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Formulation nanoemulsion based commercial pyrethroid insecticides: characterization, efficiency, safety profiling, and field study

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Abstract
Commercial pyrethroid insecticides use a high dosage that causes many side effects and accumulates as well. Nanoformulation has a promising efficacy in solving such fatal problems. Four commercial pyrethroid insecticides (Axon, Spanner, Cyperco, and Karilot) were formulated into nanoemulsions using the two-phase method. Nanoemulsion stability was checked and characterized using DLS and transmission electron microscopy (TEM) and tested for their physicochemical properties. The insecticidal efficiency of both the commercial and nanoemulsion insecticides was evaluated against *Spodoptera littoralis* under laboratory and field conditions. The cytotoxicity of the most effective commercial and nanoemulsion insecticides was also checked on normal human skin cells (BJ1). The nanoemulsions demonstrated good stability and had mean droplet sizes ranging from 166.7 to 221.7 nm. The nanoemulsions also exhibited higher insecticidal activity against *Spodoptera littoralis*, with lower LC_{50} (166.93–226.24 mg/L) and LC_{90} (284.11–336.65 mg/L) values and a higher percentage of decrease in the larval population (77.5–86.5%) than the commercial insecticides. The nanoformulations of Axon and Spanner were the most effective insecticides. The commercial and nanoformulations of Axon did not harm normal human cells at concentrations below 500 µg/mL. The study demonstrates that nano insecticides can be considered as potential replacements for conventional insecticides. Indeed, this study highlights the potential of nano-insecticides as efficient and safer alternatives to conventional formulations for pest management.

*Keywords***:** Nanoemulsions; Pyrethroid insecticides; cotton leafworm; Insecticidal efficiency; Cytotoxicity; Cell viability.

1. Introduction

Insect pests are one of the major threats to agricultural production and food security worldwide. Among the insect pests is the cotton leafworm, *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae) is a polyphagous and cosmopolitan pest that causes significant damage to various crops, especially cotton (*Gossypium* spp.) [1]. This pest originated in Egypt and is currently found in Africa, the Canary Islands, the Middle East, and parts of Mediterranean Europe [2]. *S. littoralis* can feed on more than 40 plant families, including economically important crops such as cotton, soybean, maize, tomato, potato, and legumes [3]. *S. littoralis* larvae feed voraciously on the leaves, this affects plant growth and often leads to bolls wilting and falling. They also damage the plants by eating the buds and flowers and boring into the bolls which leads to reduced yield and quality [4]. The pest has a high reproductive potential and can develop resistance to insecticides, making its management challenging [1, 5].

Pyrethroids are synthetic analogs of natural pyrethrins extracted from the chrysanthemum plant's flowers. Pyrethroids act on the sodium channels of the insect nerve cells, causing paralysis and death [6]. So far, synthetic pyrethroids play an important role in controlling *S. littoralis* [7]. The global usage of pyrethroids has been estimated to be 6.45 billion dollars in 2021 [8]. Overusing conventional pesticides led to environmental pollution, human

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health hazards, and the development of insecticide resistance [9]. Therefore, there is a need to develop novel and eco-friendly insecticides for the management of *S. littoralis* and other insect pests.

One of the promising approaches to enhance the efficacy and safety of insecticides is the use of nanotechnology. Nanotechnology is a promising field that offers new opportunities and benefits for various applications, including pest control [10]. Nanotechnology can help to improve the efficiency, specificity, and safety of pest control agents by using nanoparticles, nanocapsules, nanocrystals, and other nanomaterials that can deliver, enhance, or modify the activity of insecticides, biopesticides, pheromones, and other pest control agents [11]. It can also help to reduce environmental pollution and the adverse effects of pest control agents on non-target organisms, humans, and soil fertility by providing controlled or delayed release, targeted delivery, and lower doses of the active ingredients [12]. Nanoemulsions can be used for low water-soluble compounds, such as pesticides [13]. It's easy to formulate, handle, and obtain at a relatively low cost [8]. Nanoemulsions typically exhibit better stability to gravitational separation and droplet aggregation (flocculation and coalescence) than microemulsions or conventional emulsions. They also flow better than conventional emulsions [14]. These advantages make nanoemulsions a desirable system for many industrial applications. Nanoemulsions are also called mini or ultrafine emulsions with small droplet sizes. They are typically characterized by a size range of less than 500 nm. However, various size ranges have been reported in the literature, including ultrafine nanoemulsions (less than 10 nm), fine nanoemulsions (10–100 nm), and coarse nanoemulsions (100–500 nm) [15]. One of the primary challenges in formulating nanoemulsions is maintaining their stability over time. Nanoemulsions can be susceptible to factors like temperature, pH, and ionic strength, leading to phase separation or changes in droplet size [16]. Specifically for pyrethroids, which can degrade under certain environmental conditions, ensuring the long-term stability of the formulation is critical for effective pest management [17]. Nanoemulsions can be prepared using high- and low-energy techniques. The latter is influenced by the behavior and properties of the constituents, where the system's stored energy is utilized to form nanodroplets [18]. Several studies have reported preparing and characterizing nano insecticides using different active ingredients, such as pyrethroids, organophosphorus compounds, neonicotinoids, avermectins, etc. [19-22].

