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Synthesis of green, multicomponent, pH-responsive lipid-chitosan nanocapsule loaded with curcumin against West Nile virus vector, *Culex pipiens* (Diptera: Culicidae)

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ABSTRACT

Mosquitoes, among the most lethal creatures on Earth, result in millions of deaths annually by transmitting various human diseases. This work aims to synthesize novel, efficient, safe, and environmentally sustainable natural substances to enhance mosquito vector management based on nanotechnology applications of the chitosan biopolymer. A nanoscale chitosan-coated nanocapsule containing linalool, α-terpinene, and curcumin was synthesized and characterized using several analytical methods, which confirmed the incorporation of the active ingredients to the vicinity of nanoscale lipid molecules coated with chitosan biopolymer. Under acidic conditions, an initial rapid release of active compounds was observed within the first two days, subsequently exhibiting a gradual decline by the fourth day. Conversely, under alkaline conditions, the release commenced at a lower rate and progressively increased over time. The efficacy of α-terpinene, linalool, curcumin, and their nanoformulations against the third instar larvae of *Culex pipiens* was evaluated in both acidic and alkaline media at 24- and 48-h post-treatment. The NLC-CLA-CS nanocapsule demonstrated promising (the most potent) insecticidal effects, with notable potency observed for combinations of α-terpinene, linalool, and curcumin. The LC_{50} values for these formulations ranged from 8.78 to 61.33 ppm at 24 h post-treatment and from 6.08 to 29.54 ppm at 48 h post-treatment, highlighting the potential of these compounds in mosquito control. The tested compounds demonstrated significant antimicrobial activity against a broad spectrum of bacterial strains, showcasing its potent efficacy in inhibiting microbial growth. Reduced toxicity against normal cells (WI38) compared to individual compounds. Minimal impact on most non-target insects, apart from *Gambusia affinis*.

1. Introduction

Pesticides play a major role in ensuring that food production keeps pace with the growing population. It also reduces the risk of disease-carrying insects to human health [1–3]. However, traditional pesticides that lack efficiency and specificity in their targets, coupled with

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frequent use, lead to significant environmental concerns and potential risks to human well-being. Furthermore, reports indicate that the effectiveness of pesticides used against the intended organisms is less than 0.1 % [4]. As a result, the negative effects of public health pesticides and other pesticides on the environment and human health pose significant limitations to their use [5,6]. Hence, there is an urgent need to develop pesticide systems that can improve their efficiency and prolong their effect on insects.

Among the medical insects that negatively affect human health are mosquitoes. Mosquitoes are responsible for transmitting various detrimental diseases to both humans and animals [7]. These diseases include malaria, dengue fever, yellow fever, filariasis, Japanese encephalitis, chikungunya, and Streptococcus epidermidis in livestock. Vector-borne diseases affect every part of the world, however mosquito-borne diseases have significant economic consequences, such as reduced commercial production and employment; increased disease burden and mortality; poverty; and social vulnerability worldwide, particularly in impoverished nations. Hemalatha, Elumalai, Janaki, Muthu, Babu, Velu, Velayutham and Kaleena [8] have identified regions characterized by tropical and subtropical climates. Mosquitoes frequently present hazards, prompting the use of various methodologies. People have created synthetic pesticides over time, and they have generally been successful. However, they compel insects to adjust to them, leading to the eventual development of resistance to insecticidal substances. According to Sudo, Takahashi, Andow, Suzuki and Yamanaka [9], the regular utilization of pesticides presents potential hazards to the ecosystem.

Most mosquito larvae inhabit ponds and water bodies that are inherently acidic due to alterations in water composition, compounded by pollution that further acidifies aquatic ecosystems. The review demonstrates that extreme pH levels, whether acidic or alkaline, negatively impact larval survival rates, developmental duration, and adult emergence success. Larvae subjected to severely acidic surroundings demonstrated diminished growth and increased mortality rates, whereas those in alkaline circumstances experienced developmental delays and decreased fitness. The best growth happened in neutral to slightly acidic conditions (pH 6.5–7.5), where larvae had higher survival rates, grew faster, and successfully became adults [10,11]. The results indicate that pH values in larval environments are a crucial determinant of mosquito population dynamics and may be utilized in vector control techniques. Understanding how pH levels affect mosquito growth could help create targeted methods to reduce mosquito-borne diseases by changing their living conditions to stop them from breeding [12].

Nanopesticides are tiny particles that have a pesticide's active ingredient and other specially designed features that help with pest control [13]. Nanopesticides made from essential oils and /or natural products are a new technology that is growing quickly. They have many potential benefits, such as better safety for people and the environment, better stability, longer effectiveness, and less need for active ingredients [14]. Various researchers proposed that plants could be the best applicants and are suitable for large-scale production of nanoparticles [15]. Nanoformulation can enhance the solubility and bioavailability of essential oils (EOs) while also providing protection against environmental agents and facilitating controlled release. Studies by Asbahani, Miladi, Badri, Sala, Addi, Casabianca, Mousadik, Hartmann, Jilale, Renaud and Elaissari [16] and Cimino, Maurel, Musumeci, Bonaccorso, Drago, Souto, Pignatello and Carbone [17] have demonstrated these improvements.

Nanoparticles have greatly advanced in medical uses because the body can more easily absorb them and can keep certain medicines working longer at the right spot [18]. Chitosan nanoparticles (CS NPs) have unique physical and chemical properties, such as being minimal, dense, and having a large surface area [19]. These features have led to many important uses in medicine, like how drugs are released, how they spread in the body, and how they stick to mucus. The carriers must meet the fundamental conditions of being non-toxic, biocompatible, and bioactive. As a result, the CS NPs containing glycosaminoglycans have garnered significant attention. Making polymer nanoparticles often involves using natural carbohydrate polymers such as alginate and chitosan because they are safe for the body and can break down naturally [20].

Chitosan is a polycationic polymer that is both non-toxic and biodegradable, with a low level of immunogenicity. The ionic gelation method is a simple way to make CS NPs and get the right particle size so that they can pass through the epithelial membrane [21]. Polymer-based nanoparticles can be made by connecting polymer chains with covalent bonds or using physical interactions such as hydrogen bonds, electrostatic forces, or hydrophobic associations [22]. The pharmaceutical industry has investigated the use of sodium tripolyphosphate (TPP) in the synthesis of CS NPs as a potential nanocarrier. The CS nanoparticles could deeply infiltrate tissues via small capillaries, resulting in the effective transportation of drugs and proteins [19].

In this study, we plan to create lipid-chitosan nanocapsules that can deliver several natural drugs together, specifically curcumin, linalool, and α-terpinene, which are known to work as insecticides. Our goal is to combine these substances into one small formula to enhance their effects when used together. This smart nanocapsule is expected to release its active ingredients within both acidic and basic environments in different patterns, creating unique effects that would only have occurred by using more than one insecticide at the same time. By creating a new type of pesticide that responds to pH levels, we hope to provide an eco-friendlier way to control pests that is less harmful to other insects, making pesticide use more effective.

2. Materials and methods

2.1. Chemicals

Curcumin 97 %, oleic acid 90 %, steric acid 97 %, polysorbate 20 (Tween 20), sodium taurocholate 96 %, sodium glycocholate 97.5, low molecular weight chitosan, deionized water was procured from Alfa Aesar (Thermo Fisher Scientific, Dreieich, Germany). Linalool (L) and α-terpinene (A) were procured from Acros Organics (Thermo Fisher Scientific, Dreieich, Germany). All chemical reagents were utilized without being purified.

2.2. Nano drug delivery

2.2.1. Synthesis of nanostructure lipid carrier encapsulated curcumin and monoterpenes

A Nanostructure lipid carrier was synthesized using the hot homogenization-sonication method with modification [23–27]. A lipid mixture consisting of stearic acid (880 mg) and oleic acid (1000 mg) was heated until fully melted. In a different container, 150 mg of curcumin was mixed with 1.5 mL of a methanol/chloroform mixture (1:3) while being heated and then added to the melted lipid mixture. Heating was maintained until the solvent was completely evaporated. Following this, equal amounts (200 mg each) of linalool and α-terpinene were introduced beneath the lipid layer. The lipid phase was then swiftly transferred into the aqueous phase and stirred vigorously for two minutes. To stabilize the emulsion, ice-cold water was added, followed by 15 min of sonication. The resulting emulsion was lyophilized at −48 °C for 48 h to yield a semi-solid nanostructured lipid carrier (NLC) containing co-encapsulated curcumin, α-terpinene, and linalool, referred to as NLC-CLA.

2.2.2. Synthesis of nanostructure lipid carrier (NLC) coated chitosan nanocapsule

According to Gartziandia, Herran, Pedraz, Carro, Igartua, and Hernandez [28] and Radwan, El-Sherbiny, Selim, and Metwally [29], the prepared NLC-CLA suspension (10 mL) was combined with 30 mL of a 0.25 % (*w/v*) chitosan solution and stirred for 2 h. A 1 % (w/v) solution of sodium tripolyphosphate (STPP) was then added dropwise over the

course of one hour, and stirring was maintained overnight. The mixture was then frozen and dried at - 48 °C for 48 h to create a thick, chitosan-coated nanostructured lipid carrier that includes curcumin, α-terpinene, and linalool (NLC-CLA-CS). Lyophilization changes the liquid emulsion into a solid, making it easier to measure concentrations for biological tests and concentrating the sample for analyses like FTIR and UV–V is spectroscopy.

2.2.3. Fourier transform infrared

Fourier transform infrared spectroscopy (FTIR) was done using a Thermo Scientific NicoletTM iS50 FTIR spectrometer, and the sample was placed directly without a KBr disc, scanning the wave numbers from 400 to 400 cm^{-1}.

2.2.4. Particle size and the surface charge

The quality of the made nanoparticles was checked by looking at their size and how varied they are, using dynamic light scattering (DLS) at a 173° angle at room temperature. Their zeta potential, which helps understand how stable the particles are by measuring changes in scattered light due to their charge at a 12° angle, was also tested. All measurements, including zeta potential, PDI, and particle size, were carried out at the Egyptian Petroleum Research Institute using a Zeta Silver Nano Series (HT) Nano ZS instrument (Malvern Instruments, UK). Before testing, 5–10 mg of each nanoformulation was mixed in 10 mL of distilled water using a sonication bath and then placed into a quartz cuvette. Three independent readings were recorded for both the PDI and size distribution, and the most representative values were reported.

2.2.5. Surface morphology estimation by transmission electron microscope (TEM)

The morphology and stability of NLC nanoparticles were examined using Transmission Electron Microscopy (TEM). High-resolution TEM (HR-TEM) analysis was conducted at the Egyptian Petroleum Research Institute (EPRI) using a JOEL Jem-2100-115 system operating at 200 kV. To prepare samples, NLC nanoparticles were diluted with double-distilled water, applied to a carbon-coated grid, and negatively stained with 2 % phosphotungstic acid (PTA). The TEM images revealed consistent shapes and provided insight into the nanoparticles' stability and aggregation behavior.

2.3. The drug encapsulation efficiency (EE) and loading capacity (LC)

The encapsulation efficiency (entrapment) of NLC-CLA-CS is measured by using the indirect ultrafiltration centrifugation method [29–31]. A 1 ml freshly prepared NLC-CAL-CS NP was placed in a vivspin 20 centrifugal concentrator tube (MWCO 5 k Da), then centrifuged at 4500 rpm for 20 min at a temperature of 4 °C. The obtained supernatant, which contained free or non-encapsulated drugs, were separated and dissolved in DMSO by probe sonication (Scientz, ultrasonic homogenizer-HD, Ningbo Scientz Biotechnology Co., Ltd., China). The concentration of the non-encapsulated drugs of curcumin was measured using a UV spectrophotometer (Genway spectrophotometer 6305, Japan), DMSO as a blank, and a standard concentration of the target compounds (curcumin, Linalool and α-terpinene) in DMSO at 425 nm using a 1 cm glass cuvette at room temperature. Subsequently, the non-encapsulated drug (free drug) was calculated from this equation:

$$\frac{Absorbance\ of\ the\ sample\ (free\ drug\ or\ supernatent)}{Absorbance\ of\ standard\ curcumin} \times Standard\ concentration$$

The amount of entrapped drug is estimated by subtracting the free drug from the total amount of drug contained in 1 ml dispersion, as follows: Entrapped drug is calculated as the total amount of drug incorporated minus the amount of free drug found in the supernatant. The following equations are used to calculate the entrapment efficiency (EE) and the drug loading capacity (LC):

$$EE\% = \frac{Amount\ entrapped\ drug}{Amount\ of\ total\ drug\ added} \times 100$$

$$LC\% = \frac{weight\ of\ the\ entrapped\ Drug}{Nonoparticles\ weight} \times 100$$

Due to the high hydrophobicity of certain drugs, they can become adsorbed onto the ultrafiltration membrane, potentially leading to inaccuracies in calculating the amount of drug entrapped [32]. To evaluate the extent of drug adsorption on the membrane, a solution with a known drug concentration was passed through, and the concentration in the filtrate was analyzed. All experiments were conducted in a manner that prevented adsorption.

