å and 3.76Å) interactions with Asp *C*233 and Asp *A*233. The C22 atom of cypermethrin forms two conventional hydrogen bond (3.66, 3.59), SO_4 808 with Leu *C*444 and Leu *A*444, respectively. In addition, cypermethrin surrounded by 21 amino acids through Van der Waals interactions (Figure 2A).

Deltamethrin interacted in the binding pocket with six vital amino acids (Phe *A*490, Phe

*C*490, Gly *A*445, Gly *C*445, Glu *A*448, Glu *C*448) via hydrogen bonding. The bromine atom of deltamethrin forms two HBD, Phe *A*490 with (Br 20- 3.54 Å) and Phe *C*490 with (Br- 3.54 Å). While Br 21 forms four hydrogen bonds (Br 21-3.25 Å) with Gly *A*445, (Br 21- 3.47 Å) with Glu *C*448, (Br21 -3.25 Å) with Gly *C*445, and (Br21- 3.47Å) with Glu *A*448. 24 amino acids also surround deltamethrin through Van der Waals interactions (Figure 2B). In the binding pocket of lambda-cyhalothrin, the nitrogen atom formed 2 hydrogen bonds with tyrosine amino acid, (N50-3.66Å) with Tyr *A*494 and (N50-3.66 Å) with Tyr *B*494 through H- acceptor interaction (HBA). Twenty-six amino acid residues in the binding pocket interact with lambda-cyhalothrin via Van der Waals interactions (Figure 2C).

3.4.2. Docking onto ATPase

The results of the intermolecular interaction energy values obtained from the docking calculation on ATPase enzyme also are shown in Table 6, study of the docking poses showed that the insecticides had good binding affinity to the site of the target enzyme with docking energy -9.69, 9.68 and -8.82 kcal mol^{-1} for cypermethrin, deltamethrin, and lambda-cyhalothrin, respectively. Figure 3 shows the binding modes and orientations of insecticides.

Two key amino acids (Thr *A*804 and Thr *A*804) in the binding pocket interrelate with cypermethrin via HBA. The N atom of the cyano group formed two hydrogen bonds (N47-3.87Å and N47-3.87Å) with Thr *A*804 and Thr *A*804. The non-bonded contacts formed by many hydrophobic groups, the O23 linked with Ala *A*330 and Ala *A* 330 protein residues with 2.54 Å and 2.54 Å, respectively, via HBA interaction. Cypermethrin attached 38 amino acids to the ATPase enzyme's active site through van der Waals interactions, as shown in Figure 3A.

Deltamethrin interacted with two amino acids Lys *A*912 and Arg *A*893, via HBA. The N47 atom of deltamethrin formed two hydrogen bonds (N-3.82Å) with Lys *A*912 and (N-3.44 Å) with Arg *A*893. The non-bonded contacts were noticed in deltamethrin, the O14 atom connected with Thr*A*804 and Thr*A*804 protein residues with 2.51 Å and 2.23 Å, respectively. The O23 is linked with Ala*A*330 and Ala *A* 330 protein residues with 2.54 Å and 2.54 Å via H-acceptor interaction. The C12 atom of deltamethrin forms contact with 6-ring Phe *A*790 of protein residues with 4.44Å via H-pi interaction. Deltamethrin interacts with 45 amino acids in the ATPase enzyme's active site through van der Waals as interactions (Figure 3B).

In the binding pocket of lambda-cyhalothrin with ATPase enzyme, the insecticide interacted with two amino acids (Thr *A*804 and Thr *A*804) via HBA. The nitrogen atom formed two hydrogen bonds (N47-3.38Å and N47-3.38 Å) with Thr *A*804 and Thr *A*804, respectively. The non-bonded contacts formed by many hydrophobic groups were detected, the O14 atom related with Thr*A*804 and Thr*A*804 protein residues with 2.51 Å and 2.42 Å via H-acceptor interaction, respectively. The O23 is linked with Ala*A*330 and Ala*A*330 protein residues with 2.54 Å and 2.54 Å via H-acceptor interaction, respectively. The C12 atom of lambda-cyhalothrin forms contact with 6-ring Phe*A*790 of protein residues with 4.47 Å via H-pi interaction. Lambda-cyhalothrin attached 46 amino acids to the ATPase enzyme's active site through van der Waals interactions (Figure 3C).

