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Preparation, Characterization, and Efficiency of Nano-emulsion Chlorpyrifos and Methomyl and Determination of their Residues in Pepper Fruits

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Abstract

The current study aimed to prepare nano-emulsions of chlorpyrifos and methomyl insecticides as well as evaluate their efficiency against cotton leafworm (*Spodoptera litoralis*) compared with their commercial formulation under laboratory conditions. Also, residues in pepper fruits were determined after field application. Results revealed that the resultant particles of the chlorpyrifos 10% nano-emulsion particle size distribution ranged between 50 and 500 nm; with an average particle size of 187.3 nm. In the case of methomyl nano-emulsion, the resultant particle size distribution was ranged between 30 and 2000 nm with an average particle size of 336.4 nm. The results revealed that the nano-formulations of both insecticides were more toxic and effective against the 4th instar larvae than their commercial formulations. According to the LC_{50} values, the toxicity of the chlorpyrifos and methomyl 10% nano-emulsion were 1.71 and 2.1 times higher toxic than that of chlorpyrifos 48% EC and methomyl 90% SP, respectively. The sub-lethal doses (LC_{25} and LC_{10}) of both nano and commercial formulations of chlorpyrifos and methomyl affected pupation % and adult emergence%. The highest effect was observed in larvae treated with LC_{25} of nano-emulsion of both insecticides. Moreover, after 15 days of fruit spray with the two tested insecticides and their nano emulsions, the lost amount of chlorpyrifos EC and nano-emulsion forms reached 96.5 and 99.2 % of the initial deposit, respectively. In contrast, it reached 0.08 ppm of Methomyl SP (loss % was 98.8), and no residues were detected in methomyl nano-emulsion-treated fruits. This means that the nano-emulsion formulations of both insecticides were faster degradable and more effective against *S. litoralis* larvae than their commercial formulations. Accordingly, these formulations may improve efficiency and reduce the residues that may decrease the adverse impacts on the environment and non-target organisms.
Keywords: Preparation, Nano-pesticides, *Spodoptera litoralis*, Chlorpyrifos, Methomyl, Efficiency.

1. Introduction

Pest control is necessary to enhance outcomes by safeguarding resources while minimizing the environmental impact of sustainable agricultural practices as a consequence of the scarcity of natural resources [1, 2, 3]. Pesticides have been applied without following proper recommendations and have a major negative impact on the environment and the development of resistance. Furthermore, only 1% or less of the applied pesticides reached the target pest, and the remaining 99% negatively affected non-target organisms [3, 4]. In addition to the rapid increase in pesticide prices, it is difficult for farmers to afford them. Therefore, new pest management alternatives are required to address these problems. Chlorpyrifos (organophosphorous class) and methomyl (carbamate group) are pesticides that are widely used against

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numerous insect pests worldwide [5, 6]. Although these insecticides are widely used to control different insects, particularly lepidopterous insects, including *S. littoralis*, on different crops and vegetables, their residues may affect the environment and human health. *Spodoptera littoralis* (Lepidoptera: Noctuidae) is a highly polyphagous destructive pest that has economic importance in Egypt [7]. Pepper is an important vegetable with high economic value and nutrition worldwide, including in Egypt [8, 9]. It is affected by numerous pests, causing loss of yield, quantity, and quality wherever it is planted (open fields or greenhouses) [10]. Accordingly, it is necessary to apply pesticides to control these pests, unfortunately, pesticide residues may persist on pepper fruits [11]. Therefore, and these pesticide residues should be monitored in pepper fruits to confirm their safety for consumption.

To decrease the undesirable effects of traditional pesticides, controlled-release formulations have been developed using numerous techniques, including nano-emulsions and micro-encapsulation [12, 13]. Nano-pesticides are a recent technology that provides many benefits, such as enhanced efficiency and decreased application rates. Different types of nano-pesticides such as metal and metal oxide nanoparticles, nano-spheres, nano-gels, nano-capsulated, and nano-emulsion formulations have been developed [14]. Owing to the exceptional properties of nanoparticles, they can be used to develop novel insecticides. However, due to the lack of studies on the toxicity and sub-lethal effects on pests, the extent of action and fate of these nanoformulations is still not fully understood, limiting their widespread use [15]. Therefore, this study aimed to 1) prepare nano-emulsions of chlorpyrifos and methomyl insecticides, 2) evaluate their efficiency against cotton leafworm (*S. litoralis*) compared with their commercial forms under laboratory conditions, and 3) determine their residues in pepper fruits after field application.

2 Materials and Methods

Insecticides used:

Chlorpyrifos (Chlorizan 48% EC) and methomyl (Jeto 90 % SP); insecticides were purchased from Kafr El Zayat Pesticides & Chemical Co., Egypt.

Reagents and solvents: Certified reference standard of chlorpyrifos and methomyl (purity > 98%) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). All solvent and reagents were HPLC grade or analytical grade and used without further purification.