2.4. Drug release study

The in vitro release profile of the encapsulated NLC-CLA-CS nanocapsules was assessed using the dialysis bag technique [33,34]. Approximately 10 cm of dialysis tubing was soaked in 20 ml of phosphate buffer (pH 7.4) and left overnight to equilibrate before the release study. Exactly 5 ml of the NLC-CLA-CS nanoformulation was put into a dialysis bag (with a molecular weight cut-off of 12 kDa from Sigma Aldrich), sealed at one end, and then tightly closed at the other end before being placed in a 250 ml beaker that contained 100 ml of phosphate-buffered saline (PBS, pH 7.4). To help it dissolve better, 1 ml of Tween 20 was mixed in, and the mixture was stirred gently at 150 rpm with a battery-operated magnetic stirrer (IKA® C-MAG, HS4 Digital, India). The entire setup was maintained in an incubator at 37 °C. Samples (1 ml each) were withdrawn at specified intervals over 72 h, and each removal was followed by the addition of an equal volume of fresh buffer to maintain constant volume. Each sample taken was divided: one part was tested for curcumin levels. In contrast, the other part was processed with n-hexane to measure the amounts of alpha-terpinene and linalool using gas chromatography with flame ionization detection (GC-FID). The analysis was done using an Agilent 7890B gas chromatography system with a DB-624 column, using hydrogen gas at a flow rate of 1.5 ml/min in a 1:5 split mode. The injection volume was 1 μl, with a temperature program starting at 40 °C (0 min), ramping at 10 °C/min to 200 °C (0 min), then increasing at 20 °C/min to 220 °C (0 min), and finally rising at 30 °C/min to 250 °C, where it was held briefly. The injector and FID were maintained at 250 °C and 300 °C, respectively.

2.5. Atomic force microscopy

An atomic force microscope (AFM, Flex Axiom Nanosurf) was used for the visualization of the surface topography, particle size, and roughness of the nanostructure lipid carrier encapsulated curcumin, linalool, and a-terpinene, and its coated nanocapsules deposited on a silicone substrate operating in the Phase Contrast mode and Cantilever type NCLR. The thin film of the nanomaterial was acquired for a surface 625 × 625 nm and 1.25 × 1.25 μM.

2.6. Antimicrobial evaluation

The well-diffusion method is among the most applied techniques for assessing antimicrobial susceptibility. It provides both quantitative data (measured as the diameter of inhibition zones in millimeters) and qualitative results (susceptible or resistant). The Clinical and Laboratory Standards Institute (CLSI) recommends this technique for evaluating mold susceptibility. One of the key benefits of the disk diffusion method is its relatively quick turnaround time, with results available after 16 to 48 h of incubation [33,34]. According to CLSI guidelines, it is best to use Mueller-Hinton agar without extras or standard bacteriology-grade Mueller-Hinton agar with a pH of 7.2 to 7.4 after it has set, because this helps fungi grow well in 24 to 48 h. However, each new batch of

agar needs to be checked for compatibility according to CLSI rules, because some batches may not allow all species to grow well, resulting in larger inhibition zones that could go beyond acceptable limits. For this method, the inoculum should be prepared to match the concentration used in broth dilution procedures and must be applied to agar plates within 15 min of adjustment. The plate surface is streaked uniformly in three directions. After drying the surface for no more than 15 min, a sterile cork borer or pipette tip is used to punch a 6–8 mm diameter well. A volume of 20 to 100 μL of the antimicrobial agent or test extract, at the desired concentration, is then carefully added into each well.

2.7. In-vitro cytotoxicity assessment

The cytotoxicity assessment of the synthesized nanoparticles of NLC-CLA and NLC-CLA-CS was assessed using lung fibroblast WI38, American Type Culture Collection CCL-75 (Vacsera, Cairo, Egypt). The cell line was seeded using RPMI media with 10 % fetal bovine serum (FBS) and an equivalent amount of antibiotics of streptomycin and penicillin (100 units per milliliter) to inhibit bacterial growth. The incubation protocol was done using a CO_2 incubator at 37 °C with a 5 % humidity rate. Cells were transferred to 96-well plates and seeded at a density of 1.0×10^5 per well, and the incubation was completed in 48 h. As for the tested compounds, serial dilutions of the synthesized nanoparticles of 1000, 500, 250, 125, 62.5, and 31.25 were prepared. After 48 h of incubation, cells were subsequently treated with several doses of the prepared dilutions. The treated plates were re-incubated for 48 h under the same conditions of humidity and temperature. After achieving suitable confluency, each seeded well received 20 μL of MTT solution (5 mg/mL), and the additional hours of incubation were processed under the same conditions before cell viability determination and consequently, IC_{50} calculations. Optical densities were measured after the addition of 100 μL DMSO to ensure complete solubility of the purple color, and the absorbance was measured at a wavelength of 570 nm using a plate reader (EXL 800, California, CA, USA) [27,35].

2.8. Mosquito larvicidal bioassay

2.8.1. Culex pipiens rearing

Cx. pipiens larvae were reared in an insectary environment set to a constant temperature of 27 ± 2 °C, relative humidity of 75 ± 5 %, and a 12-h light/dark photoperiod. The larvae were fed a diet consisting of Tetramin fish food mixed with powdered bread in a 3:1 ratio. Upon reaching the pupal phase, they were moved from enamel trays into containers filled with dechlorinated water and housed in mesh cages (35 × 35 × 40 cm), where adult mosquitoes eventually emerged. Female adults were regularly provided with hamster blood meals, while a 10 % sugar solution was available to all adults throughout the study. All tests, involving both larval and adult mosquitoes, were conducted within the same laboratory setting [36].

2.8.2. Assessment of larvicidal effectiveness

The larvicidal potential of selected natural products (α-terpinene, linalool, and curcumin) and their nanoparticles in pH 6 and pH 8 media were evaluated under controlled lab conditions against the 3rd larval instar of *Cx. pipiens*. Larvae were exposed to various concentrations of the oils: 6.3, 12.5, 25, 50, and 100 ppm, adjusted to pH levels of 6 and 8. For each treatment, twenty-five larvae were placed in 250 mL of distilled water within glass beakers, and each concentration was tested in four replicates. Mortality was recorded at 24 and 48 h post-exposure and during subsequent observation periods.

2.9. Efficacy of tested materials against non-target insects

Curcumin, α-terpinene, and linalool—and their conjugate nanoparticles were examined against common water-dwelling arthropods in the mosquito breeding areas of Kafr Saad village, which has many water sources. Fish and insects like *Stratiomys longicornis, Cybister tripunctatus, Sphaerodema urinators, Gambusia affinis, Eristalis tenax,* and *Ephydra* sp. were caught using a nylon net trap and a dipper, then placed in plastic bags with some water and taken to the lab for testing. They were subsequently placed in plastic bags partially filled with natural water and transported to the laboratory for assessment. Before the test, predators were deprived of food for 24 h and subsequently administered LC_{50} concentrations of the tested oils and their nanoparticles. Three predators were introduced into a 5-l tank containing 300 larvae per liter of dechlorinated water. The impact of the selected oil extracts was also evaluated over the survival rate of these aquatic insects that remained in these oil extracts for two days, where 10 predators were included for each oil under the LC_{50} concentration. Each test comprised three replicates, and controls and observations were performed following a 24-h exposure. Dr. Yasser El-Sayed identified the aquatic insects based on the classifications of Haggag, Mahmoud, Bream and Amer [37], Department of Entomology, Faculty of Science at Benha University in Egypt.

2.10. Statistical analysis

The data were analyzed by the software, SPSS V23 (IBM, USA), for doing the Probit analyses to calculate the lethal concentration (LC) values and the one-way analysis of variance (ANOVA) (Post Hoc/Turkey's HSD test). The significant levels were set at $P < 0.05$.

3. Results

3.1. Drug delivery

3.1.1. Particle size (DLS) and Zeta potential (ζ)

According to (Table 1) and (Fig. 1), the size of the nanostructure lipid carrier encapsulated curcumin, α-terpinene, and linalool co-delivery (NLC-CLA) and its conjugate chitosan-coated nanocapsule (NLC-CLA-CS) was 29 and 162 nm, while the polydispersity index manifested 0.31 and 0.33, respectively. Zeta potential of the two nanoformulations revealed (−7.37 mV) and (+50.4 mV), respectively.

3.1.2. Transmission electron microscope

The internal morphology of transmission electron microscopes presented in Fig. 2 showed spherical and semispherical nanoparticles from less than 100 nm up to 200 nm.

3.1.3. Fourier transform infrared (FTIR)

Fourier transform infrared was performed to clarify the interactions resulting from the encapsulation process and the extent of the association between linalool and alpha terpinene with the lipid matrix, which were then encapsulated by chitosan. The curves presented in Fig. 3 were combined for ease of comparison and to reveal the changes occurring because of the encapsulation process.

3.1.4. Drug encapsulation efficiency (EE) and loading capacity (LC)

The encapsulation efficiency and loading capcity were assessed and the results presented in Table 2 confirmed the encapsulation efficiency of curcumin, α-terpinene and linalool were 72.6, 81.50 and 74.00 and loading capacity of 10.38, 14.09 and 15.52 %, respectively.

3.1.5. Drug release

The amount of curcumin released in the acidic (at pH = 6, Fig. 5) and

Table 1
Size (DLS) and zeta potential of the synthesized nanoparticles.

Nanoformulation	Size(nm)	PDI	Zeta(mV)
NLC-CLA	29 ± 1.99	0.31	− 7.37 ± 1.99
NLC-CLA-CS	162 ± 1.99	0.333	+ 50.4 ± 1.99

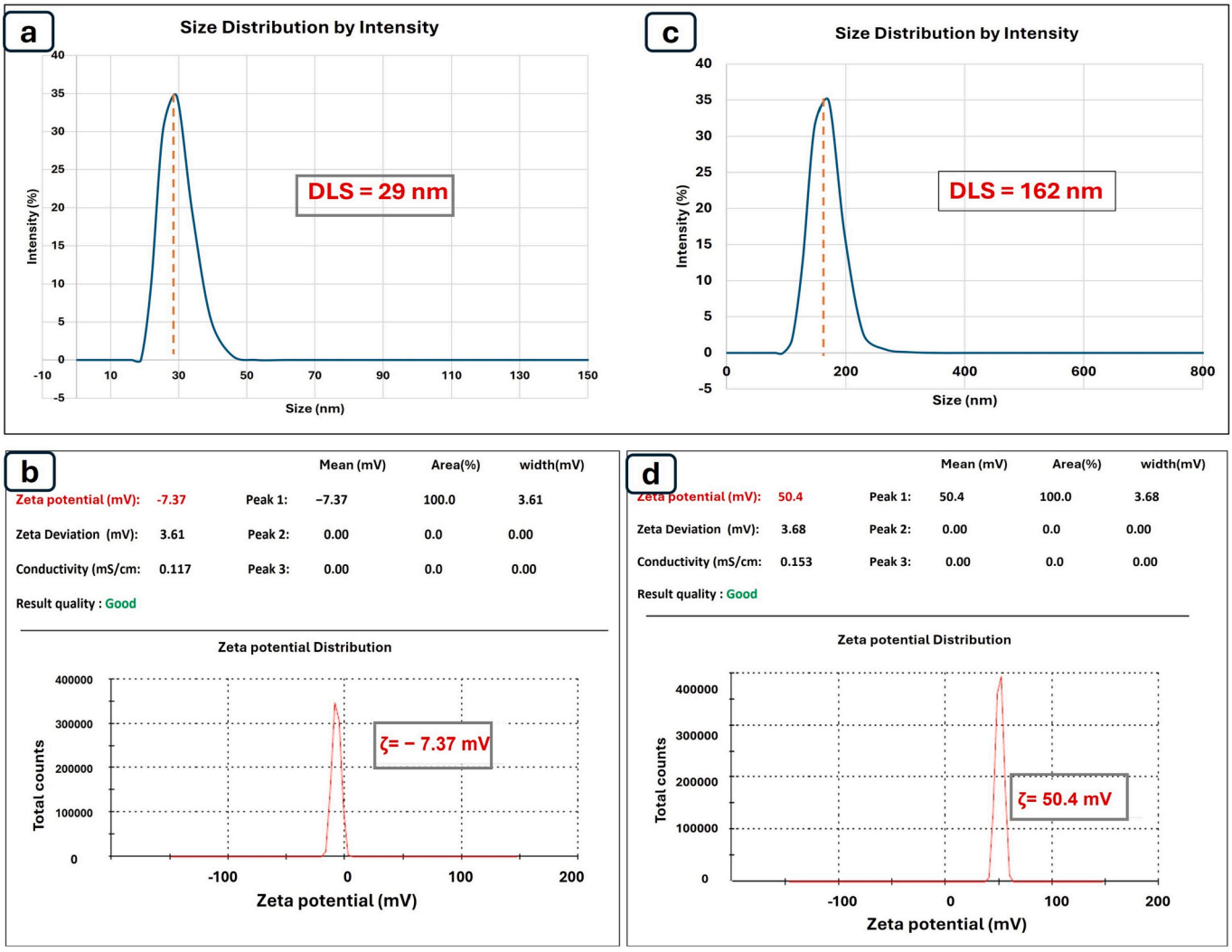

Fig. 1. DLS and zeta potential of synthesized nanoparticles: a, b) NLC-CLA; c, d) NLC-CLA-CS.