The cytotoxicity of insecticides on normal cells is a subject of critical concern in the field of pesticide research and regulation. Insecticides are designed to target pests by disrupting their nervous systems or metabolic processes, but there is an inherent risk of their potential harm to non-target organisms, including humans [23]. Several studies have been conducted to understand the specific mechanisms through which insecticides exert cytotoxic effects on normal cells [24-26]. The cytotoxicity of pyrethroids to human cells was evaluated using different cell lines, such as hepatocytes, lymphocytes, keratinocytes, fibroblasts, and neurons [27-29]

This study aimed to prepare and characterize nanoformulations of four conventional insecticides (Axon, Spanner, Cyperco, and Karilot) and evaluate the insecticidal efficiency of these insecticides against the cotton leafworm, *S. littoralis*, under laboratory and field conditions. The cytotoxicity of the most efficient commercial and nano insecticide was also investigated.

2. Materials and Methods

2.1. Rearing the cotton leafworm

The study used a homogenous and susceptible strain of *S. littoralis*, a laboratory strain of the cotton leafworm. The strain was grown on castor leaves (from the Faculty of Agriculture farm, Cairo University) in a controlled environment with a temperature of 25 ± 2 ºC and a relative humidity of 65 ± 5%. The strain was not exposed to any chemicals before the study.

2.2. Preparation of insecticide nanoemulsions

Four commercial pyrethroid insecticides (Table 1) were converted to nanoemulsions according to Elnabi et al ., 2021 [8] . The nanoemulsions consisted of two phases: organic and aqueous. The organic phase contained 10 mL toluene, 10 mL insecticides, and 1 mL butanol. The aqueous phase contained 9 mL of tween 80 and 70 mL water. The organic phase was added drop by drop to the aqueous phase while stirring at 4000 rpm for 30 min. Then, the nanoemulsions were made by ultrasonic for 15 min, using 50% ultrasonicate power (20 kHz) and 7 cycles/sec pulses. The temperature change from the first coarse emulsion to the final emulsion was less than 25 °C.

Table 1. List of insecticides with their trade names, common name, IRAC classification, and their producers.

Trade names	Common name	Manufacturer	IRAC MoA
Axon 5% EC	Lambda-cyhalothrin	Jiangsu Zhongqing Agrochemical - China	3A
Spanner 4.9% CS	Lambda-cyhalothrin	Haihang Industry Co., Ltd. China	3A
Cyperco 20% EC	Cypermethrin	UPL Ltd., - India	3A
Karilot El Nasr 2.5% EC	Lambda-cyhalothrin	El-Nasr Co. for Intermediate chemical - Egypt	3A

2.3. Characterizations of insecticide nanoemulsions

2.3.1. Centrifugation test

The samples were centrifuged (50 mL) at 5000 rpm for 30 min and checked for phase separation, creaming, or cracking. Nanoemulsions should be very stable, with no phase separation. The measurements were done in triplicate.

2.3.2. Freeze-thaw cycle

The test exposed the formulation to quick and extreme temperature changes that it could face during regular handling without affecting its physical properties. The formulation was kept at -21 °C for a day and then at 21 °C until it melted for another day. The separation, or creaming layer, was checked. The measurements were done three times.

2.3.3. Heating and cooling tests

The nanoemulsions were stored at 4 and 40 °C for two days each to test their stability at different temperatures. The ones that remained stable were further studied.

2.3.4. Stability at a temperature of 25 °C

The nanoemulsions of 25 mL were moved to a glass tube and kept at 25 °C. The change from stability to aggregation and cohesion was monitored for four months.

2.3.5. Droplet Diameter and Size Distribution Analysis

The dynamic light scattering instrument (Santa Barbara, CA, USA) was utilized to ascertain the average diameter, size distribution, and zeta potential of the final nanoemulsions. This was done at 23 °C, with the incident light being the 632.8 nm line of a HeNe laser at an angle of 13.9°.

2.3.6. Examining the nanoemulsions with TEM

The stable nanoemulsions were studied by TEM (JEM-1230, JEOL, Tokyo, Japan) with specifications as a resolution up to 0.2nm resolution. - Uses a tungsten filament for beam generation - Uses HT voltage up to 120kV. The nanoemulsion was diluted with deionized water and placed on a carbon-coated copper grid. Phosphotungstic acid was used as a stain for 1 min. The sample was dried at room temperature, observed, and photographed with the TEM.

2.4. Insecticidal efficiency of the tested insecticides against *Spodoptera littoralis*

2.4.1. Laboratory experiments

The leaf-dipping method was used, where castor leaves were dipped in different insecticide solutions (100–850 mg/L) for 10 sec and then dried for 2 min at ambient temperature. The control treatment was water only. The leaves were given to newly molted 4th instar larvae of *S. littoralis*. Each concentration and the control had five replicates. The mortality was measured 48 h after treatment. Abbott's formula [30] was used to calculate the corrected mortality percentages. The LC_{50} and LC_{90} and their slope values were determined by Probit analysis of the mortality data based on the regression lines [31].