Preparation of nano-emulsion: Vigorous homogenization was used to fashion the pesticide nano-emulsions. The Ultra Turrax homogenizer (IKA T 10 B S25 basic Ultra-Turrax; IkaWerke, Germany) was used to mix 0.2 g of pesticides dissolved in 5 mL methanol and 0.2 g of Tween 80 poured in 100 mL water. The mixture was agitated at 28 000g for 5 minutes to generate pesticide nano drops. Following a brief time of stabilisation, nano-drops of pesticides were filled with the 0.5 g PEG solution mixed in 20 mL ethanol while being stirred magnetically. The third solution, which consisted of 0.2 g of Tween 80 in 20 mL of water, was added to the first solution and stirred with a magnetic stirrer for 10 minutes after the mixture above had been stirred for 10 minutes. After that, an ethanolic pesticide solution (10 mg mL^{-1}) was applied drop by drop while being continuously stirred for 60 minutes at room temperature. The resultant emulsion was sonicated for 15 minutes.

Next, an appropriate volume of $CaCl_2$ (2 mg mL^{-1}) was added dropwise for an additional 60 minutes. Finally, theemulsion was agitated for an additional 60 minutes [16].

Characterizations of the prepared pesticides nano-emulsion:

a. Transmission electron microscopy (TEM): The morphology and particle size of the obtained insecticide nano-emulsions were measured using a TEM (JEM-1230; JEOL Ltd., Tokyo, Japan) at an 80 kV accelerating voltage. A drop of the formulated insecticide nano-emulsion was inserted onto a carbon-coated copper grid for the transmission electron microscopy specimens.

b. Dynamic light scattering (DLS): The variation in particle size of the developed insecticide nano-emulsion was measured using a NICOMP 380 ZLS dynamic light scattering (DLS) equipment (PSS, Santa Barbara, CA, USA). 10 mg of the nano-emulsion was dispersed in 100 mL of distilled water and sonicated for 30 minutes then the variation was measured. For water, the refractive index is 1.333 and the viscosity is 0.995 cP.

Evaluation of the efficiency of the insecticides and their nano-emulsion formulations against *S. litoralis*:

Tested insect: A laboratory strain of *S. litoralis*was used. The larvae were reared on fresh castor leaves and maintained at 25 ± 1°C, 75 ± 5% RH without exposure to any insecticides as mentioned by El-defrawiet al. [17].

Toxicity bioassay: Leaf dip technique was performed to evaluate the toxicity of the commercial and nano-emulsion formulations ofeach insecticide. A serial concentration of an aqueous solution of each insecticide wasprepared. Fresh castor-bean leaves were immersed into each insecticide solution for 10 s, air-dried, and then placed in petri dishes. Ten fourth-instar larvae were fed on the treated leaf, whereas control was dipped only in water and three replicates were used for each concentration. Percentage of mortalities was calculated after 48 h post treatment and the mortality was corrected using Abbott's formula [18] if needed. LC_{50}& $LC_{90,}$ sublethal concentrations (LC_{10} and LC_{25})and slope values of the tested insecticides were estimated according to Finney [19], through software computer programLdp Line ®. The efficiency of insecticides and their nano formulations were compared, referring to the most effective insecticide according to Sun equations [20].

Latent Effects of the sublethal concentrations on certain biological aspects of S. *littoralis*: Five groups of the 4^{th}instar larvae (one-day-old) 10 larvae/ group were fed for 48 h on castor bean leaves treated with two sublethal concentrations ($LC_{10}$and LC_{25}) of chlorpyrifos, methomyl and their nano emulsion formulations. After 48h, the treated leaves were replaced by fresh castor-bean leaves ones to fed the larvae for the rest of the experiment. Duration of larval and pupal stages, percentages of pupation, pupal & adult malformation and emergence of adults were recorded. The data were subjected to ANOVA analysis using Costat Statistical Software, 1990, https://www.cohort.com [21].

Residue determination:

Field trail: The field experiment was carried out at the National Research Centre farm (Al-Nobariah region, Al-Beharah governorate, Egypt) during the summer 2022.The experiment area was divided into 4 treatments; each contained 3 plots (21 m^2), each plot had 5 rows with 20 plants/row. The plots were distributed in a completely randomized blockdesign. Seedlings of pepper (*Capsicum annuum*L.) were transplanted 30 days after sowing. Tested insecticides were applied after two months of the planting date. Commercial forms were applied at the recommended rates; while nano-emulsion formulations of both tested insecticides were applied at the LC_{90} against *S.littoralis* according to the laboratory studies.

Residue analysis:

Sampling: Pepper fruit samples were collected randomly at 0 time (1 h), 1, 3, 7, 10 and 15 days after spraying. The collected samples were kept under freezing conditions (-20ºC) until analysis.

Extraction and clean-up: The QuEChERS method was used as it is the most common technique due to its simplicity, good purification efficiency, and low organic solvent and chemical consumption [22, 23]. The frozen homogenized pepper fruit (10 g) was weighed into a 50 mL centrifuge tube and 10 mL of acetonitrile (ACN) was added. Samples were extracted by vortex for 2 min after adding a piece of ceramic homogenizer to the tube. 1 g of sodium chloride and 4 g of anhydrous magnesium sulfate were added. The sample was shaken again for 30 s. After centrifugation at 5000 rpm for 5 min, 0.2 mL of the top layer ACN was 5x diluted using ACN, then vortexed for 30 s. Finally, the tubes were filtered through a 0.22 μm syringe filter for HPLC analysis.