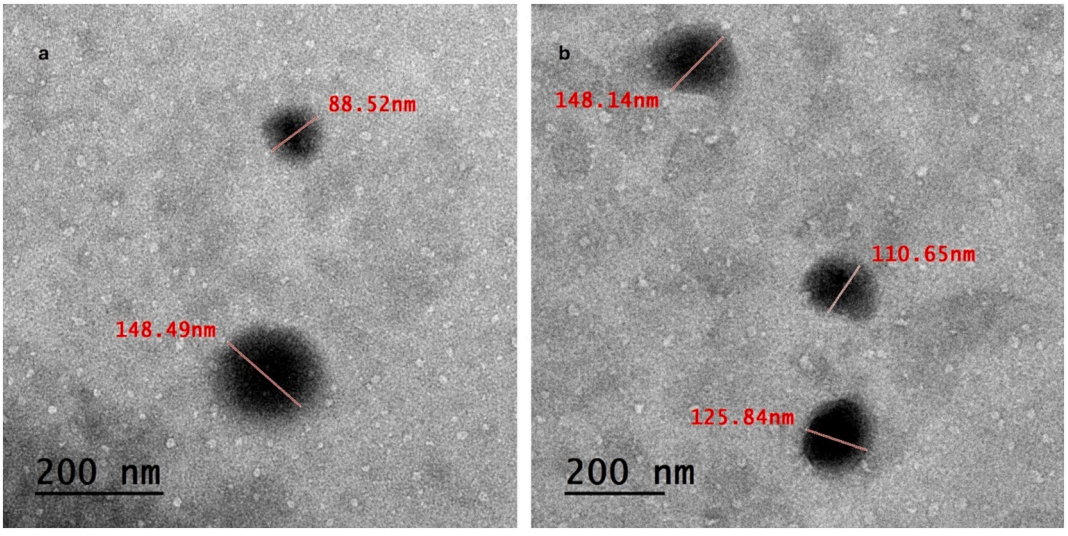

Fig. 2. Internal Morphology manifestation by TEM of the curcumin-based nanoformulations: a) NLC-CLA, b) NLC-CLA-CS nanocapsule.

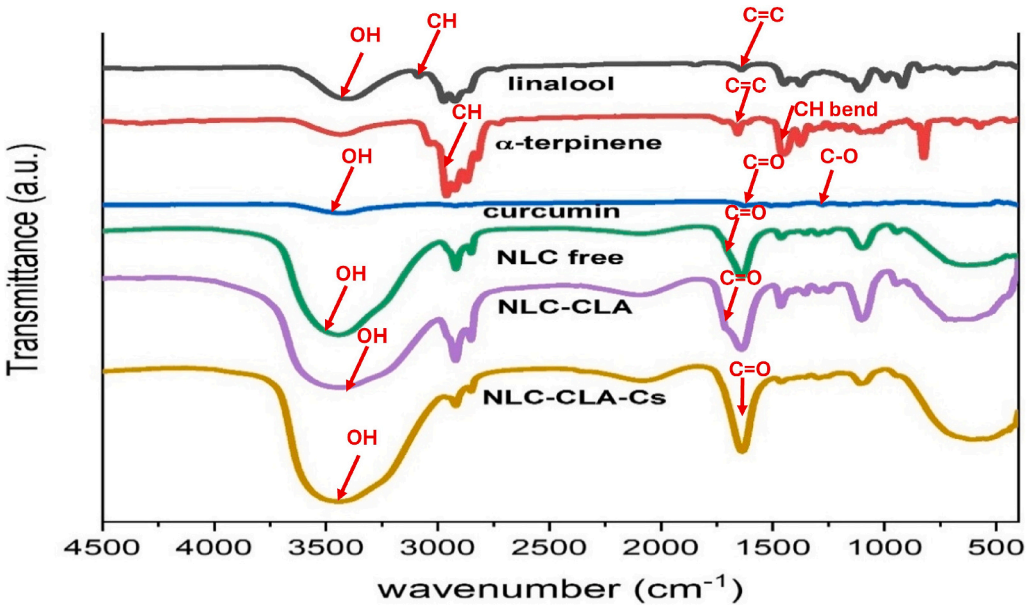

Fig. 3. FTIR of the free and encapsulated curcumin, linalool, a-terpinene and their conjugate nanoformulations.

Table 2
Encapsulation efficiency and loading capacity of the synthesized NLC-CLA-CS.

ID	Encapsulation efficiency (%)			Loading capacity (%)		
Drug	Curcumin	α-terpinene	Linalool	Curcumin	α-terpinene	Linalool
NLC-CLA-CS	72.6 ± 1.99	81.50 ± 1.18	74.00 ± 1.06	10.38 ± 0.93	14.09 ± 1.21	15.52 ± 1.15

Table 3
The curcumin concentration released from NLC-CLA-CS in acidic and alkaline mediums.

Release in acidic medium		Release in alkaline medium	
Time (h)	Conc (μg/mL)	Time (h)	Conc (μg/mL)
6	10,980	6	280
12	12,850	12	543
18	12,995	18	924
24	11,980	24	1002
30	12,210	30	1789
36	12,880	36	2566
42	11,943	42	4670
48	10,625	48	4823
54	8980	54	3980
60	7567	60	4620
66	5360	66	5011
72	5217	72	7534
78	3297	78	8016
84	2894	84	7640
90	1894	90	9426
96	1937	96	10,889

alkaline conditions (at pH = 8, Fig. S1 and S2) were studied and measured during the first 96 h and the released concentrations were combined in Table. 3. The release of the drug in both media was compared to determine which one ensured faster or longer release and achieved better efficacy. The release study of both linalool and α-terpinene in acidic medium was completed for technical reasons that will be covered in detail in the discussion section using GC-FID (Fig. S3-S110) chromatography by injecting standards for these materials to determine their retention time and from there, and by comparing them with the samples of the release study, the concentrations of each of them

Table 4
α-Terpinene and linalool concentrations released from NLC-CLA-CS in acidic medium.

	α-Terpinene conc.			Linalool conc		
Time (h)	Conc (mg/mL)	Conc (μg/mL)	±SD	Conc (mg/mL)	Conc (μg/mL)	±SD
Standard	2.07	2070	106.3	2.716	2716	123.4
Release at 12 h	0.9	900	45.5	7.12	7120	97.8
Release at 24 h	0.98	980	67.1	4.47	4470	112.3
Release at 36 h	1.15	1150	98.9	3.72	3720	145.1
Release at 48 h	1.05	1050	38.5	1.92	1920	22.5
Release at 72 h	0.89	890	9.5	1.78	1780	37.2
Release at 96 h	0.36	360	2.3	1.63	1630	6.9

are calculated through the beak area. The concentration of the released linalool and α-terpinene has been added to Table 4 and Figs. S11 and S12.

3.1.6. Atomic force microscopy

Atomic force microscopy was used to investigate the surface topography, particle size and roughness. Considering the AFM two and three-dimensional images at scale 1.25 × 1.25 μM presented in Figs. 4 and 5, both NLC-CLA and NLC-CLA-CS have particle sizes in the range below 100 nm. The area roughness depicted by NLC-CLA and NLC-CLA-CS was 9.089 nm and 1.079 nm, respectively.

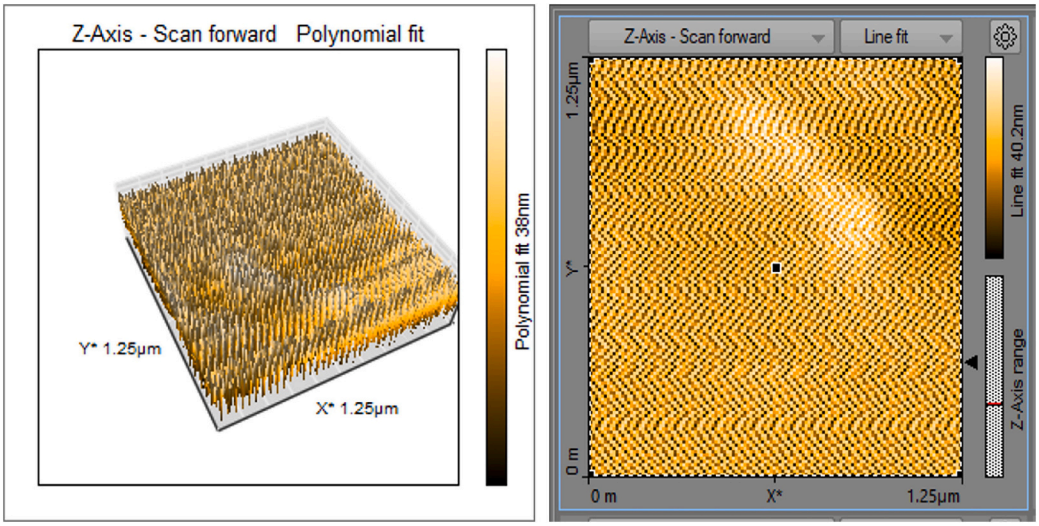

Fig. 4. Two and Three-dimensional AFM of the nanostructure lipid carrier loaded curcumin, linalool and alpha terpinene.

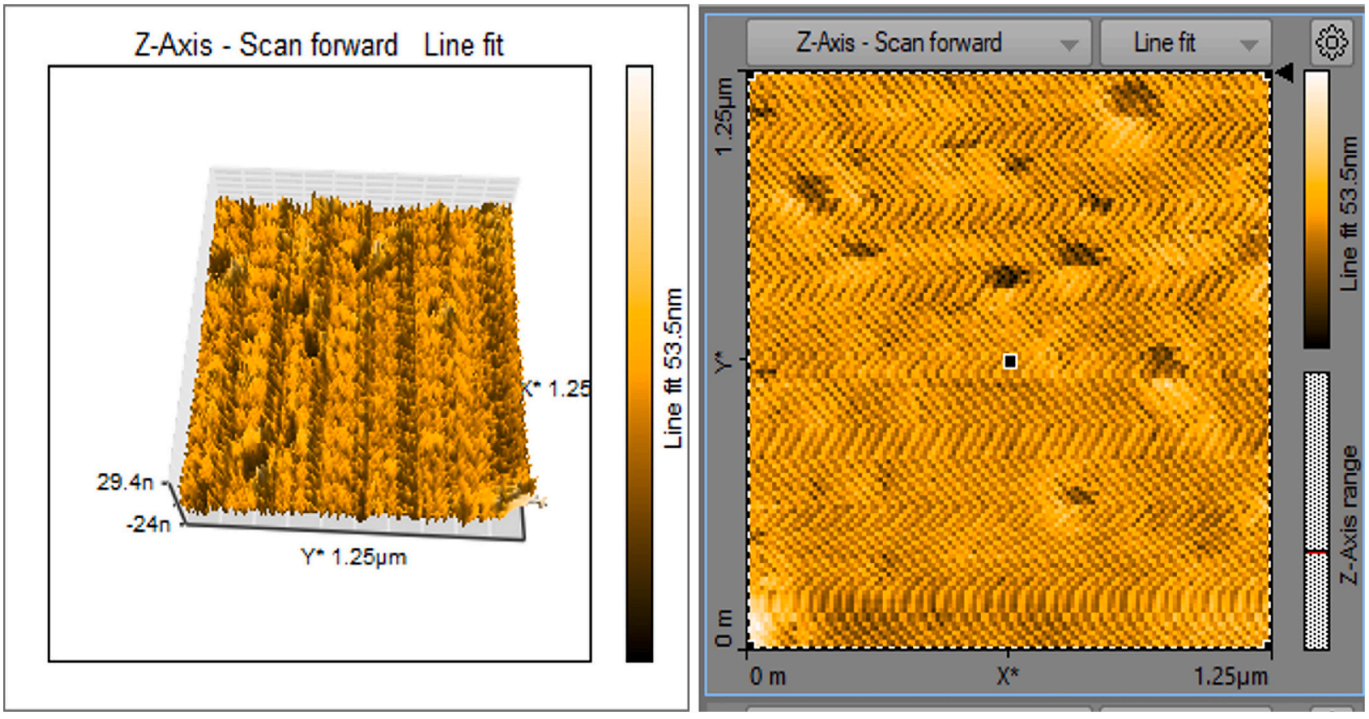

Fig. 5. Two and Three-dimensional AFM of the nanostructure lipid carrier loaded curcumin, linalool and alpha terpinene coated with chitosan.