2.4.2. Field experiments

The field experiments were done in Kerdasa village, Giza Governorate, Egypt, in the 2021 and 2022 cultivation seasons. The experiment area was 1/2 feddan. (2100 m^2) split into 4 equal blocks. Each block had 9 replicates (58 m^2 each). The LC_{90} values that were calculated before were multiplied by three and used to spray each compound. The control group received only water. A knapsack sprayer (CP-3) with one nozzle was used. The spray solution was applied at 200 liters per feddan. Before spraying, larvae were counted at five points (four corners and one center) along one meter on each plot. This was repeated after 1, 3, 5, 7, 10, and 14 d of spraying. Henderson and Tilton [32] equation was used to estimate the reduction percentages of the *S. littoralis* population.

2.5. Cytotoxicity on human cells

The cytotoxic activity test (*in vitro* bioassay on human tumor cell lines) of the most potent commercial and nanoemulsion insecticides was done and measured by the Bioassay-Cell Culture Laboratory, National Research Centre, Dokki, Egypt. The two tested compounds were checked for their cytocompatibility with the normal cell line, BJ1 (normal skin fibroblast) [33], using the MTT procedure. Cells were cultured in a mixture of DMEM-F12 medium supplemented with 1% antibiotic-antimycotic solution (10,000 U/mL

penicillin, 10,000 µg/mL streptomycin, and 25 µg/mL amphotericin B) and 1% L-glutamine. The culture was maintained at 37 °C under 5% CO_2 for 10 days. After this period, cells were seeded in a new complete growth medium at 10,000 cells per well in a 96-well plate. The plate was incubated for 24 hours at 37 °C under 5% CO_2 in a water-jacketed carbon dioxide incubator. Subsequently, the medium was removed, and fresh serum-free medium was added. Cells were then treated with different sample concentrations (62.5, 125, 250, 500, and 1000 µg/mL) or left untreated as a negative control. After 48 hours of incubation, the medium was again aspirated, and 40 µL of MTT solution (2.5 µg/mL) was added to each well. The plate was incubated for another 4 h at 37 °C under 5% CO_2 [34]. Finally, the absorbance was measured at 595 nm with a reference wavelength of 620 nm using a microplate reader. The viable cells' percentage was calculated using the following equation:

$$\text{Viability \%} = \frac{\text{Tested O.D}}{\text{Control O.D}} \times 100$$

Where: the tested O.D. is the obtained absorbance of the sample, and the control O.D. is the obtained absorbance of the control.

2.6. Data analysis

The concentration–mortality data of the laboratory evaluation was analysed by Probit analysis to get the LC_{50} and LC_{90} values using the SPSS 27.0 program. Non-overlapping 95% confidence intervals suggest a statistically significant difference between the estimated LC_{50} and LC_{90} values. The field experiment used a randomized complete block design. The mortality data were transformed by arcsine before the analysis to make the variance homogeneous. A one-way ANOVA with Duncan's multiple range test (α = 0.05) was used to assess the significance between mean values. The Shapiro-Wilk and Levene tests checked the variances' normality and homogeneity, respectively.

3. Results and discussion

3.1. Physical and chemical characteristics of nanoemulsions

The results in Table 2 display the thermodynamic stability of the examined insecticide nanoemulsions (5000 rpm centrifugation, 25°C storage, heating cycle, cooling cycle, and freeze-thaw cycle). The results demonstrated that all nanoemulsions passed the centrifugation investigation. The nanoemulsions were stable for alterations in their physical characteristics at 25 °C for up to three months. The heating and cooling test results confirmed that all samples were stable by keeping a homogenous state.

The size distribution of nanoparticles within the prepared nanoemulsions, obtained through dynamic light scattering, is visualized in Figure 1. Results demonstrated that the mean droplet size diameter was 166.7, 189.5, 221.7, and 172.4 nm for the insecticide nano forms of Axon, Cyperco, Karilot, and Spaner, respectively. Moreover, the PDI ranged from 0.07 to 0.259. The zeta potential of the estimated nanoemulsions was highly negative. They extended between -32.5 and -36.9 (Figure 2).

TEM of insecticide nanoemulsions is exhibited in Figure 3. The mean droplet sizes of the insecticide nanoemulsions were 117.35 nm for Axon Nano, 122.91 nm for Cyperco Nano, 160.03 nm for Karilot nano, and 135.08 nm for Spaner Nano.

Table 2. Thermodynamic stability of the tested insecticide nanoemulsions.