Chromatographic analysis: Quantitative analysis of chlorpyrifos and methomyl was performed using HPLC (Thermo Ultimate 3000 system) with a quaternary pump, an automated injector, and a thermostat compartment for the column and UV detector. The chromatographic column was a C18 Zorbax XDE (25 mm x 4.6 mm. 5 µm). The column was maintained at room temperature. The flow rate of the mobile phase (methanol/deionized water = 95/5 v/v) was 0.8 mL/min, and the injection volume was 20 µL. Under these conditions, the retention times of chlorpyrifos and methomyl were 5.0 and 11.23 min, respectively.An external standard method was used. Accurately measured about 0.05 mg of chlorpyrifos and methomyl standards were transferred into a 50 ml volumetric flask and made up to volume with methanol. Concentrations of the tested insecticides were determined, and the peak areas of the standards were recorded. The slope and intercept of the calibration graph were obtained by linear regression of peak area versus concentration; $y = ax + b$, where a is the slope, b is the intercept, x is the concentration and y is the peak area.

Recovery studies were performed by spiking fresh samples without (non -treated) with 1 mL of 1 mg/L of both chlorpyrifos and methomyl standards in a solution of acetonitrile. Before homogenization, these standard solutions were added to chopped pepper samples in a blender jar. The same extraction procedure and HPLC conditions were used for recovery studies.

Half-life calculated: The residual half–lives ($t_{1/2}$) values of chlorpyrifos and methomyl were calculated using Moye et al. [24].

3. Results and Discussion

Characterization of the prepared pesticides nano-emulsion:

1. Chlorpyrifos 10% nano-emulsion

a. Particle size distribution and Zeta potential: Using a dynamic light scattering (DLS) instrument (NICOMP 380 ZLS, USA), the particle size of the prepared chlorpyrifos 10% nano-emulsion was investigated. One widely used technique for establishing out the size of the particles in a colloidal solution is the dynamic light scattering. The chlorpyrifos 10% nano-emulsion showed a size distribution ranging from 50 to 500 nm (Fig. 1a). The obtained data showed that the prepared nano-emulsion chlorpyrifos 10% had an average particle size of roughly 187.3 nm. The chlorpyrifos 10% nano-emulsion was found to have good stability against gravitational separation, as evidenced by Fig.1b where the zeta potential was around -28 mV. Accordingly, zeta potential investigations represent an important classification technique for determining the surface charge of the prepared nano-emulsion, which may be used to assess its physical stability. Because of the electrostatic repulsion of the individual particles, a high positive or negative value of zeta potential of the particles indicates strong physical stability of the nano-emulsion. It generally indicates that a zeta potential value between -30 mV and +30 mV has sufficient repulsive power to produce good physical stability for colloids. This resulted in structures that were either optically clear or somewhat muddy due to modest light scattering [25].

b. Transmission electron microscope (TEM): Transmission electron microscopy (TEM) was used for evaluating the morphology of the prepared chlorpyrifos 10% nano-emulsion.The prepared chlorpyrifos 10% nano-emulsion was spherical according to morphological analysis (**Figure 2**). Furthermore, the droplet size of the chlorpyrifos nano-emulsionwas in a range of 50–100 nm.

2. Methomyl 10% nano-emulsion:

a. Particle size distribution and Zeta potential: Dynamic light scattering (DLS) equipment (NICOMP 380 ZLS, USA) was used to measure the particle size of the prepared methomyl 10% nano-emulsion. The particle size of methomyl 10% nano-emulsion showed the size distribution starting at 30 and going up to 2000 nm **(Fig. 3a**). The obtained results showed that methomyl 10% nano-emulsion had an average particle size of roughly 336.4 nm. The zeta potential, was around -5 mV, (Fig. 3b) indicating that the prepared methomyl 10% nano-emulsion was stable enough to stand with gravity separation and produce structures that were either optically clear or somewhat muddy due to modest light scattering. The individual particles' electrostatic repulsion, a high positive or negative value of the nano-emulsion' zeta potential indicates the methomyl 10% nano-emulsion's strong physical stability. It is typically reported that a zeta potential value between -30 mV and +30 mV has sufficient repulsive power to produce good physical colloidal stability [26]. Moreover, the methomyl nano-emulsion has developed quite noticeable through effects that the nano-emulsion effectiveness of 10% methomyl was a definite technique with a high degree of stability.

b. Transmission electron microscope (TEM): The prepared methomyl 10% nano-emulsion was in spherical form according to morphological examination (Fig. 4). Furthermore, the droplet size of the methomyl 10% nano-emulsion was in the range of 20–50 nm. Small methomyl 10% nano-emulsion particles can display respectable surface area, [27], which reveals its efficacy as a decent active component to increase its efficiency and reduce the residues that may decline the adverse impacts on the environment and non-target organisms.