Table 5
Inhibition zone diameter (mm) of the synthesized curcumin-based nanocapsules against different microbial strains.

Microorganism	NLC-CLA-CS	NLC-CLA	Cont.
Bacillus subtilis (ATCC 6633)	31 ± 0.2	29 ± 0.8	28 ± 0.5
Staphylococcus aureus (ATCC 6538)	24 ± 0.8	22 ± 1	24 ± 1
Escherichia coli (ATCC 8739)	23 ± 0.6	21 ± 0.5	20 ± 0.5
Klebsiella pneumoniae (ATCC13883)	23 ± 0.4	20 ± 0.5	23 ± 0.8
Candida albicans (ATCC 10221)	26 ± 0.6	26 ± 0.7	26 ± 0.6

3.2. Antimicrobial evaluation

Antimicrobial evaluations were conducted (Tables 5, 6 and Fig. 6) to show the activity of the synthesized nanocapsule against different strains of bacteria which probably may grow in stagnant water.

3.3. In vitro cytotoxic effect

The cytotoxicity effect was performed and the results listed in Table 7 and Fig. 7

Table 6
Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the curcumin-based nanocapsules.

Microorganism	NLC-CLA-CS			NLC-CLA		
	MIC	MBC	index	MIC	MBC	index
Bacillus subtilis (ATCC 6633)	15.62 ± 0.0	15.62 ± 0.0	1	15.62 ± 0.0	31.25 ± 0.0	2
Staphylococcus aureus (ATCC 6538)	31.25 ± 0.0	62.50 ± 0.0	2	62.50 ± 0.0	62.50 ± 0.0	1
Escherichia coli (ATCC 8739)	125.0 ± 0.0	250.0 ± 0.0	2	250.0 ± 0.0	250.0 ± 0.0	1
Klebsiella pneumoniae (ATCC13883)	250.0 ± 0.0	500.0 ± 0.0	2	250.0 ± 0.0	500.0 ± 0.0	2
Candida albicans (ATCC 10221)	15.62 ± 0.0	31.25 ± 0.00	2	31.25 ± 0.0	31.25 ± 0.00	1

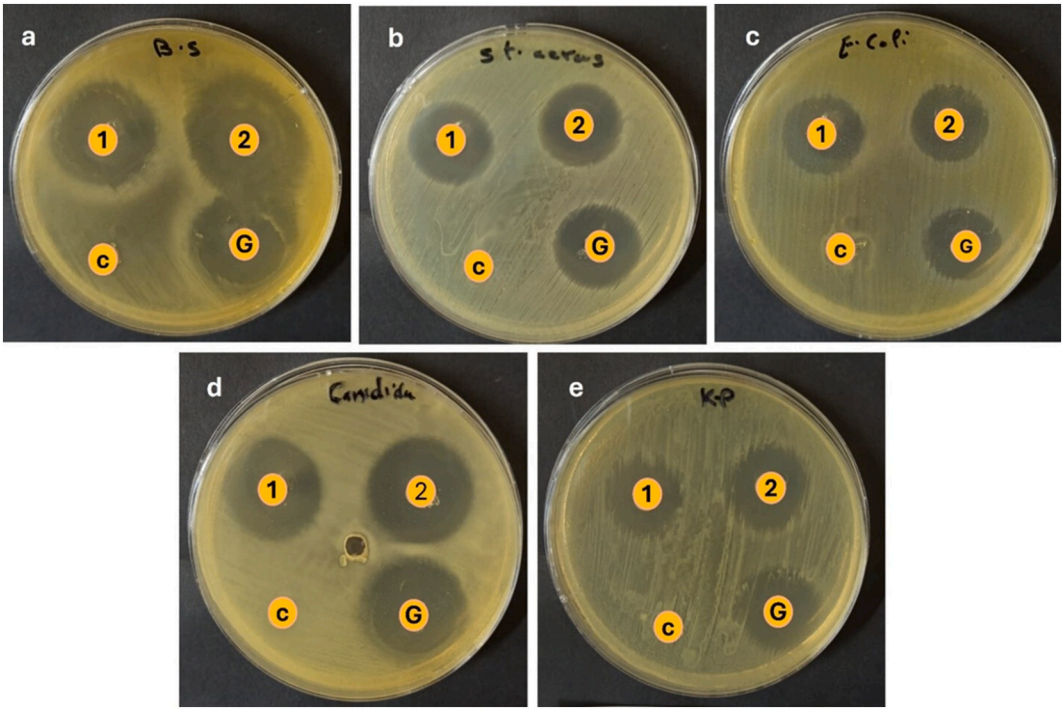

Fig. 6. The inhibition zones diameter of curcumin-based nanocapsule 1 (NLC-CLA), and 2 (NLC-CLA-CS) of the untreated negative control (C), and gentamycin positive control (G) (10.0 mg/ml) for bacterial strains (a) *Bacillus subtilis*, (b) *Staphylococcus aureus*, (c) *Escherichia coli*, (d) *Klebsiella pneumoniae*, (e) and *Candida albicans*.

3.4. Mosquito larvicidal activity

The larvicidal effects of tested curcumin, α-terpinene, and linalool active ingredients and their conjugate nanoparticles were evaluated against the early 3rd larvae of *Culex pipiens* across various concentrations, as shown in Tables 8–11. The active ingredients tested in this study showed strong potential to kill *Cx. pipiens* larvae, indicating they could be useful for controlling mosquitoes. Data showed that the most effective mosquito killer was nanoformulations (α-terpinene, linalool, and curcumin), followed by a blend of the three active ingredients (α-terpinene, linalool, and curcumin) in a special tiny droplet form, followed by the same oils mixed physically without formulation as nanoparticles, then a mix of just α-terpinene and linalool, and finally, α-terpinene alone. The most effective concentrations were 25, 50, and 100 ppm. At pH 6 and a concentration of 100 ppm, most of the tested materials and their nanoformulations resulted in 100 % mortality. All tested larvicides were more effective at pH 6 than at pH 8.

The results showed that mortality rates of the 3rd larval instars at pH 6, 24 h post-treatment (PT) with α-terpinene, linalool, curcumin, a mixture of α-terpinene and linalool, a mixture of α-terpinene and curcumin, a mixture of linalool and curcumin, a mixture of α-terpinene, linalool, and curcumin, and a nanocapsule (NLC-CLA-CS) mixture of α-terpinene, linalool, and curcumin were 55, 40, 24, 68, 47, 48, 80, and 93 % at 25 ppm and 90, 73, 44, 100, 86, 81, 100, and 100 % at 50 ppm, respectively. The corresponding mortality rates at pH 6, 48 h PT were 72, 59, 43, 86, 77, 61, 94, and 100 % at 25 ppm and 100, 91, 64, 100, 100, 98, 100, and 100 % at 50 ppm, respectively (Table 8**)**.

The results in Table 9 showed that the mortality rates of *Cx. pipiens* at pH 8, 24 h PT with α-terpinene, linalool, curcumin, a mixture of α-terpinene and linalool, a mixture of α-terpinene and curcumin, a mixture of linalool and curcumin, a mixture of α-terpinene, linalool, and curcumin, and a nanocapsule (NLC-CLA-CS mixture of α-terpinene, linalool, and curcumin were 42, 32, 18, 56, 49, 41, 64, and 86 % at 25 ppm; 76, 57, 36, 89, 80, 72, 92, and 100 % at 50 ppm; and 96, 88, 64, 100, 100, 95, 100, and 100 % at 100 ppm, respectively. The corresponding values at pH 8, 48 h PT were 65, 48, 29, 80, 68, 55, 85, and 97 % at 25 ppm; 95, 77, 53, 95, 91, 92, 100, and 100 % at 50 ppm; and 100, 98, 82, 100, 100, 100, 100, and 100 % at 100 ppm, respectively.

The LC_{50} values at pH 6 for α-terpinene, linalool, curcumin, and their various mixtures were 19.17, 26.43, 61.33, 15.75, 20.83, 21.89, 12.55, and 8.78 ppm after 24 h, and 12.35, 17.51, 29.54, 9.47, 11.82, 15.69, 8.56, and 6.08 after 48 h, respectively (Table 10). The corresponding values at pH 8 were 25.99, 37.31, 70.14, 19.09, 22.08, 27.65, 16.53, and 10.57 at 24 h PT and 15.79, 23.62, 41.62, 12.64, 15.64, 18.81, 10.33,

Table 7
Cytotoxic effect of curcumin, linalool and α-terpinene and their conjugat nanoformulations

ID	Conc. µg/ml	O·D (R1)	O·D (R2)	O·D (R3)	Mean O·D	±SE	Viability %	Toxicity %	IC_{50} ± SE
wi38	0(cont.)	0.735	0.739	0.731	0.735	0.0023	100	0	
	1000	0.062	0.067	0.075	0.068	0.0037	9.251	90.748	302
NLC-CLA-CS	500	0.093	0.098	0.084	0.091	0.0040	12.471	87.528	±4.06
	250	0.559	0.564	0.552	0.558	0.0034	75.963	24.036	
	125	0.68	0.675	0.672	0.675	0.0023	91.927	8.072	
	62.5	0.733	0.735	0.732	0.733	0.0008	99.773	0.226	
	31.25	0.739	0.738	0.728	0.735	0.0035	100.00	0.0	
NLC-CLA	1000	0.044	0.049	0.042	0.045	0.0020	6.122	93.877	198.08
	500	0.17	0.175	0.0169	0.120	0.0518	16.412	83.587	±0.751
	250	0.309	0.315	0.317	0.313	0.0024	42.675	57.324	
	125	0.523	0.532	0.528	0.527	0.0026	71.791	28.208	
	62.5	0.701	0.705	0.709	0.705	0.0023	95.918	4.081	
	31.25	0.729	0.733	0.735	0.732	0.0017	99.637	0.362	
Curcumin	1000	0.032	0.036	0.031	0.033	0.0015	4.489	95.510	56.69
	500	0.056	0.055	0.059	0.056	0.0012	7.709	92.290	±1.4
	250	0.079	0.084	0.078	0.080	0.0018	10.929	89.070	
	125	0.16	0.169	0.171	0.166	0.0033	22.675	77.324	
	62.5	0.336	0.339	0.344	0.339	0.0023	46.213	53.786	
	31.25	0.588	0.595	0.599	0.594	0.0032	80.816	19.183	
Linalool	1000	0.029	0.033	0.032	0.031	0.0012	4.263	95.736	42.62
	500	0.049	0.053	0.058	0.053	0.0020	7.256	92.743	±3.62
	250	0.089	0.08	0.095	0.088	0.0043	11.972	88.0272	
	125	0.152	0.15	0.156	0.152	0.0017	20.770	79.229	
	62.5	0.304	0.309	0.312	0.308	0.0023	41.950	58.049	
	31.25	0.485	0.494	0.498	0.492	0.0038	66.984	33.015	
Terpinene	1000	0.023	0.03	0.028	0.027	0.0020	3.673	96.326	81.86
	500	0.059	0.055	0.062	0.058	0.0020	7.981	92.018	±3.05
	250	0.108	0.114	0.119	0.113	0.0030	15.464	84.535	
	125	0.243	0.24	0.238	0.240	0.0014	32.698	67.301	
	62.5	0.395	0.41	0.403	0.402	0.0043	54.784	45.215	
	31.25	0.533	0.539	0.544	0.538	0.0031	73.287	26.712	