Nano-insecticides	Stability after centrifugation at 5000 rpm	Stability at room temperature				Freeze thaw cycles	Heating-cooling cycle
		30 days	60 days	90 days	120 days		
Axon	√	√	√	√	√	√	√
Spanner	√	√	√	√	√	√	√
Cyperco	√	√	√	√	√	√	√
Karilot El Nasr	√	√	√	√	√	√	√

√ it means passed the test

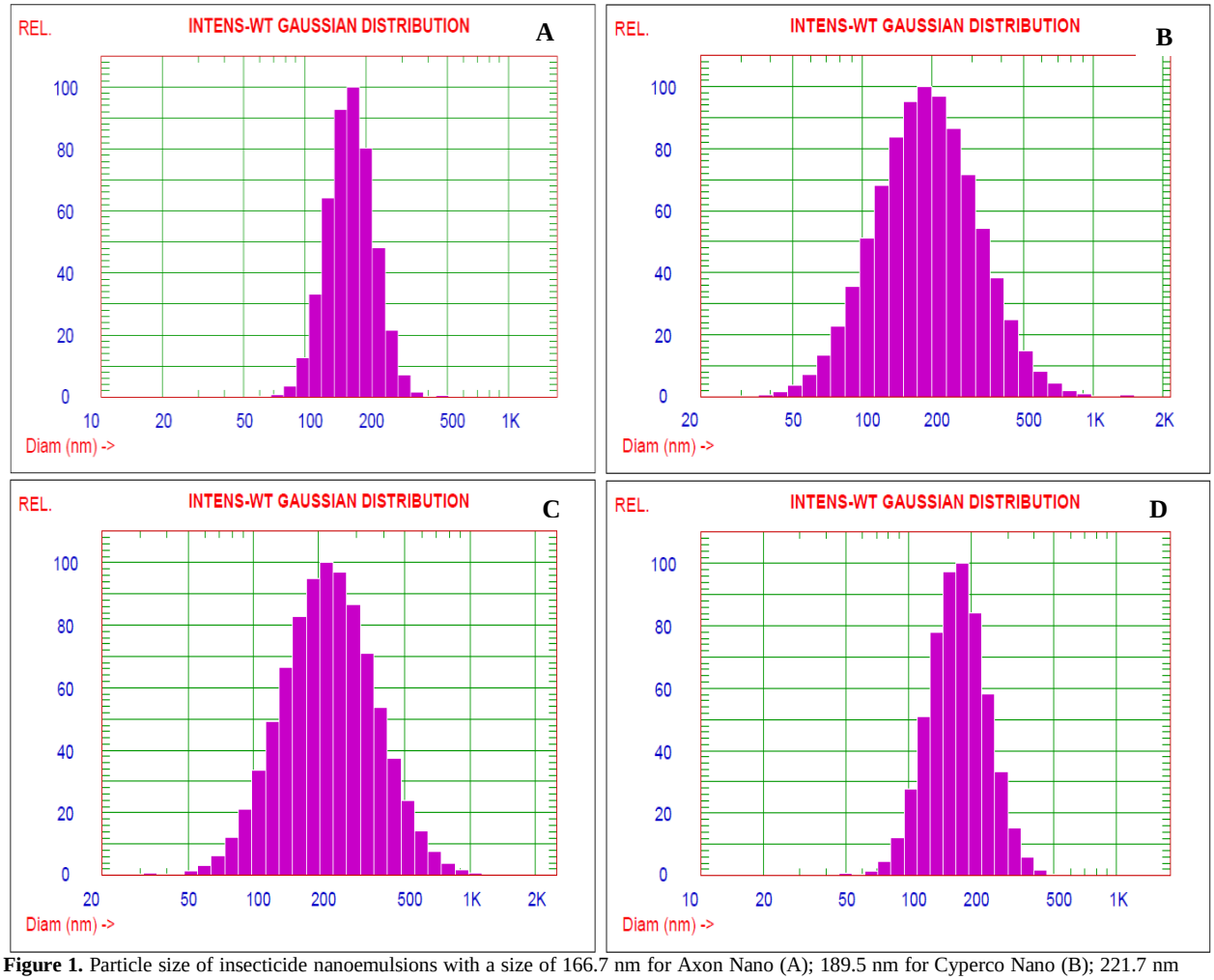

Figure 1. Particle size of insecticide nanoemulsions with a size of 166.7 nm for Axon Nano (A); 189.5 nm for Cyperco Nano (B); 221.7 nm for Karilot Nano (C); 172.4 nm for Spaner Nano (D). The nano formulations showed PDI values of 0.259, 0.104, 0.070, and 0.250 nm for Axon, Cyperco, Karilot, and Spanner, respectively.