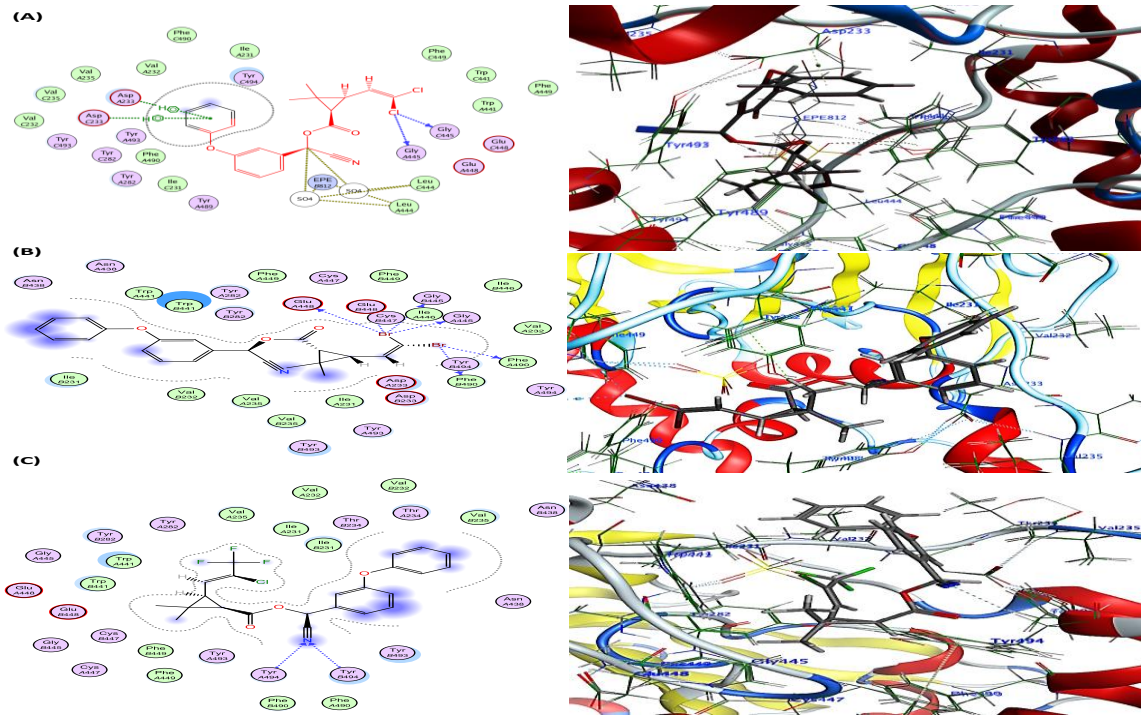

Figure 2. Docking view of cypermethrin (A), deltamethrin (B), and lambda-cyhalothrin (D) in the binding sites of AChE (PDB: 5X61). The left is a 2D Interaction diagram of the insecticides-5X61 complex and the 3D of the complex structure.

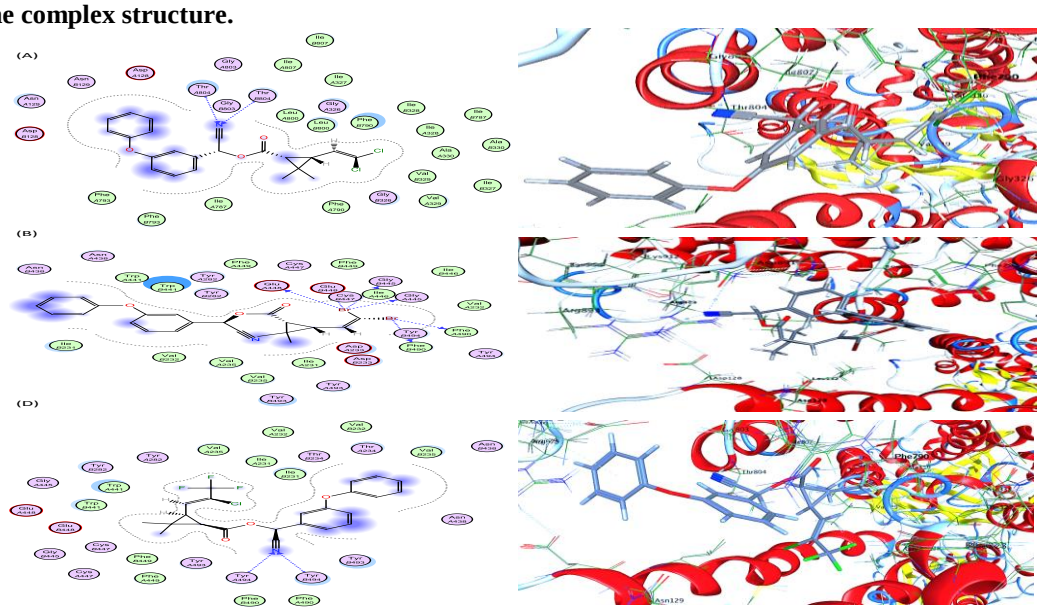

Figure 3. Docking view of cypermethrin (A), deltamethrin (B), and lambda-cyhalothrin (D) in the binding sites of ATPase (PDB: 3A3Y). The left is a 2D Interaction diagram of the insecticides-3A3Y complex and the 3D of the complex structure.