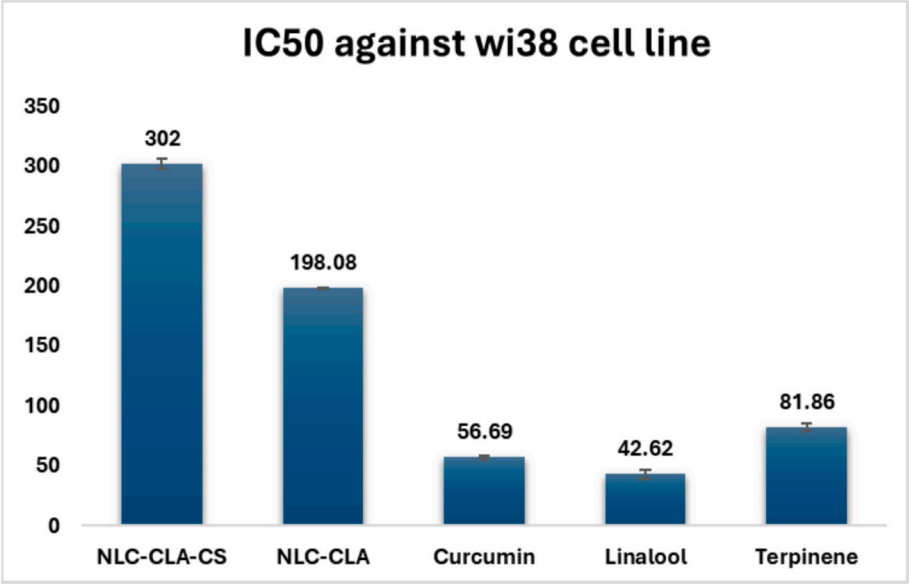

Fig. 7. Cytotoxic effect of curcumin, linalool, α-terpene, NLC-CLA, and NLC-CLA-CS.

and 7.55 at 48 h PT, respectively (Table 11).

3.5. The efficacy of active ingredients and their conjugate nanoformulations against non-target insects

The efficacy of the active ingredients and their conjugate nanoformulations were evaluated against several predators as nontarget insects, including *Stratiomys longicornis, Cybister tripunctatus, Sphaerodema urinators, Gambusia affinis, Eristalis tenax,* and *Ephydra* sp., after being treated with LC_{50}, as shown in Table 12, Fig. 8. The data showed that the selected active ingredients (α-terpinene, linalool, curcumin, and nanocapsule (NLC-CLA-CS) did not significantly affect the efficiency of some predators, such as *Stratiomys longicornis* and *Cybister tripunctatus*, as their predation rates remained close to those of the control. However, *G. affinis* showed a decrease in predation in how well it could catch prey when using nanocapsule (NLC-CLA-CS) compared to the control (81.00 and 64.67 larvae, respectively). On the other hand, *S. urinators* had increased in their ability to predate with nanocapsule (NLC-CLA-CS)

Table 8
Efficacy of the active ingredients and their conjugate nanoformulation on the mortality of *Culex pipiens* larvae at pH 6 media, 24, and 48, h post-treatment (mean ± SE).

Time (hr)	Tested material	Concentration (ppm)					
		0	6.3	12.5	25	50	100
24	α-terpinene	0 ± 0^{aF}	13 ± 1.22^{dE}	27 ± 2.55^{dD}	55 ± 2.74^{dC}	90 ± 2.74^{bB}	100 ± 0.00^{aA}
	Linalool	0 ± 0^{aF}	9 ± 1.00^{eE}	20 ± 2.24^{fD}	40 ± 1.58^{fC}	73 ± 2.55^{eB}	98 ± 2.00^{bA}
	Curcumin	0 ± 0^{aF}	4 ± 1.00^{fE}	11 ± 1.00^{gD}	24 ± 1.87^{gC}	44 ± 2.45^{fB}	75 ± 2.24^{cA}
	α-terpinene - Linalool	0 ± 0^{aE}	17 ± 1.22^{cD}	38 ± 2.55^{cC}	68 ± 2.55^{cB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	α-terpinene - Curcumin	0 ± 0^{aF}	12 ± 1.22^{dE}	28 ± 2.00^{dD}	47 ± 2.55^{eC}	86 ± 4.30^{cB}	100 ± 0.00^{aA}
	Linalool - Curcumin	0 ± 0^{aF}	12 ± 1.22^{dE}	25 ± 2.24^{eD}	48 ± 2.00^{C}	81 ± 1.87^{dB}	100 ± 0.00^{aA}
	α-terpinene- Lin - Cur	0 ± 0^{aE}	20 ± 2.24^{bD}	46 ± 2.92^{bC}	80 ± 3.16^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	NLC-CLA-CS	0 ± 0^{aE}	34 ± 1.87^{aD}	66 ± 1.87^{aC}	93 ± 2.00^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
48	α-terpinene	0 ± 0^{aE}	21 ± 1.00^{eD}	51 ± 2.92^{eC}	72 ± 2.55^{EbB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	Linalool	0 ± 0^{aF}	14 ± 1.00^{gE}	34 ± 1.00^{gD}	59 ± 3.67^{gC}	91 ± 2.45^{cB}	100 ± 0.00^{aA}
	Curcumin	0 ± 0^{aF}	8 ± 1.22^{hE}	23 ± 1.22^{hD}	43 ± 2.55^{hC}	64 ± 2.92^{dB}	90 ± 1.58^{bA}
	α-terpinene - Linalool	0 ± 0^{aE}	30 ± 1.58^{cD}	66 ± 3.67^{cC}	86 ± 2.92^{cB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	α-terpinene - Curcumin	0 ± 0^{aE}	23 ± 2.00^{dD}	53 ± 2.55^{dC}	77 ± 1.22^{dB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	Linalool - Curcumin	0 ± 0^{aF}	17 ± 1.22^{fE}	37 ± 2.55^{fD}	61 ± 4.30^{fC}	98 ± 2.00^{bB}	100 ± 0.00^{aA}
	α-terpinene- Lin - Cur	0 ± 0^{aE}	29 ± 1.87^{bD}	77 ± 1.22^{bC}	94 ± 4.00^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	NLC-CLA-CS	0 ± 0^{aD}	54 ± 3.67^{aC}	84 ± 1.87^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}

a, b & c: There is no significant difference ($P > 0.05$) between any two means for each time, within the same column have the same superscript letter; A, B & C: There is no significant difference ($P > 0.05$) between any two means, within the same row have the same superscript letter.

Table 9
Efficacy of the active ingredients and their conjugate nanoformulation on the mortality of *Culex pipiens* larvae at pH 8 media, 24, and 48, h post-treatment (mean ± SE).

Time (hr)	Tested material	Concentration (ppm)					
		0	6.3	12.5	25	50	100
24	α-terpinene	0 ± 0^{aF}	9 ± 2.45^{deE}	20 ± 2.74^{eD}	42 ± 2.00^{eC}	76 ± 1.87^{eB}	96 ± 2.92^{bA}
	Linalool	0 ± 0^{aF}	5 ± 2.24^{fE}	14 ± 3.67^{fD}	32 ± 2.55^{fC}	57 ± 3.39^{gB}	88 ± 2.55^{dA}
	Curcumin	0 ± 0^{aF}	2 ± 1.22^{gE}	8 ± 1.22^{gD}	18 ± 2.55^{gC}	36 ± 2.92^{hB}	64 ± 2.92^{eA}
	α-terpinene - Linalool	0 ± 0^{aF}	12 ± 2.00^{cE}	29 ± 1.87^{cD}	56 ± 1.87^{cC}	89 ± 1.87^{cB}	100 ± 0.00^{aA}
	α-terpinene - Curcumin	0 ± 0^{aF}	11 ± 1.87^{cdE}	25 ± 4.18^{dD}	49 ± 5.10^{dC}	80 ± 2.24^{dB}	100 ± 0.00^{aA}
	Linalool - Curcumin	0 ± 0^{aF}	7 ± 2.00^{efE}	20 ± 3.16^{eD}	41 ± 1.87^{eC}	72 ± 2.55^{fB}	95 ± 3.16^{cA}
	NLC-CLA-CS	0 ± 0^{aF}	16 ± 1.87^{bE}	34 ± 1.87^{bD}	64 ± 5.79^{bC}	92 ± 2.55^{bB}	100 ± 0.00^{aA}
	NLC-CLA-CS	0 ± 0^{aE}	26 ± 3.32^{aD}	57 ± 4.36^{aC}	86 ± 3.67^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
48	α-terpinene	0 ± 0^{aF}	15 ± 2.74^{dE}	38 ± 2.55^{dD}	65 ± 4.18^{eC}	95 ± 3.16^{bB}	100 ± 0.00^{aA}
	Linalool	0 ± 0^{aF}	9 ± 2.92^{fE}	25 ± 2.74^{fD}	48 ± 4.06^{gC}	77 ± 2.55^{dB}	98 ± 2.00^{bA}
	Curcumin	0 ± 0^{aF}	5 ± 1.58^{gE}	16 ± 1.87^{gD}	29 ± 4.00^{hC}	53 ± 3.39^{eB}	82 ± 3.39^{bA}
	α-terpinene - Linalool	0 ± 0^{aF}	23 ± 2.00^{cE}	46 ± 1.00^{cD}	80 ± 3.54^{cC}	95 ± 3.16^{bB}	100 ± 0.00^{aA}
	α-terpinene - Curcumin	0 ± 0^{aF}	16 ± 1.87^{dE}	39 ± 1.87^{dD}	68 ± 3.39^{dC}	91 ± 2.45^{cB}	100 ± 0.00^{aA}
	Linalool - Curcumin	0 ± 0^{aF}	12 ± 3.39^{eE}	29 ± 3.32^{eD}	55 ± 4.18^{fC}	92 ± 5.15^{cB}	100 ± 0.00^{aA}
	α-terpinene- Lin - Cur	0 ± 0^{aE}	28 ± 2.00^{bD}	58 ± 2.55^{bC}	85 ± 6.71^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	NLC-CLA-CS	0 ± 0^{aE}	40 ± 3.16^{aD}	76 ± 2.92^{aC}	97 ± 3.00^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}

a, b & c: There is no significant difference ($P > 0.05$) between any two means for each time, within the same column have the same superscript letter; A, B & C: There is no significant difference ($P > 0.05$) between any two means, within the same row have the same superscript letter.

Table 10
Lethal concentrations (ppm) of the active ingredients and their conjugate nanoformulation on the mortality of *Culex pipiens* larvae at pH 6 media, 24, and 48, h post-treatment (mean ± SE).