Table 1: Toxicity of chlorpyrifos and its nano-emulsion on 4th instar larvae of *S. littoralis*

Treatment	Lc_{10} (ppm)	Lc_{25}(ppm)	Lc_{50}(ppm)	Lc_{90} (ppm)	Slope	Toxicity index (%)*	Relative potency *
Chlorpyrifos 48%E(	2.22 (1.56 -2.79)	4.41 (3.67-25.09)	9.49 (67.36 -11.01)	40.56 (29.7 -64.20)	2.03	58.37	1
Chlorpyrifos 10% nano-emulsion	1.03 (0.47-1.54)	2.29 (1.53-2.87)	5.54 (4.69-6.86)	29.79 (18.29-156.6)	1.76	100	1.71

***Toxicity Index and Relative potency based on LC50**

Table 2: Toxicity of methomyl and its nano-emulsion on 4th instar larvae of *S. littoralis*

Treatment	Lc_{10} (ppm)	Lc_{25} (ppm)	Lc_{50} (ppm)	Lc_{90} (ppm)	Slope	Toxicity index (%) *	Relative potency *
Methomyl 90 %	29.25 (20.76-36.68)	64.55 (52.39-83.26)	155.50 (113.27-226.3)	826.47 (423.3-2808.1)	1.76	47.71	1
Methomyl 10% nano-emulsion	31.39 (23.29-37.59)	47.18 (39.94-52.69)	74.19 (68.0-81.94)	175.3 (141.7-250.3)	3.43	100	2.01

***Toxicity Index and Relative potency based on LC_{50}**

Table 3: Latent effect of sub lethal dose of chlorpyrifos and its nano-emulsion on some biological parameters of 4th larval instar of *S. littorals*

Treatment		Larval duration (day)	% Pupation	Pupal duration (day)	% Pupal malformation	% adult emergence	%adult malformation
Chlorpyrifos48%EC	LC_{10}	$11.6^a \pm 0.60$	$74.0^b \pm 5.09$	$8.0^b \pm 0.54$	$2.2^c \pm 0.97$	$59.9^{bc} \pm 4.92$	$0.0^c \pm 0.0$
	LC_{25}	$11.8^a \pm 0.80$	$60.0^c \pm 3.16$	$8.6^a \pm 0.24$	$5.0^b \pm 0.0$	$56.6^{bc} \pm 3.15$	$16.0^b \pm 1.89$
Chlorpyrifos-nano-emulsion 10%	LC_{10}	$10.8^a \pm 0.20$	$82.0^b \pm 2.00$	$8.6^a \pm 0.24$	$7.5^{bc} \pm 1.58$	$58.6^b \pm 5.58$	$13.0^b \pm 2.00$
	LC_{25}	$10.8^a \pm 0.80$	$58.0^c \pm 3.70$	$8.8^a \pm 0.20$	$19.5^a \pm 2.93$	$45.7^c \pm 1.97$	$26.6^a \pm 1.87$
Control		$10.2^a \pm 0.48$	$96.0^a \pm 2.44$	$7.2^b \pm 0.48$	$0.0^c \pm 0.0$	$91.5^a \pm 2.13$	$0.0^c \pm 0.0$
$LSD_{0.05}$		1.82	9.32	1.10	10.53	11.33	10.1

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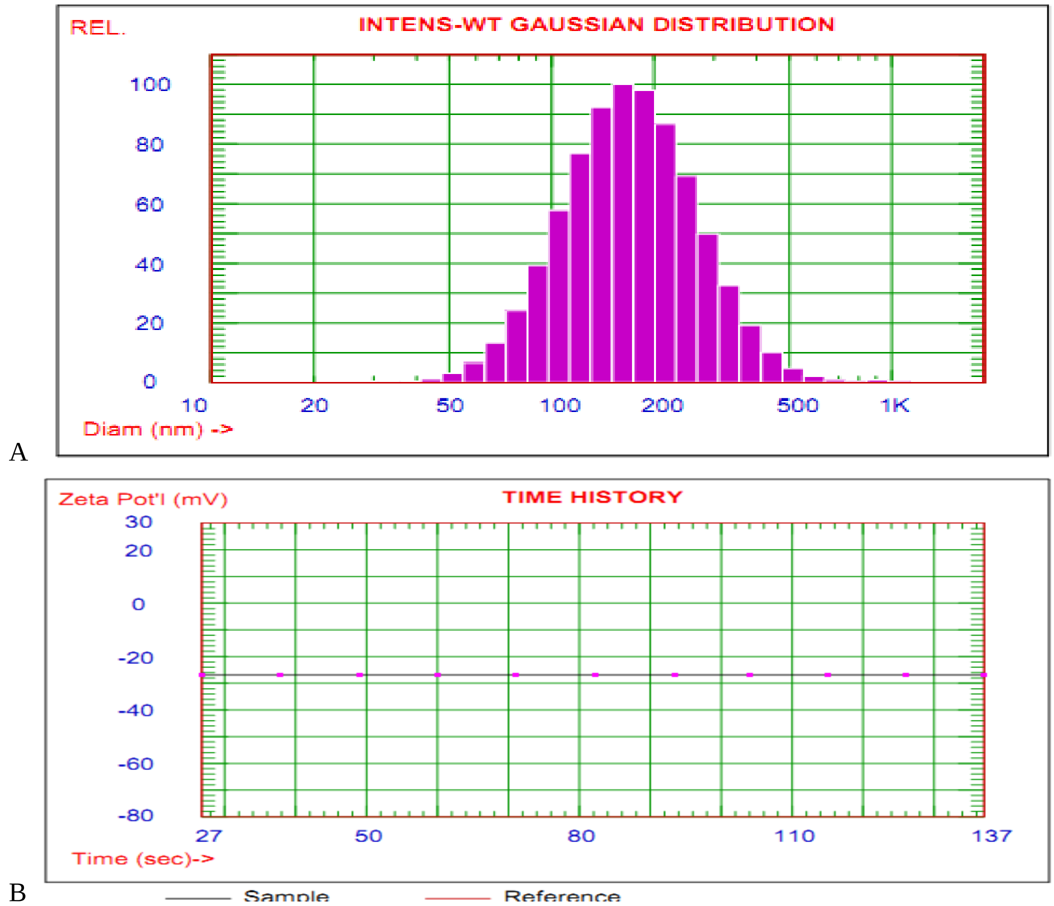

Fig. 1): Particle size distribution (A) and Zeta potential (B) of chlorpyrifos10% nano-emulsion.