Table 6. Binding energy and amino acids interactions of cypermethrin, deltamethrin and lambda-cyhalothrin docked into AChE and ATPase enzymes

Pesticides	AChE							ATPase						
	Docking score (kcal mol−1	H-bonds Amino Acid -Ligand Atom			Hydrophobic interaction		rmsd	Docking score (kcal mol−1	H-bonds			Hydrophobic interaction		rmsd
		Acceptor	Donner	Distance A◦	Amino acid-ligand Atom	Distance A◦			Acceptor	Donner	Distance A◦	Amino acid-ligand atom	Distance A◦	
Cypermethrin	-9.52	-	Gly *C*445-(Cl21), Gly *A*445,(Cl21), Leu *C*444-(C22), Leu *A*444-(C22)	2.96, 2.96, 3.66, 3.59,	Asp*C*233(6-ring),Asp*A*233(6-ring)	3.76, 3.76	2.08	-9.69	Thr *A*804(N47), Thr *A*804(N47)	-	3.87, 3.87	-	-	1.89
Deltamethrin	-8.83	-	Phe *A*490-(Br20) Phe *C*490-(Br20) Gly *A*445-(Br21) Glu *A*448-(Br21) Gly *C*445-(Br21) Glu *C*448-(Br21)	3.54, 3.54, 3.25, 3.47, 3.25, 3.47	-	-	2.09	-9.68	Arg *A*893-(N47) , Lys *A*912-(N47)		3.82, 3.44	-	-	1.24
Lambda-cyhalothrin	-9.25	Tyr *A*494(N50), Tyr *B*494(N50)		3.66, 3.66	-	-	2.56	-8.82	Thr *A*804-(N47), Thr *A*804-(N47)	-	3.38, 3.38	-	--	1.60

rmsd: The root mean square deviation of the pose, in Å, from the original ligand

4. Conclusion

Pyrethroids are effective insecticides against a wide variety of pests belonging to different orders of insects. Cypermethrin, deltamethrin, and lambda-cyhalothrin are broad-spectrum pyrethroid insecticides with low water solubility. O/W nanoemulsions are a good tool for solubilization and delivery of hydrophobic pesticides. By high energy method, cypermethrin, deltamethrin, and lambda-cyhalothrin nanoemulsion were prepared with droplet diameter 84.99, 24.42, and 79.05 nm, respectively. Compared with EC and active ingredient, nanoemulsions have enhanced the toxic effects against 4th instar larvae of *S. littoralis* and some of its enzymatic systems by leaf-dip and topical application techniques. MOE was used to predict how a proteins (AChE and ATPase) interact with small molecules (cypermethrin, deltamethrin, and lambda-cyhalothrin) to form an enzyme-insecticide by induced fit protocol. Nano pesticide delivery systems (O/W) nanoemulsions containing selected insecticides were practical and could replace conventional EC, thus reducing the organic solvent content in agricultural formulations. Many studies focus on the formation of nanoemulsions, ignoring the interaction between plant surfaces and pesticide and toxicity on non-target organisms. Further work is needed to study the interaction between plant surfaces and nano pesticide droplet under field conditions and its effects on the ecosystem.

5. Conflicts of interest

There are no conflicts to declare.

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فعالية بعض مبيدات البيروثريد النانويه ضد دودة ورق القطن سبودوبترا ليتوراليس

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على الرغم من أن مبيدات الآفات تستخدم لمكافحة الآفات حيث أن أكثر من 30-40٪ من إنتاج الغذاء يفقد بسبب الآفات ، إلا أن الاستخدام المكثف لمبيدات الآفات له آثار ضارة على البيئة والصحة. في الآونة الأخيرة ، تستخدم تقنيات النانو في تطوير تركيبات الكيماويات الزراعية. مستحلبات النانو جذبت اهتمامًا كبيرًا في توصيل العديد من المركبات النشطة في الماء ضعيفة الذوبان. في هذه الدراسة ، تمت تحضير بعض المبيدات الحشرية البيرثرويدية (سايبرمثرين ، ديلتاميثرين ولامبداسيهالوثرين) كمستحلبات نانوية بطريقة (ultrasonication). تم فحص أحجام قطرات المستحلبات النانوية المحضرة بواسطة تشتت الضوء الديناميكي (DLS) الميكروسكوب الإلكتروني النافذ (TEM). اوضحت نتيجة التوصيف أن حجم الجسيمات في النطاق 42.24-84.99 نانومتر. تم تقييم مستحلبات النانويه بالمقارنة مع الماده الفعاله (ai) ومركزات قابله للاستحلاب (EC) ضد يرقات الطور الرابع من دوده ورق القطن *S. littoralis* ، وتم دراسة مستويات النشاط الانزيمي ، أستيل كولين أستراز (AChE) والأدينوزين ثلاثي الفوسفات (ATPase) بطريقتي (غمس الأوراق والتطبيق الموضعي). أظهرت النتائج التي توصلنا إليها أن مستحلب النانو سايبرمثرين له أعلى نشاط مع LC_{50} = 19.92 ملجم / لتر و LD_{50} = 2.11 نانوجرام / يرقه علاوة على ذلك، أظهرت تركيبة النانو المطورة مستويات سمية جيدة على AChE و ATPase مقارنة مع المادة الفعالة وتجهيزه ال EC. تمت دراسة اطريقه ارتباط المبيد مع الانزيم البروتيني وأظهرت النتائج أن المبيدات الحشرية لها صلة ارتباط جيدة بالموقع النشط للأنزيمات المستهدفة. على هذا الأساس، تشير هذه النتائج إلى أنه يمكن استخدام المستحلبات النانوية (زيت في الماء) لتوصيل المبيدات الحشرية.