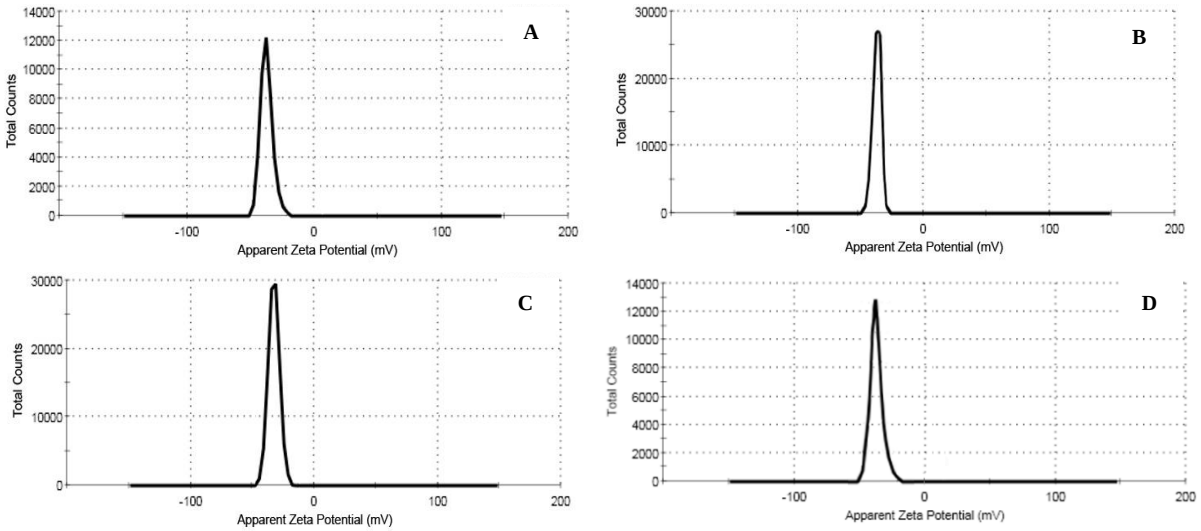

Figure 2. Zeta potential of insecticide nanoemulsions with values of -35.5 for Axon Nano (A); -36.9 nm for Cyperco Nano (B); -32.5 nm for Karilot Nano (C); -34 nm for Spaner Nano (D).

Nanoemulsions have been addressed with great concern for their characteristic features in several sectors, such as long-term kinetic stability [35], transparent or translucent appearance [19], and gravitational sedimentation or creaming compared to conventional emulsions. Previous studies have successfully prepared and characterized different pyrethroid nanoemulsions, notably alpha-cypermethrin, deltamethrin, lambda-cyhalothrin, and permethrin [36, 37]. The current research revealed that the prepared insecticide nanoemulsions exhibited good stability under various conditions, including centrifugation, storage at 25 °C, heating and cooling cycles, and freeze-thaw cycles. This indicates that the nanoemulsions are physically and chemically stable. The droplet size of the prepared nanoemulsions indicated that the nanoformulations had a narrow particle size distribution and a mean droplet size of less than 200 nm, which is consistent with previous studies on nanoemulsions. According to Mishra, et al., the average size of permethrin nanoparticles was 175.3 nm. Similarly, four pyrethroid nanoemulsions (alpha-cypermethrin, deltamethrin, lambda-cyhalothrin, and permethrin) were prepared by, and their mean droplet diameters varied from 72.00 to 172.00 nm. PDI is an indication of the particle size distribution of a nanoemulsion. The present research showed that the PDI values were relatively low. These low values imply that the droplets in the nanoemulsions have a small polydispersity index, which is evidence of the stability of the formulations. Values of PDI greater than 0. 3 suggest that there is a lower level of homogeneity of the droplet nanoemulsions [38]. In the present study, the zeta potential of the nanoparticles was found to be less than −30 mV. A zeta potential below −30 ensures a stable system and prevents the formation of large particles [39, 40].

The TEM images showed that the nano droplets had a spherical shape and a smooth surface, which is desirable for enhancing the penetration and adhesion of the insecticides to the insect cuticle.

3.2. Insecticidal efficiency against *S. littoralis*

The toxic effects of the commercial insecticides and their nano forms are shown as LC_{50} and LC_{90} in Table 3. All tested insecticides exhibited statistically significant differences in LC_{50} and LC_{90} values, indicating variations in their effectiveness against *S. littoralis*. Notably, the nano formulations demonstrated lower LC_{50} and LC_{90} values than their commercial counterparts. The LC_{50} values for the nano formulations ranged from 166.93 to 226.24 mg/L, while the LC_{50} values for the commercial insecticides ranged from 251.49 to 395.75 mg/L. Similarly, the LC_{90} values for the nano formulations ranged from 284.11 to 336.65 mg/L, while the LC_{90} values for the commercial insecticides ranged from 369.12 to 561.83 mg/L. Axon Nano demonstrated the highest efficacy with LC_{50} of 166.93 mg/L, while Karilot commercial insecticide displayed the highest LC_{50} and LC_{90} values, translating to the lowest toxicity towards *S. littoralis* larvae. The percentage of increased insecticidal efficiency for Axon, Spanner, Cyperco, and Karilot nano forms was 33.62, 35.20, 41.28, and 42.83%, respectively, compared to their counterpart commercial insecticides.