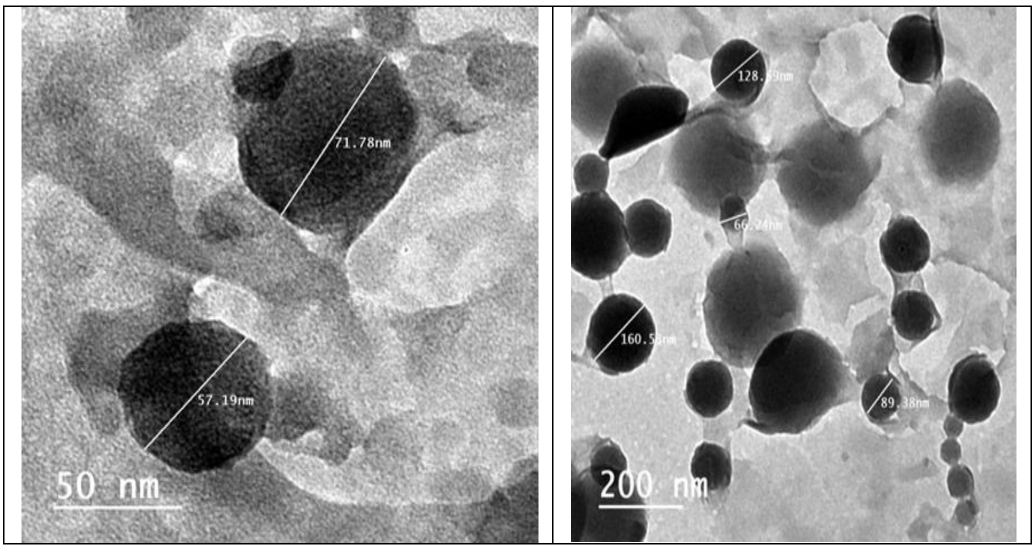

Fig. 2): TEM images of chlorpyrifos10% nano-emulsion at different magnification.

A

B

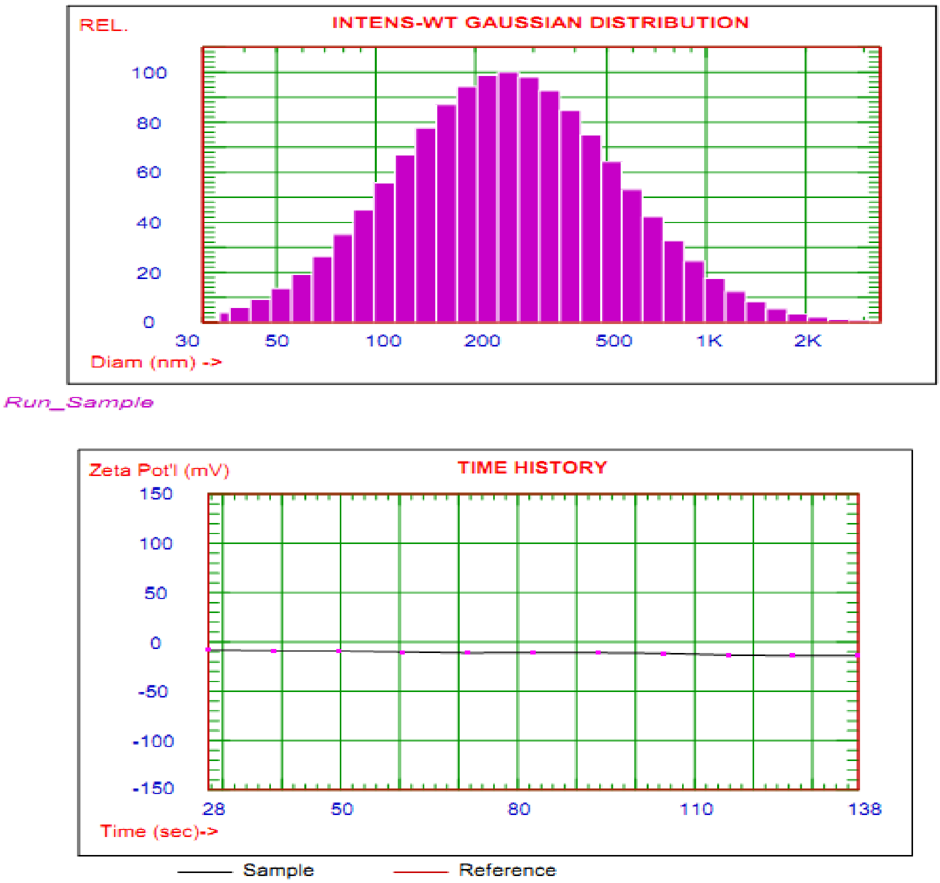

Fig (3): Particle size distribution (A) and Zeta potential (B) of methomyl 10% nano-emulsion.