Time (hr)	Tested material	LC_{50} (Low-Up.)	LC_{90} (Low-Up.)	LC_{95} (Low-Up.)	Slope ± SE	Chi (Sig.)
24	α-terpinene	19.17 (13.74–26.15)	54.21 (42.14–97.19)	72.78 (56.26–145.11)	2.839 ± 0.209	7.935 (0.047)
	Linalool	26.43 (18.40–38.27)	83.36 (64.20–171.97)	115.43 (88.79–271.26)	2.569 ± 0.1902	8.940 (0.030)
	Curcumin	61.33 (46.86–93.93)	322.48 (178.63–898.56)	516.24 (259.16–1716.69)	1.777 ± 0.251	0.013 (0.993)
	α-terpinene - Linalool	15.75 (13.81–17.91)	48.72 (39.83–63.97)	67.10 (52.67–93.70)	2.6133 ± 0.237	3.318 (0.345)
	α-terpinene - Curcumin	20.83 (13.77–30.67)	62.08 (48.66–133.04)	84.61 (67.51–207.91)	2.701 ± 0.199	10.896(0.012)
	Linalool - Curcumin	21.89 (14.85–31.69)	67.33 (52.21–138.91)	92.58 (72.29–217.83)	2.626 ± 0.194	9.683 (0.021)
	α-terpinene- Lin - Cur	12.55 (11.19–13.96)	31.19 (26.92–37.65)	40.37 (33.90–50.79)	3.240 ± 0.266	4.737 (0.192)
	NLC-CLA-CS	8.78 (7.64–9.87)	21.92 (18.90–26.70)	28.41 (23.75–36.42)	3.224 ± 0.316	1.281 (0.781)
48	α-terpinene	12.35 (8.16–17.56)	34.29 (27.17–69.28)	45.54 (36.80–106.15)	2.949 ± 0.241	9.813 (0.020)
	Linalool	17.51 (15.54–19.65)	50.91 (43.17–62.67)	68.89 (56.65–88.64)	2.765 ± 0.208	5.389 (0.145)
	Curcumin	29.54 (25.75–34.03)	118.84 (93.64–162.57)	176.34 (132.63–257.84)	2.119 ± 0.170	2.005 (0.871)
	α-terpinene - Linalool	9.47 (8.21–10.69)	25.50 (21.84–31.25)	33.76 (27.99–43.60)	2.978 ± 0.277	2.948 (0.399)
	α-terpinene - Curcumin	11.82 (10.42–13.25)	31.76 (27.21–38.75)	42.03 (34.93–53.71)	2.985 ± 0.251	6.430 (0.092)
	Linalool - Curcumin	15.69 (9.66–23.49)	43.05 (34.70–100.55)	51.32 (48.26–156.92)	2.922 ± 0.223	13.186(4.395)
	α-terpinene- Lin - Cur	8.56 (7.54–9.54)	19.66 (17.38–22.96)	24.89 (21.49–30.18)	3.550 ± 0.310	2.198 (0.532)
	NLC-CLA-CS	6.08 (5.02–6.96)	13.76 (11.98–16.79)	17.34 (14.61–22.60)	3.613 ± 0.476	2.208 (0.530)

Table 11
Lethal concentrations (ppm) of the active ingredients and their conjugate nanoformulations on the mortality of *Culex pipiens* larvae at pH 8 media, 24, and 48, h post-treatment (mean ± SE).

Time (hr)	Tested material	LC_{50} (Low-Up.)	LC_{90} (Low-Up.)	LC_{95} (Low-Up.)	Slope ± SE	Chi (Sig.)
24	α-terpinene	25.99 (23.02–29.37)	83.31 (69.03–105.81)	115.90 (92.75–154.61)	2.533 ± 0.188	4.680 (0.196)
	Linalool	37.31 (32.76–42.85)	133.02 (105.78–179.66)	190.73 (145.41–27,354)	2.321 ± 0.183	2.878 (0.410)
	Curcumin	70.14 (58.79–87.53)	313.07 (217.23–528.89)	478.42 (311.59–889.35)	1.972 ± 0.192	0.677 (0.878)
	α-terpinene - Linalool	19.09 (17.00–21.37)	54.21 (46.06–66.52)	72.88 (60.12–93.30)	2.827 ± 0.208	5.606 (0.132)
	α-terpinene - Curcumin	22.08 (15.52–31.00)	67.34 (51.87–129.29)	92.36 (70.86–199.73)	2.647 ± 0.195	8.392 (0.038)
	Linalool - Curcumin	27.65 (24.47–31.28)	89.40 (73.78–114.22)	124.69 (99.32–167.47)	2.514 ± 0.187	2.944 (0.340)
	α-terpinene- Lin - Cur	16.53 (14.65–18.55)	47.96 (40.70–58.99)	64.86 (53.37–83.43)	2.770 ± 0.210	4.346 (0.226)
	NLC-CLA-CS	10.57 (9.32–11.83)	26.87 (23.14–32.65)	35.02 (29.27–44.55)	3.161 ± 0.280	2.363 (0.500)
48	α-terpinene	15.79 (14.04–17.66)	43.61 (37.23–53.24)	58.16 (48.22–74.10)	2.904 ± 0.222	4.636 (0.200)
	Linalool	23.62 (20.96–26.57)	90.57 (63.45–93.98)	105.09 (85.43–136.69)	2.537 ± 0.178	3.200 (0.631)
	Curcumin	41.62 (36.05–48.78)	172.62 (131.24–249.71)	258.35 (186.61–402.41)	2.074 ± 0.175	2.168 (0.538)
	α-terpinene - Linalool	12.64 (11.05–14.26)	39.03 (33.74–45.57)	53.72 (45.16–66.76)	2.618 ± 0.187	0.768 (0.856)
	α-terpinene - Curcumin	15.64 (13.82–17.59)	46.38 (39.28–57.26)	63.12 (51.78–81.61)	2.715 ± 0.210	2.026 (0.567)
	Linalool - Curcumin	18.81 (13.58–25.47)	51.55 (40.25–90.80)	68.62 (53.27–133.85)	2.926 ± 0.215	7.868 (0.048)
	α-terpinene- Lin - Cur	10.33 (9.04–11.62)	27.48 (23.56–33.57)	36.26 (30.13–46.53)	3.015 ± 0.269	2.861 (0.413)
	NLC-CLA-CS	7.55 (6.50–8.51)	17.77 (15.40–21.60)	22.64 (19.03–29.08)	3.448 ± 0.376	2.063 (0.559)

Table 12
The mean number (± SE) of *Culex pipiens* larvae consumed by some predators treated with the active ingredients and their nanoformulation under laboratory conditions.

Parameters	Insects	Tested materials				
		Control	α-terpinene	Linalool	Curcumin	NLC-CLA-CS
Predation	*S. longicornis*	46.00 ± 1.15^{cA}	44.67 ± 0.88^{cAB}	43.67 ± 0.67^{cC}	45.33 ± 0.33^{cA}	44.33 ± 0.33^{cB}
	C. tripunctatus	59.00 ± 1.53^{bC}	64.00 ± 1.00^{bA}	63.67 ± 1.20^{bA}	61.33 ± 0.67^{bB}	46.00 ± 1.15^{bD}
	S. urinators	24.00 ± 0.00^{dD}	27.00 ± 0.58^{dB}	25.00 ± 0.58^{dC}	24.67 ± 0.67^{dCD}	45.17 ± 0.65^{bA}
	G. affinis	81.00 ± 1.00^{aA}	74.00 ± 1.15^{aC}	72.00 ± 1.00^{aD}	78.00 ± 1.53^{aB}	64.67 ± 0.67^{aE}
Survival	*S. longicornis*	10.00 ± 0.00^{aA}	9.67 ± 0.33^{bB}	9.33 ± 0.33^{cC}	10.00 ± 0.00^{aA}	9.00 ± 0.58^{cD}
	C. tripunctatus	9.67 ± 0.33^{bB}	9.33 ± 0.33^{cC}	9.33 ± 0.33^{cC}	10.00 ± 0.00^{aA}	9.33 ± 0.33^{bC}
	S. urinators	10.00 ± 0.00^{aA}	10.00 ± 0.00^{aA}	10.00 ± 0.00^{aA}	10.00 ± 0.00^{aA}	9.67 ± 0.33^{aB}
	G. affinis	10.00 ± 0.00^{aA}	9.00 ± 0.58^{dB}	9.00 ± 0.58^{dB}	10.00 ± 0.00^{aA}	8.67 ± 0.33^{dB}
	E. tenax	10.00 ± 0.00^{aA}	10.00 ± 0.00^{aA}	9.67 ± 0.33^{bB}	10.00 ± 0.00^{aA}	9.33 ± 0.33^{bB}
	Ephydra sp.	10.00 ± 0.00^{aA}	9.33 ± 0.33^{cB}	9.00 ± 0.00^{dB}	10.00 ± 0.00^{aA}	8.67 ± 0.33^{dB}

a, b & c: There is no significant difference ($P > 0.05$) between any two means, within the same column have the same superscript letter; A & B: There is no significant difference ($P > 0.05$) between any two means, within the same row have the same superscript letter.

compared to the control (45.17 and 24 larvae, respectively). Furthermore, results showed that most predators maintained high survival rates for 48 h with slight variation. Reduced survival rates were observed for some species when using nanocapsule (NLC-CLA-CS) in tested cups, where the survival rate of *G. affinis* ($n = 8.67$) and *Ephydra* sp. ($n = 8.67$) decreased compared to a control rate ($n = 10.0$). This reduction indicates the potential toxicity of this nanoformulation towards some sensitive aquatic insects. On the other hand, *Sphaerodema urinator*s and *Stratiomys longicornis* showed no significant effect on survival.

4. Discussion

Although scientific understanding of insects has advanced considerably, the demand for insecticides continues to rise, largely due to the growing resistance these pests have developed against existing chemical agents. Moreover, the ecological harm caused by synthetic insecticides has necessitated limiting their application, especially at higher concentrations, because of their toxicity to the environment. In response, there has been a notable shift towards exploring natural, eco-friendly alternatives for pest control [38]. In this context, lipid-chitosan nanocapsules incorporating curcumin and co-delivered with linalool and α-terpinene (NLC-CLA-CS) were prepared through a two-step process. First, nanostructured lipid carriers (NLCs) with curcumin, linalool, and α-terpinene were made by mixing them at high temperatures and then using ultrasound waves. The above process was then followed by encapsulating the NLCs with a chitosan layer through the addition of sodium tripolyphosphate (STPP), a safer crosslinking agent, and usually used in the food industry according to the United States Food and Drug Administration.

FTIR study was performed to characterize the chemical structure of each single component separately and combine them at the same nanostructure lipid carrier, and consequently, its chitosan-coated nanostructure lipid carrier. To determine the interactions of excipients with the drugs, firstly, the FTIR of the pure drugs curcumin, linalool, and a-terpinene were analyzed. The FTIR spectrum of curcumin revealed broad absorption at 3425 cm^{-1} for the OH (hydroxyl), C═O (ketone): around 1628 cm^{-1}, and C—O (enol): 1245 cm^{-1}. Furthermore, FTIR spectrum of the linalool includes 3380 cm^{-1} for the (OH), C═C (alkene) at 1675 cm^{-1}, and C—H (alkyl) at 2950 cm^{-1}. Also, FTIR spectrum of the a-terpinene contains C—H stretching at 2880 cm^{-1}, C═C stretching at 1650 cm^{-1}, and C—H bending at 1580 cm^{-1}. Meanwhile the nanostructure lipid carrier before loading (free) is composed of steric acid, oleic acid, tween 20, and sodium glycocholate. Such components have comparable and similar bands: carboxylic acid O—H stretching around 2500–3300 cm^{-1}, (stearic and oleic acids), C—H stretching: around 2800–3000 cm^{-1}, (stearic and oleic acids), C═O stretching: round 1690–1710 cm^{-1} (stearic and oleic acids), and C—H bending: around 1400–1500 cm^{-1}. After loading, the NLC-CLA spectrum differs a little, and most of the significant peaks of each single component exist in addition to a broad band of the hydroxyl group (-OH) in an FTIR spectrum, which typically indicates the presence of hydrogen bonding or intermolecular interactions involving the hydroxyl group. Also, after chitosan-coating, NLC-CLA-CS FTIR spectrum revealed O—H and N—H stretching at 3550 cm^{-1}, C—H stretching at 2910 cm^{-1}, Amide *C*═O stretching, and 1680 cm^{-1} confirmed the succession of coating NLC with chitosan polymer [39]-

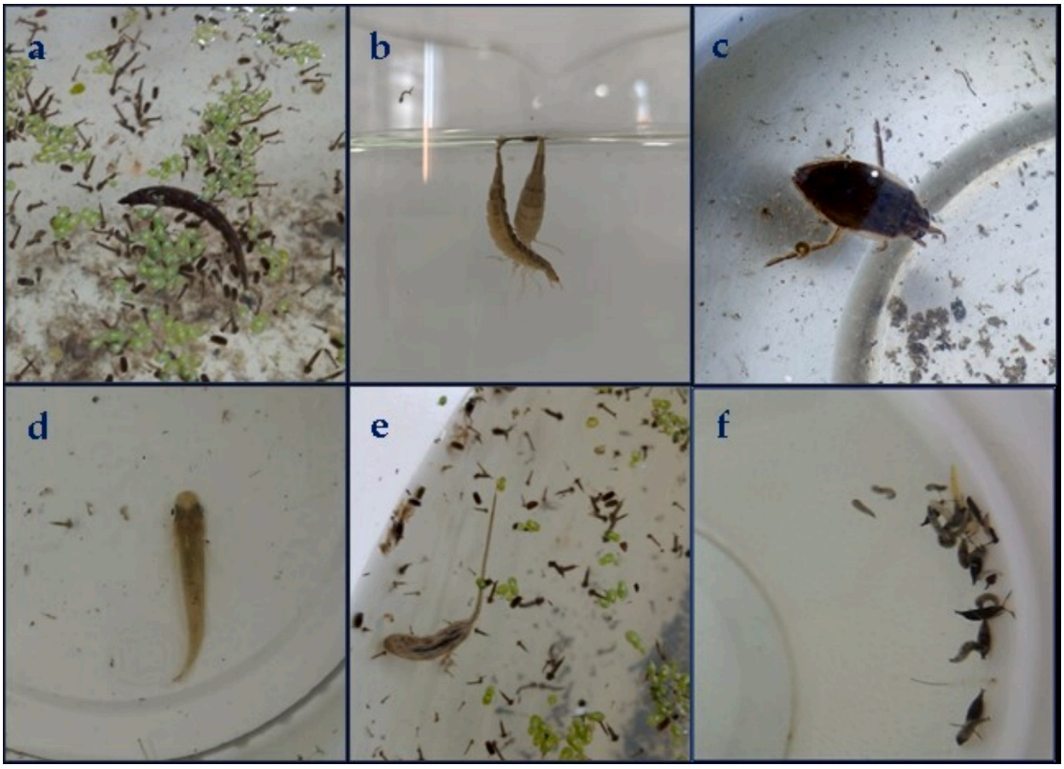

Fig. 8. Comparative efficacy of the tested active ingredients and their nanoformulations against non-target predatory species: *Stratiomys longicornis* (a), *Cybister tripunctatus* (b), *Sphaerodema urinator* (c), *Gambusia affinis* (d), *Eristalis tenax* (e), and *Ephydra* sp. (f).