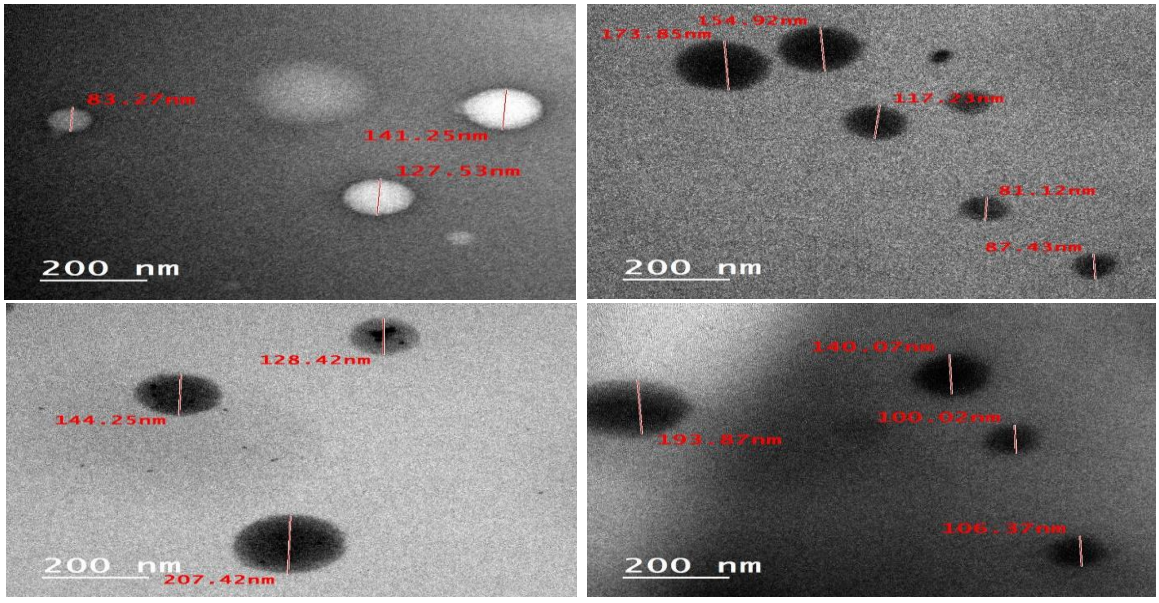

Figure 3. Transmission electron micrograph (TEM) of insecticide nanoemulsions: (A) Axon Nano; (B) Cyperco Nano; (C) Karilot Nano; (D) Spaner Nano.

Table 3. Toxicity of the tested insecticides against *Spodoptera littoralis* larvae.

Insecticides	LC_{50} (mg/L)[a]	95% Confidence limits (mg/L)	LC_{90} (mg/L)[b]	95% Confidence limits (mg/L)	Slope ± (SE)[c]	Intercept ± (SE)[d]	$(\chi^2)^e$
Axon	251.49	240.44-261.87	369.12	345.07-406.39	7.69±0.80	-18.46 ± 194	0.40
Spanner	289.01	274.24-301.49	432.43	407.97-468.83	7.32±0.74	-18.02 ± 1.86	0.57
Cyperco	329.08	309.90-346.54	527.68	488.81-586.74	6.25±0.63	-15.73 ± 1.61	1.52
Karilot	395.75	377.83-411.21	561.83	529.84-611.66	8.42±0.93	-21.8 7± 2.45	2.44
Axon Nano	166.93	156.26-176.79	284.11	259.90-322.15	5.55±0.57	-12.33 ± 1.30	1.34
Spanner Nano	187.27	173.46-199.18	336.65	309.41-378.40	5.03±0.51	-11.43 ± 1.19	1.06
Cyperco Nano	193.23	181.25-204.08	323.76	295.88-369.38	5.72±0.63	-13.07 ± 1.48	0.03
Karilot Nano	226.24	170.12-257.61	330.84	281.71-727.06	7.77±0.90	-18.28 ± 2.16	4.04

[a]concentration triggering 50% mortalities; [b]concentration triggering 90% mortalities; [c]slope of concentration fatality regression line; [d]intercept of regression line; [e]chi square value

Data in Table 4 presents the results of a field study evaluating the efficacy of commercial insecticides (Axon, Cyperco, Karilot, and Spanner) and their nanoformulations at different time intervals against *S. littoralis* during season 2021. Both commercial and nanoformulations exhibited significant insecticidal activity against *S. littoralis* compared to the control.

Table 4. Effect of insecticides and their nano form against *Spodoptera littoralis* in the cotton field during 2021.

Treatments	Population before spraying ± SE[a]	Percentage of reduction ± SE at different days					GP ± SE[b]	GA[c]
		3	5	7	10	14		
Axon	23.3± 0.7a	96.3±0.1c	86.1±1.8bcd	82.2±0.3bc	72.5±1.3bcd	56.0±3.5bc	7.2±0.2cd	77.2±0.8cd
Cyperco	22.7± 1.2a	95.1±1.0c	82.1±1.2de	78.5±2.1cd	66.6±1.8de	51.2±0.6cd	8.3±0.5c	73.1±0.7ef
Karilot	24.7± 0.3a	94.2±1.2c	80.1±1.8e	75.1± 2.9d	63.8±2.0e	45.4±4.2d	10.0±0.1b	70.0±1.2f
Spanner	22.3± 0.7a	96.2±0.1c	83.0±0.7cde	79.1±1.4cd	69.1±1.9cde	53.2±3.3cd	7.7±0.2c	74.6±0.9de
Axon Nano	22.7± 1.2a	100.0±0.0a	94.0±1.1a	91.3±1.1a	81.9±1.3a	68.9±3.4a	4.3±0.47f	86.1±1.3a
Cyperco Nano	23.3± 0.9a	98.7±1.3ab	88.3±1.4b	85.1±2.7b	76.2±3.9ab	60.6±2.8abc	6.1±0.4de	80.5±2.0bc
Karilot Nano	25.0± 0.6a	97.7±1.1bc	87.0±1.4bc	84.4±0.5b	74.3±1.5bc	58.2±2.0bc	7.1±0.2cd	79.0±0.4c
Spanner Nano	24.7± 0.9a	8.9±1.1ab	90.2±1.6b	86.2±0.2b	78.5±2.0ab	65.2±3.5ab	5.8±0.1e	82.6±0.7b
Control	24.0± 0.6a	0.0± 0.0d	0.0± 0.0f	0.0±0.0e	0.0± 0.0f	0.0±0.0e	32.5±0.8a	0.0±0.0g
F value	1.44	159.24	394.37	343.70	228.08	98.43	462.59	779.01
P value	0.24	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[a]Mean number of live larvae per plant ± standard error (SE), [b]Mean general population after spraying, [c]Mean general reduction.
Means with the same letter within a column are not statistically different at $P < 0.05$, df = 8.