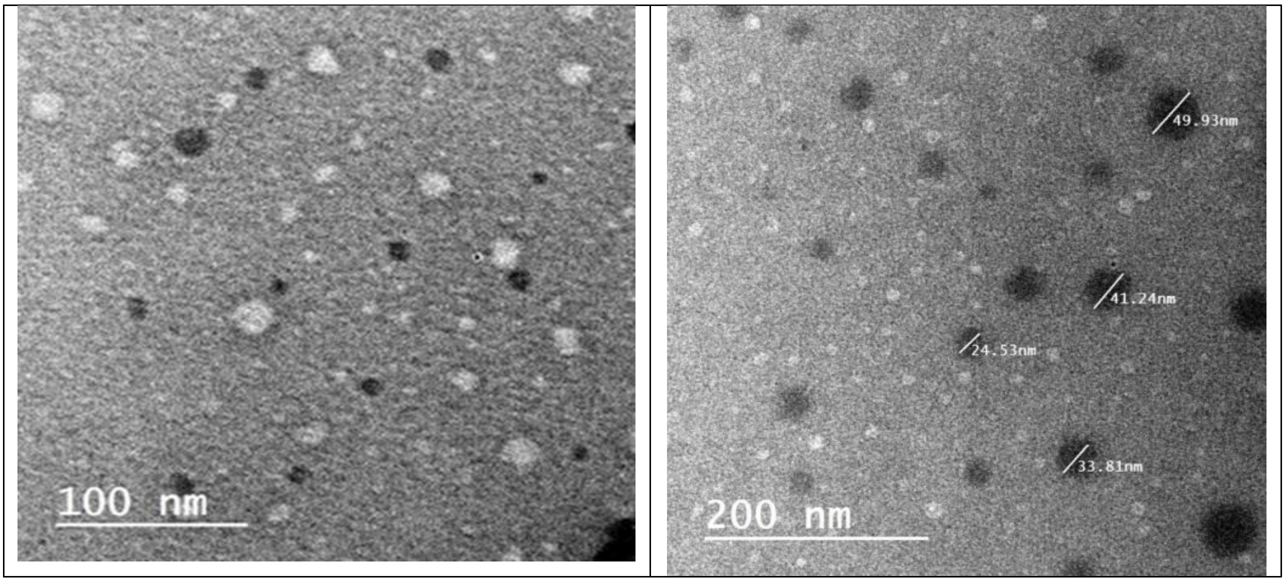

Fig (4): TEM images of methomyl 10% nano form at different (fields) magnification.

Toxicity ofinsecticides and their nano-emulsions against the 4th instar larvae of the *S. littoralis*: Efficacy of chlorpyrifos and its nano-emulsion against the 4th instar larvae of *S. littoralis*under laboratory conditions after 48 h of treatments present in Table(1). Data showed that, chlorpyrifos nano-emulsion (10%) was more toxic 1.71 times with LC_{50}value 5.54 ppm than the commercial chlorpyrifos 48% EC with LC_{50} value 9.49 ppm. The toxicity index (based on the most effective insecticide) value of the commercial chlorpyrifos 48% EC was 58.37% which confirms that the nano-emulsion formulation (100%) elevated the activity of chlorpyrifos.

Data in Tables 2 summarized the efficacy of methomyl and its nano-emulsion against the4thlarval instar of *S. littoralis*. Likewise, the methomyl 10% nano-emulsion was more effective (LC_{50}= 74.19) than methomyl 90% SP (LC_{50}=155.50 ppm). Relative potency of methomyl 10% nano-emulsion was 2.1-fold comparing with Methomyl 90% SP. Also, the calculated toxicity index of methomyl 90% SP was 47.71% referring to the high toxicity of the methomyl 10% nano-emulsion (100%).

Our results are in accordance with those obtained by Assemi et al., [28], who found that the toxicity of nano-imidacloprid against tobacco aphids was 1.84 times higher compared with the conventional imidacloprid after 72 h of exposure (LC_{50}= 37.919 and 69.623 μl/ ml, respectively). However, data obtained by Bhan et al. [29] revealed that the efficacy of non-capsulated temephos and imidacloprid against *Culex quinquefasciatus* larvae was high compared with their encapsulated (nano-forms).While, after encapsulation, there were no differences in mortality rate as a result of the slow release of lower quantities of the pesticides e.g., (0.019 and 0.003 mg/L)of 4% imidacloprid and 8% temephos formulations compared with their non-capsulated forms (0.021 and 0.004 mL/L) after 72 h of exposure. Due to the controlled slow release of nano-particles of encapsulated forms they are more economical as well as they are more eco-friendly. In addition, Memarizadeh et al. [4] reported that, the nano form of imidacloprid showed improved efficacy over the bulk imidaclopridagainst larvae of *Glyphodespyloalis,* since the LC_{50} of nano Imidaclopridagainst the 5th instar larvae decreased as the exposure time increased in a rate higher than the bulk imidacloprid. Therefore, four- and five-days post exposure,theLC_{50} values of nano- imidacloprid decreased 4.82 and 9.05 times less than the bulkform, respectively. Also, Ahmed et al. [30], reported that, lambda-cyhalothrinnano-composites was more effective against cotton leaf worm larvae compared with their conventional form. Furthermore, Sabry et al. [31]stated that, indoxacarb and imidaclopridnanoforms can be used as more effective forms against the 2ndinstar larvae of cotton leaf worm compared with the conventional formulations.