Determining the size of nanomaterials is a crucial parameter, as it influences not only their behavior but also their functional mechanisms and overall effectiveness. In the case of NLC-CLA, dynamic light scattering (DLS) analysis indicated that the average particle size was below 100 nm prior to chitosan encapsulation. Additionally, the polydispersity index (PDI) (which reflects the variation in particle size) was found to be above 0.2, indicating a relatively broad size distribution. This type of size heterogeneity is commonly observed in nanostructured lipid systems, as reported in several earlier studies [29,32,40].

After covering the lipid-based particles with chitosan, the average size of the particles increased significantly (over 150 nm) because a coating layer formed around them. This process also showed an increase in the polydispersity index (PDI), meaning there was more variation in size and differences among the particles. These changes signify the effectiveness of the encapsulation and indicate a clear alteration in particle morphology. Measuring the surface charge of these nanoparticles is also important, as it offers information about their stability; the farther the charge value is from zero, whether positive or negative, the stronger the electrostatic repulsion between particles, which helps prevent aggregation, which reduces the chances of flocculation and thus keeps the particles separated, which ensures greater stability for longer periods [26]. We found that NLC-CLA particles were characterized by a type of stability that reflected the negative zeta value of −7.37 mV. However, after the encapsulation process, we observe a complete reversal of the surface charge value to become more electropositive. Such a large positive value confirmed the successful occurrence of the encapsulation process. In the beginning, the NLC-CLA, which was composed of carboxylic acid ends, was predominantly negatively charged, so the zeta value was negative. The NLC-CLA-CS formulation exhibited a shift in zeta potential to a more positive value, indicating successful surface modification with a chitosan layer around the NLC-CLA nanoparticles [41,42].

The particle shape analysis revealed valuable insights into the formation and stability of nanoparticles. Both NLC-CLA and NLC-CLA-CS nanoparticles were predominantly spherical or oval in shape, with sizes under 200 nm, consistent with DLS results. Notably, the uncoated NLC-CLA particles displayed a uniform spherical morphology, whereas chitosan coating led to a morphological shift towards an oval shape and a slight size increase, likely due to the uniform distribution of chitosan on the particle surface [26].

Being NLC-CLA is the first step of preparation, followed by coating with chitosan, so the encapsulation efficiency (EE) and drug loading capacities (LC) of the incorporated curcumin, linalool and α-terpinene will be measured in the NLC-CLA nanoparticles. The total incorporated percentage of curcumin, α-terpinene, and linalool was performed with with an encapsulation efficiency (EE) exceeding 70 %. Whereas the loading capacity (LC) was found to be between 10-15 % for each single active ingredient. Such high EE and LC ratios indicate high compatibility between drug and carrier due to electrostatic attraction and donor–receptor coordination between drug molecules and carrier material [43,44].

Chitosan is a preferred material for encapsulating lipid nanostructures, due to its high safety rating, not only for humans but also for animals and the environment. Its natural origin has made it a target for researchers as well as its biodegradability, non-toxic, controlled drug release, and compatibility. The designated nanocapsule, combined with the lipid nps which contain curcumin, linalool, and α-terpinene active ingredients. The drug release study was conducted directly using our target nanocapsule NLC-CLA-CS, which contains curcumin, linalool, and α-terpinene, fully immersed in the vicinity of the NLC lipid matrix, consequently coated with chitosan.

At first, curcumin release was studied using both acidic and basic mediums. In the acidic medium, chitosan, a weak polybasic biopolymer, started to protonate due to the presence of amino groups, and the polymer became more positively charged. As a result, chitosan became more swollen and consequently more soluble and that permits active ingredients to release faster [45,46]. This explains the increased concentrations of curcumin released at the beginning of the study, during

the first two days, and then starting on the third day, the amount of the drug released begins to decrease significantly until we reach the fourth day, when curcumin release continues at a much lower rate. In the alkaline medium (7.5–8), chitosan chains became less protonated (lost their positive charges) and less soluble, leading to chitosan chain aggregation and a compact structure. Such behavior potentially slows down the drug release.

In other words, the chitosan coating slowed down the release of the active ingredients, resulting in a reduced concentration of detected compounds. This delayed release profile explains the decreased release rate of curcumin from the chitosan-coated formulation, particularly when compared to the faster release observed in acidic medium. Not all chitosan chains are necessarily negatively charged; in most cases, some chitosan amino groups have both positive and negative charges, especially if the medium is weakly basic, as in this study. The formation of zero charges on chitosan chains makes them more soluble, but at a slower rate than in the weak acidic mediums. This low solubility results in slower but longer-term release of compounds from the capsule, which explains the results obtained in the weak basic conditions. Unlike the weak acidic condition, the release of small concentrations of active ingredients at the beginning of the study, which increase over time, [47–49].

The release of both linalool and alpha terpinene in acidic media by GC/MS did not differ significantly from the results obtained by curcumin. The released concentrations began to increase to reach their maximum value within the first 24 h (36 h for α-terpinene) and the release of the drugs continued decreasing until reaching the lowest concentration on the fourth day for both compounds, after most of the loaded amount had been released.

Atomic Force Microscopy (AFM) is an excellent tool for characterizing nanoparticles due to its ability to provide high-resolution, three-dimensional images of their size, morphology, and surface texture. Three- dimensional AFM illustrations presented in Figs. 9 and 10 manifested that the nanostructure lipid carrier loaded curcumin, linalool and a-terpinene (NLC-CLA) have particle size in the nanoscale. The particles exhibited a solid and rough topography. Such roughness confirms the successful preparation of the nanostructure lipid carrier, because of the sudden crystallization of its main component (solid lipids), during the preparation process. The Root Mean Square Roughness (sq) value of 9.08 nm, indicates the presence of a significant percentage of particles with an irregular spherical structure. This is consistent with both the DLS and the polydispersity index (PDI), as well as with the results of the transmission electron microscope. Perhaps one of the advantages of this roughness is the increased surface area, which enhances interaction with the surrounding environment and leads to improved adhesion to the target cells or tissues. Conversely, this roughness may lead to negative effects such as the instability of these particles. This explains and confirms the results we obtained from the zeta potential values, which were barely moving away from zero towards negative by a factor of 7.

On the other hand, Encapsulation with chitosan (NLC-CLA-CS) resulted in, the formation of capsules in the nanoscale range with a slight size increase. AFM images confirmed a thin layer of chitosan coated the nanostructure lipid particles. The chitosan layer reduced surface roughness to 5.91 nm, resulting in a smoother surface [39,50]. This

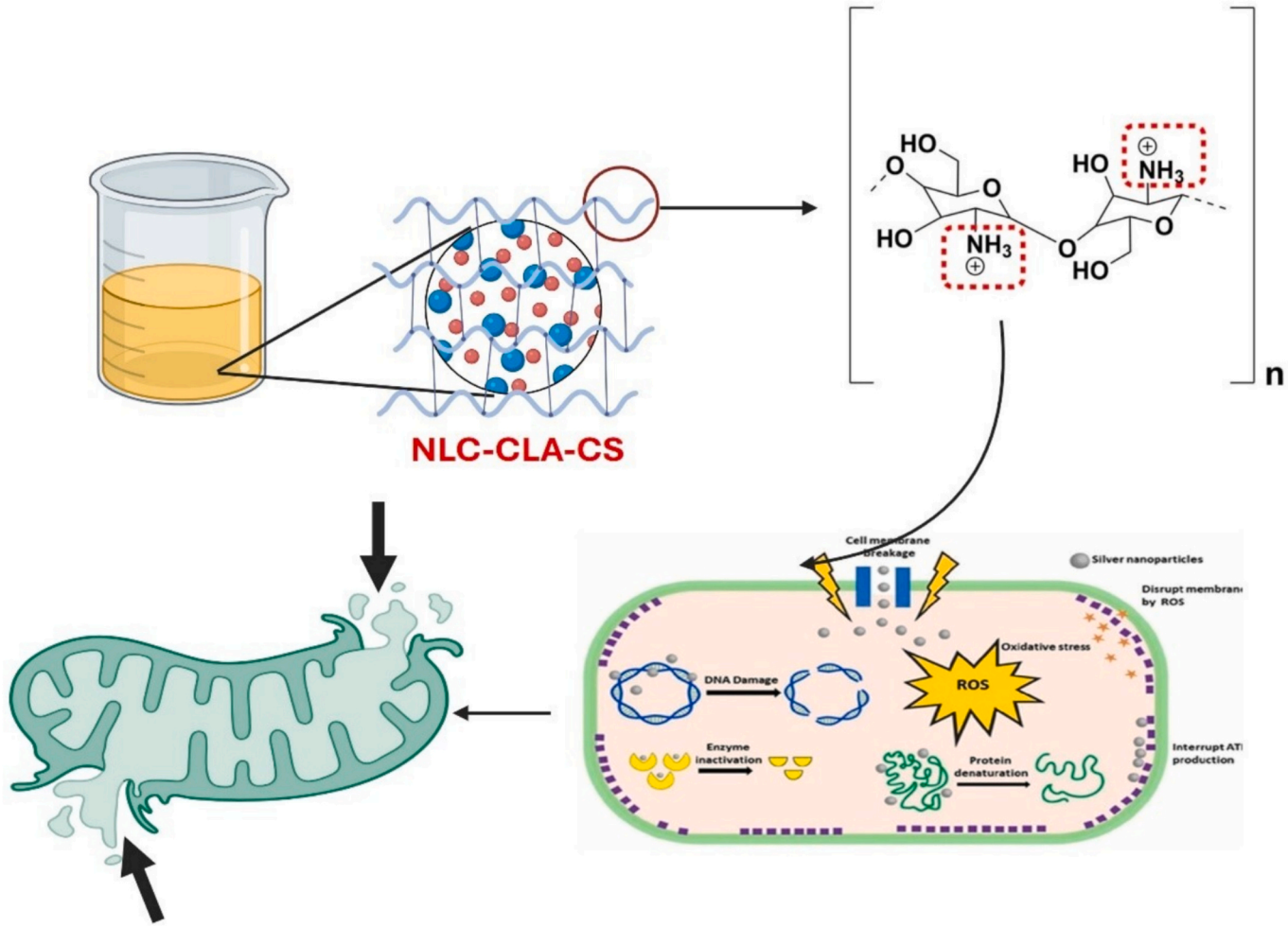

Fig. 9. Suggested mode of action of the synthesized nanocapsule, NLC-CLA-CS, and their active ingredients in antimicrobial activity.