The nanoformulations generally outperformed their commercial counterparts in terms of insecticidal efficacy. The general reduction in the larval population ranged from 79 to 86.1% for the nanoformulations and 70 to 77.2% for the commercial insecticides. Axon Nano achieved the highest percentage of reduction in the larval population and general reduction at all time intervals. The general reduction of Axon Nano was 86.1%, followed by 82.61, 80.46, and 78.96% for Spanner Nano, Cyperco Nano, and Karilot Nano, respectively.

The efficacy of commercial insecticides and their nano formulations against the cotton leafworm, *S. littoralis*, in cotton fields during 2022 is presented in Table 5. Data were similar to season 2021, as nano formulations generally outperformed their commercial counterparts in terms of insecticidal efficiency. Axon Nano and Spanner Nano consistently exhibited high percentages of reduction in the larval population and general reduction across different time intervals. The general reduction in the larval population ranged from 77.5% to 86.5% for the nano formulations and 64.9% to 73.4 for the commercial insecticides.

Table 5. Effect of insecticides and their nano form against *Spodoptera littoralis* in cotton field during 2022.

Treatments	Population before spraying ± SE[a]	Percentage of reduction ± SE at different days					GP ± SE[b]	GA[c]
		3	5	7	10	14		
Axon	21.7±0.9a	97.6±1.2abc	87.3±0.7d	74.8±2.0bcd	65.6±2.0de	53.5±1.1cd	8.5±0.4c	73.4±1.1cd
Cyperco	20.7±0.7a	95.9±0.2bc	81.6±2.5e	70.1±3.1de	59.8±2.5ef	49.2±4.7de	9.5±0.4c	69.0±2.4de
Karilot	20.7±0.9a	94.5±1.7c	78.1±1.6e	68.0±2.4e	54.9±1.9f	42.3±2.7e	10.7±0.4b	64.9±1.5e
Spanner	20.0±0.6a	97.1±1.4abc	82.3±1.5e	72.6±2.6cde	62.7±2.6e	51.3±3.3de	8.6±0.4c	70.9±2.1d
Axon Nano	21.0±0.6a	100.0±0.0a	96.4±0.1a	88.1±1.2a	83.3±1.8a	71.9±3.0a	4.2±0.3e	86.5±1.2a
Cyperco Nano	22.0±1.0a	98.7±1.3ab	91.9±1.5bc	79.3±0.8b	72.7±0.9bc	63.8±3.6ab	6.7±0.1d	79.5±1.1b
Karilot Nano	20.3±0.9a	97.2±1.4abc	90.2±0.9cd	76.7±2.3bc	70.7±2.2cd	62.0±2.3bc	6.8±0.5d	77.5±0.6bc
Spanner Nano	19.7±0.3a	100.0±0.0a	93.6±1.4b	80.3±1.5b	76.8±2.6b	66.2±3.7ab	5.3±0.2e	81.6±1.5b
Control	20.7±0.3a	0.0±0.0d	0.0±0.0f	0.0±0.0f	0.0±0.0g	0.0±0.0f	30.7±0.4a	0.0±0.0f
F value	1.06	113.54	425.70	248.30	265.70	94.42	433.55	466.28
P value	0.432	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[a]Mean number of live larvae per plant ± standard error (SE), [b]Mean general population after spraying, [c]Mean general reduction.
Means with the same letter within a column are not statistically different at P < 0.05, df = 8.

It's noteworthy that the confidence intervals for the LC_{50} and LC_{90} values are relatively narrow, indicating good precision in the toxicity estimates. However, Karilot Nano shows a notably wider confidence interval for its LC_{90} value, suggesting greater variability in its effects at higher concentrations. Wheeler, et al. [42]Wheeler, Park and Bailer [41] stated that a narrower confidence interval indicates more precise estimates of the LC_{50}. The chi-square (χ^2) values of the tested insecticides were generally low. This suggests a good fit of the data to the probit model used for analysis, lending credibility to the derived toxicity estimates.