Latent effect of sublethal dose of examined pesticides on some biological parameters of 4thlarval instar of *S. littorals*: Data in Table 3 represent the latent effects of sublethal concentrations of chlorpyrifos and its nano-emulsion on some biological parameters of the 4thlarval instar of *S. littorals.* Both sub-lethal sub concentrations of the two tested formulations didn't affect the larval duration, while the sublethal doses ($LC_{10}$and $LC_{25)}$ of chlorpyrifos and chlorpyrifos nano-emulsion significantly affected the pupation percentage compared with control. The LC_{25} of chlorpyrifos and its nano-emulsion decreased the pupation percent to 60% and 58%, respectively. Whereas, LC_{10} chlorpyrifos and its nano-emulsion showed less

Table 4: Latent effect of sub lethal dose of methomyl 90% SP and its nano-emulsion on some biological parameters of 4th larval instar of *S. littorals.*

Treatment		Larval duration (day)	% Pupation	Pupal duration (day)	% Pupal malformation	% adult emergence	% adult malformation
Methomyl 90%SP	LC_{10}	$11.0^{ab}\pm0.55$	$80.0^{b}\pm3.16$	$8.2^{ab}\pm0.37$	$6.9^{b}\pm0.97$	$62.1^{b}\pm1.42$	$8.0^{d}\pm1.22$
	LC_{25}	$11.4^{a}\pm0.24$	$74.0^{bc}\pm2.44$	$8.4^{a}\pm0.24$	$7.8^{b}\pm1.18$	$61.3^{b}\pm4.10$	$18.8^{c}\pm1.98$
Methomyl 10 %nano-emulsion	LC_{10}	$11.6^{a}\pm0.24$	$72.0^{c}\pm2.00$	$8.0^{ab}\pm0.32$	$8.2^{ab}\pm1.43$	$63.3^{b}\pm6.27$	$32.7^{b}\pm3.08$
	LC_{25}	$11.8^{a}\pm.037$	$70.0^{c}\pm3.16$	$8.0^{ab}\pm0.32$	$11.4^{a}\pm1.22$	$58.8^{b}\pm5.52$	$41.0^{a}\pm3.31$
Control		$10.4^{b}\pm0.24$	$94.0^{a}\pm2.44$	$7.4^{b}\pm0.24$	$2.0^{c}\pm0.44$	$93.8^{a}\pm2.56$	$0.0^{e}\pm0.0$
$LSD_{0.05}$		0.9	6.7	0.8	3.26	16.9	6.71

The same letters in a column are not significantly different at $P < 0.05$

effectiveness on pupation % (74% and 82%) compared with the LC_{25}.

Also, the treatment of sublethal LC_{25} chlorpyrifos-nano-emulsion showed the highest pupal malformation (19.7%). All the insecticides treatment reduced the adult emergence compared with the control.The LC_{25} of chlorpyrifos-nano-emulsion showed the least percent of adult emergence (45.7%). The highest percentages of adult's malformation (26.6%) were recorded with the LC_{25}chlorpyrifos-nano-emulsion followed by the LC_{25} chlorpyrifos EC (16%), while it was (0.0 %) in the control.

The effects of sublethal concentration of methomyl and its nano-emulsion on some biological parameters against the fourth instar larvae of*S. littoralis*are presented in Table 4. All the tested sublethal concentrations of both formulations slightly prolonged the larval duration compared with the untreated insects. The pupation percentages were significantly reduced as a result of treatment with the sub lethal concentrations (LC_{10}& LC_{25})of methomyl (80% and 74%) and its nano-emulsion (72% and 70%) compared with the control (94%). Moreover, the pupation percent decrease was significantly greeter in the insect group treated with LC_{10} of methomyl nano-emulsion compared with the decrease in the insect group treated with the LC_{10} of methomyl SP formulation. Concerning to the pupal and adult malformation, all the sublethal concentrations of both nano and SP formulations of methomyl increased the malformation percentages compared with the control. The Highest percent of pupal malformation (11.4%) was recorded with the LC_{25} of methomylnano-emulsion, which is significantly higher than that recorded with the same sublethal concentration of methomyl SP (7.8%). There were significant differences between sublethal concentration of both nano and SP formulations of methomyl. In addition, the methomylnano-emulsion was more effective than the methomyl SP. Whereas, the highest percent of the adult malformation was recorded for the insect treated withLC_{25} of methomyl nano-emulsion (41%) followed by LC_{10} of methomylnano-emulsion (32.7%), LC_{25} of methomyl SP (18.8%) and LC_{10} of methomyl SP

Table 5: Methomyl residues in pepper fruits

Treatments / Periods	Methomyl 90% SP		Methomyl 10% nano-emulsion	
	Ppm	**% loss**	**ppm**	**% loss**
Initial deposits*	6.7	__	4.3	__
1 day	4.21	37.16	2.87	33.26
3 days	2.2	67.16	1.8	58.14
7 days	0.92	86.27	0.23	94.65
10 days	0.13	98.05	0.04	99.07
15 days	0.08	98.8	ND	>99.99
$t_{1/2}$(days)	1.91		2.4	
MRL**	0.04			

Initial deposits* = residues at zero time (after 1 h); $t_{1/2}$= the pesticide half-life; ND = non-detected; MRL **= maximum residue limit according to EU codex 2023

Table 6: Chlorpyrifos residues in pepper fruits

Treatments / Periods	Chlorpyrifos 48 % EC		Chlorpyrifos 10% nano-emulsion	
	Ppm	**% loss**	**ppm**	**% loss**
Initial deposits*	5.71	…….	3.76	……
1 day	3.63	36.4	2.18	42.02
3 days	2.08	63.57	1.82	51.6
7 days	1.04	81.78	0.47	87.5
10 days	0.87	84.76	0.19	94.94
15 days	0.2	96.5	0.03	99.2
$t_{1/2}$ (days)	2.38		2.03	
MRL**	0.01			

Initial deposits* = residues at zero time (after 1 h); $t_{1/2}$= the pesticide half-life; MRL**= maximum residue limit according to EU codex 2023

(8%). Also, all the sublethal concentrations of both nano and SP formulations of methomyl reduced the adult emergence comparing with control. The highly reduced was in the insect group treated with $LC_{25}$of methomyl- nano-emulsion (58.8%) while it was (93.8%) in the control.

These results are in accordance with those obtained by Hegab et al. [32], they reported that, lufenuron, pyridalyl and chlorpyrifos insecticides caused an increase in larval &pupal mortality and decreased the larval and pupal duration, as well as the larval and pupal weight and pupation% of the American bollworm.Farag et al. [33] reported that, pyridalyl and triflumuron significantly prolonged the larval duration of cotton leafworms, followed by emamectin benzoate, and profenofos. Pyridalyl and emamectin benzoate treatments resulted in significantly higher percentages of pupaemalformed. All the compounds reduced the percentage of adult emergence. Profenofos, emamectin benzoate, and pyridalyl reduced fecundity. Pyridalyland emamectin benzoate significantly prolonged the incubation period of eggs.

Pyridalyl, emamectin benzoate, and profenofos reduce egg fertility.

Residues of tested insecticides in pepper fruits: Methomyl and chlorpyrifos restudies recovery from pepper fruit samples using the QuEChERS method were 90.2 and %91, respectively. These recovery percentages in this study were satisfactory and in agreement with the data from experiments obtained by Yang et al.; Malhat et al.; Rasolonjatovo et al. [22, 23, 34].

Data in Table 5 revealed that there is a variation in the initial deposits of methomyl90% SP and methomyl 10% nano-emulsionamounts in treated pepper fruits (6.7 and 4.3 ppm, respectively); this may be referred to the difference in the applied rate of both methomyl formulations. These amounts decreased during one day post spraying to 4.21 and 2.87 ppm, respectively; indicating that the rates of loss were 37.16 and 33.26%, respectively.The residues of both methomyl formulations loss were increased as the time increased, ten days after spraying, over 98% of methomyl initial deposits were dissipated. Moreover, the amount of methomyl SP reached 0.08 ppm after 15 days, while no residue was detectedin fruits sprayed with nano-emulsion. On the other hand, the half-life time (**$t_{1/2}$)** for methomyl nano-emulsion was longer than methomyl SP formulation (2.4 and 1.9 days, respectively). Also, the initial deposit of chlorpyrifos EC and nano-emulsion amounts was 5.71 and 3.76 ppm, these values decreased after 24 h to reach 3.63 and 2.18 ppm with loss rates of 36.4 and 42.02 %, respectively.After 15 days, the lost amount of chlorpyrifos residues reached 96.5 and 99.2% of EC and nano-emulsion formulations, respectively (Table 6).

Regarding health hazards, the MRL (maximum residue limit) for methomyl on and in pepper fruits established by EU Codex [35] was 0.04 mg/kg. Accordingly, our results revealed that, the pre-harvest interval (PHI) for pepper fruits treated withmethomyl nano-emulsion was 10 days. In comparison, it took more than 15 days in the case of the methomyl conventional formulation. While the MRL of chlorpyrifos was 0.01 mg/kg; accordingly, the PHI for pepper fruits was more than 15 days in both chlorpyrifos formulations (nano-emulsion and EC). This data indicated that chlorpyrifos nano-emulsion formulation was faster degradable than conventional formulation, hence the chlorpyrifos half-life ($t_{1/2}$)was 2.38 and 2.03 days for EC and nano-formulations, respectively. These levels are affected by various factors including the applied rate, the initial deposits, the environmental factors, and the reaction between the treated surface and the applied pesticide [36, 37]. In addition, Stevens et al. [38]reported that the uptake of a pesticide is affected by the structure and formulation as well as the rate of the pesticide, the nature of the plant surface, the type of spraying equipment, and the climatic conditions, in particularly the ambient temperature during the pesticide spraying.In the same respect, data obtained by Guan et al.[13] revealed that the nano formulation of imidacloprid was faster degradable compared with the suspension concentrate of Imidacloprid in soil, while opposite data were obtained in soybean plants. The half-life time of nano and suspension Imidacloprid formulations in soil was 2.8 and 6.2 d, while its value in soybean plants was 1.9 and 4.5 d, respectively.

The results revealed that the approaches and assumptions used to evaluate conventional pesticide risks might not be applicable to nano-pesticides.

Further research is required to better understand the environmental behavior of these nano-compounds.

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Data Availability:

The data used to support the findings of this study are available from the corresponding authors upon request.

Competing interests:

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this study.

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