The obtained results were consistent with Badawy, et al. [21]Badawy, Abd-Elnabi and Saad [21], who stated that nanoemulsions of organophosphorus compounds (chlorpyrifos-methyl, diazinon, and malathion) showed enhanced insecticidal activity against the larvae of the cotton leafworm (*S. littoralis*) compared to conventional formulations. Similarly, Taktak, Badawy, Awad, Abou El-Ela and Abdallah [42] found that the prepared pyrethroid nanoemulsions enhanced their mosquitocidal efficacy, showing significantly higher toxicity against *Culex pipiens* L. (Diptera: Culicidae) larvae compared to commercial formulations. In the same trend, Shoaib, et al. [43]Shoaib, Waqas, Elabasy, Cheng, Zhang and Shi [43] mentioned that emamectin benzoate nanoformulation showed higher effectiveness against the third instar larvae of *Plutella xylostella* (LC_{50} = 0.18 mg L^{-1}) compared to conventional emamectin benzoate (LC_{50} = 11.06 mg L^{-1}). The insecticidal efficacy of the field trials of both commercial insecticides and nano formulations appeared to be lower in 2022 compared to 2021. This could be attributed to factors such as variations in environmental conditions or pest resistance. Yang, et al. [20]Yang, Tang, Yu, Xue, Li, Rong, He and Qian [20] indicated that fenpropathrin nanoemulsion droplets' wettability and adhesion ability were better than those of conventional fenpropathrin EC on the biological targets. The results also revealed that the penetration performance of the insecticide nanoemulsion to *Tetranychus cinnabarinus* Boisd. (Acari: Tetranychidae) was 4–6 times higher than that of EC. Further, fenpropathrin nanoemulsion exhibited higher biological activity on *T. cinnabarinus.* Badawy, et al. [44]Badawy, Saad, Tayeb, Mohammed and Abd-Elnabi [44] mentioned that nanoemulsions applied at the nanoscale have high efficiency in pest control with low environmental risk. The nano formulations in the present research achieved higher reduction percentages at different time intervals post-spraying. These findings suggest that nanotechnology can enhance the efficacy of insecticides by increasing their potency, durability, and solubility. Nano insecticides can also reduce the amount of active ingredients needed and minimize the environmental and health risks associated with conventional insecticides [45, 46].

The improved insecticidal activity of the nano formulations can be attributed to several factors, such as the increased surface area and solubility of the active ingredients, the reduced degradation and evaporation of the insecticides, the enhanced penetration and retention of the nano droplets on the insect cuticle [40, 47].

3.3. Cytotoxicity on normal human cells

An *in vitro* evaluation of cytotoxicity is presented in Figure 4. The cytotoxicity assay results showed that both the commercial and nano forms of Axon insecticide caused a slight decrease in cell viability at higher concentrations (above 500 µg/mL). Commercial Axon and Axon nano caused a reduction in cell viability of approximately 20% at the highest concentration tested of 1000 µg/mL. The LC_{50} values of the commercial and nanoformulations of Axon against *S. littoralis* had no cytotoxicity on normal skin fibroblasts.

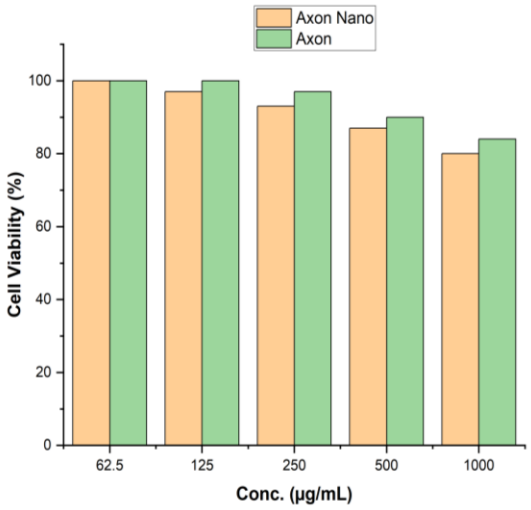

Figure 4. Cytotoxicity assay for Axon insecticide (commercial and nano form).

4. Conclusion

This study showed that the nanoformulations of four insecticides (Axon, Spanner, Cyperco, and Karilot) had superior physical and chemical characteristics and insecticidal efficiency compared to their commercial counterparts against the cotton leafworm, *S. littoralis*. The nano formulations of Axon and Spanner were the most effective insecticides. The commercial and nano formulations of Axon had slight cytotoxicity at higher concentrations. The results have important implications for developing and using nano insecticides for pest management in cotton crops. Nano insecticides may offer several advantages over conventional insecticides, such as lower dosage, reduced environmental impact, and increased safety for humans and non-target organisms. The nanoformulations of the insecticides can be considered promising alternatives to conventional insecticides for controlling *S. littoralis* and other insect pests. Further research is needed to investigate pyrethroid nanoemulsion insecticides' environmental fate and behavior, such as their degradation, bioaccumulation, and ecotoxicity, in different aquatic and terrestrial ecosystems.

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6. Declaration of competing interest

The authors declare that there is no conflict of interest.

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