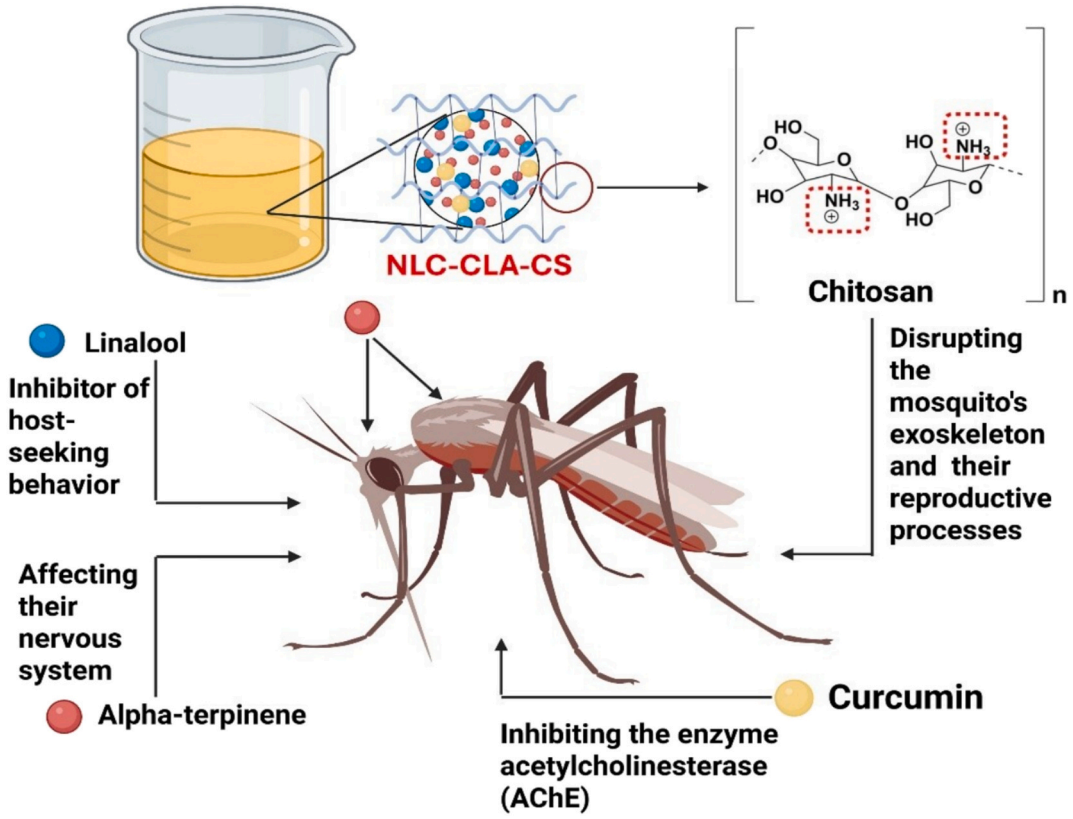

Fig. 10. Suggested mode of action of the synthesized nanocapsule, NLC-CLA-CS, to get rid of culex mosquito.

increased stability and consistent with the zeta potential values post-encapsulation.

Culex larvae thrive in both fresh, sewage and agricultural wastewater, which are often infested with various bacterial strains such as *Escherichia coli*, *Bacillus subtilis*, and other pathogens. The prepared nanocapsules were tested against five ATCC laboratory bacterial strains to measure their antimicrobial activity. Both nanomaterials were highly effective against each strain to varying degrees due to the existence curcumin, but also linalool and α-terpinene, and their antimicrobial properties [51,52]. After the encapsulation with chitosan, a significant increase in the biological activity of the various bacterial strains was observed. The MIC value also decreased significantly, reflecting the effectiveness of chitosan. This is due to a simple mechanism of action involving electrostatic interactions between the positively charged NH^{3+} sites in chitosan and the negatively charged microbial cell membranes. This interaction alters the permeability of the microbial cell (Fig. 9), leading to the release of intracellular substances [53,54].

The importance of any synthetic chemical compound lies in its effectiveness against the biological target it is designed to combat. However, these synthetic materials are always highly toxic to the living cells of the host, and they are intended to protect against a specific threat. The synthesized nanocapsules were designed to achieve safety levels against marine organisms, aquatic systems, and the primary target of care: humans. These materials are constructed from completely inert materials, represented by a mixture of fatty acids as a primary protective layer for the active compounds, preventing them from coming into direct contact with living cells until the compounds are released. To increase safety, these fatty materials were encapsulated in a thin layer of chitosan polymer, which is taken from natural sources and is completely safe for the human and aquatic system. Therefore, when the toxicity of such nanocomposites was measured on non-cancerous Wi38 cells, the results indicated very little toxicity associated with both nanomaterials, both before and after the encapsulation process with chitosan. The results also confirmed that the IC_{50} value increased several times in the case of nanomaterials compared to the individual compounds.

Natural producta and essential oils (EOs) e.g., linalool are widely used for their ability to kill bacteria, fungi, mites, and insects, and they are also important in medicine and cosmetics [55,56]. Historically, they have also been used as natural insect repellents in regions such as China and Arab countries, making them a valuable source for identifying novel natural repellent compounds. The chemical compositions and concentrations of the natural products and /or essential oils are significantly influenced by factors such as their geographical source, the part of the plant used, and the method of extraction. Since the effectiveness of the natural products is closely tied to their chemical composition, it is essential to identify the active ingredients [57].

This study aimed to test how well some natural substances, like curcumin, α-terpinene, and linalool, and their tiny particles work in solutions with pH 6 and pH 8 against *Cx. pipiens* larvae. The semi-acidic medium (pH 6) improved the efficacy of all tested materials. The best larvicide was the mixture of the three natural products (α-terpinene, linalool, and curcumin) in nanoform, followed by the insecticide mix of the same oils; then the combination of α-terpinene and linalool; and lastly, α-terpinene by itself, with LC_{50} values of 19.17, 26.43, 61.33, 15.75, 20.83, 21.89, 12.55, and 8.78 ppm at 24 h after treatment, and 12.35, 17.51, 29.54, 9.47, 11.82, 15.69, 8.56, and 6.08 ppm at 48 h.

Our findings are consistent with earlier research that reported linalool had a significant toxic effect on *Cx. pipiens* larvae, with an LC_{50} value of 14.87 μg/mL [58]. Similarly, curcumin exhibited strong activity against *Cx. pipiens* and *Ae. albopictus*, with LC_{50} values of 6.0 and 9.2 ppm, respectively [59]. Therefore, our results are in harmony with other results that recorded that the LC_{50} levels for linalool and γ-terpinene in *Musca domestica* adults were significantly lower following pre-treatment with piperonyl butoxide (PBO), indicating increased toxicity. This suggests that these compounds are normally metabolized by cytochrome P450 enzymes, which are inhibited by PBO [60].

pH-responsive nanocapsules have been used against mosquito larvae in some limited studies, but use in multiple aquatic media (e.g., different acidity and alkalinity or in fresh and polluted water) is still relatively limited.

In a similar study, Xue, Zhu, Wei, Peng, Wang, Li, Ma, Wu, He and Qian [61] developed pH-sensitive nanocapsules containing the fungicide prochloraz using the Pickering emulsion process. These capsules were better at releasing the active ingredient in acidic conditions, making them more useful for controlling aquatic insects in very acidic waters. Although this technology does not directly target *Culex pipiens* mosquitoes, it is applicable to other mosquito species. In another study, researchers evaluated the toxicity of chitosan and silica nanoparticles loaded with deltamethrin, which showed improved efficacy against *Culex* larvae. The results showed an increase in toxic efficacy against *Cx. pipiens* larvae compared to the conventional pesticide [62–66].

The mechanism of action of pH-responsive nanocapsules depends on their ability to change physically or chemically in response to variations in the pH of the surrounding medium. They remain relatively stable in neutral or alkaline water but begin to decompose or swell when the pH drops, releasing the active compounds contained within. This targeting makes them particularly effective in relatively acidic environments, which are often breeding grounds for *Culex pipiens* larvae. They absorb compounds like curcumin and monoterpenes through the body wall or enter the larval digestive tract during feeding. These active compounds interfere with physiological pathways within the larvae. Curcumin causes an imbalance in the stress levels inside cells, while monoterpenes block the enzymes that help transmit signals in the nervous system, which can result in paralysis and death [62] (Fig. 10). The nanosystem design also allows the active ingredients to be released slowly over time, making the toxic effect last longer and cutting down on the need for multiple treatments. Furthermore, the pH-based property reduces side effects on non-target organisms in aquatic environments.

Biopesticides derived from natural products and / or essential oils are known for being environmentally sustainable [67]. However, they degrade rapidly when exposed to uncontrollable environmental factors such as heat, light, and humidity. To address this limitation, the natural products have been developed into nanoformulations e.g., nanoemulsions (NEOs), nanostructure lipid carrier (NLC), and liposomes (LPs) offering multiple benefits. Compared to traditional insecticides, nanoparticle-based insecticides consist of tiny particles with enhanced stability and a larger surface area. Like their original form, nanoparticle-based insecticides are also environmentally safe. This formulation represents a modern approach to pesticide delivery. Notably, nanoparticle-based insecticides can be produced without the need for high-cost processes and the synthesis simply involves mixing the solid and liquid lipids and oils with surfactants and water in optimal ratios to achieve efficient and cost-effective results [68]. These findings align with our results, which demonstrated that the most effective larvicide was the nanocapsule blend composed of the three active ingredients: α-terpinene, linalool, and curcumin.

The study indicated that the average predation rate was similar across all the active materials tested, indicating that such active ingredients mixtures are safe for non-target organisms, but different predator species responded differently to these materials. *Gambusia* mosquitofish had the slightly decreased predation rate in all tests, but in the case of nanoformulation included α-terpinene, Linalool, and curcumin and coated with chitosan (NLC-CLA-CS) it had significantly reduced its predation rate [25]. On the other hand, *Stratiomys* and *Cybister* did not show any major changes in their health, indicating that these species might be better at handling or less influenced by the oils when they feed. In contrast, *Stratiomys* and *Cybister* did not show any significant changes in their predation, suggesting that these species may be more resistant or less affected by tested materials in their feeding behavior. Overall, most species showed relatively high survival rates, indicating that the oils were not lethally toxic to predators over the two-day period, with some minor effects for *Gambusia* and *Ephydra*. On the other hand, the other species were not significantly affected.

5. Conclusions

The results of this study showed that the the active ingredient (curcumin, α-terpinene, and linalool) and their conjugate nanocapsules are effective at killing *Culex pipiens* larvae. The different plant chemicals in some plants could kill larvae, providing a safe and effective alternative to chemical pesticides and biotechnological methods. The effectiveness of these agents was found to depend on several factors, including the applied concentration, length of exposure, and the pH of the surrounding medium. Increased concentrations and prolonged exposure improved larvicidal performance. Notably, all tested substances showed enhanced activity in a mildly acidic environment (pH 6) because of the accumulation of active ingredients released when chitosan starts to become soluble. Among the formulations, the nanoformulation containing all three active ingredients (NLC-CLA-CS) demonstrated the highest efficacy. These results suggest that such nanoformulations could serve as viable, eco-friendly alternatives to traditional chemical insecticides. It is also undeniable that insects are sensitive to the nanoformulation in a weakly alkaline medium, but to a lesser extent, since the active ingredients are released more slowly and over a longer period. The results also prove that the nanoformulation is effective in both weakly alkaline and weakly acidic media. Perhaps if the acidity increased to stronger media, the results would be completely different, depending on the chemistry of the chitosan coating polymer. Overall, by creating a new type of pesticide that responds to pH levels, we hope to provide an eco-friendlier way to control pests that is less harmful to other insects, making pesticide use more effective.

6. Future prospective

Nanostructure lipid carriers (NLCs) have revolutionized drug delivery systems, offering enhanced stability, bioavailability, skin targeting, and safer insecticides due to its natural constituents. The growing interest in lipid carrier and polymeric-based nanoacpsule systems has led to significant advancements, with over 30 commercial NLC formulations currently available. NLCs and their chitosan-coated NLCs provided prolonged release, occlusive effects and improved drug incorporation and the chitosan-coated provides additional protection in addition to being selective to release its component at a specific pH. Such functions make the promising candidate for various applications. With their qualified scale-up technology (NLC), GRAS status of excipients, and ease of large-scale production using High-Pressure Homogenization method, Membrane Contactor Technique and Microemulsion Technique, lipid nanocarriers are gaining industrial attention. Future research should focus on assessing toxicity and health hazards associated with nanostructure lipid carriers as insecticides and their impact on freshwater aquatic life.

CRediT authorship contribution statement

Ibrahim Taha Radwan: Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Noha Bagato:** Writing – original draft, Visualization, Software, Project administration, Funding acquisition, Data curation. **Shaimaa H. Mohammed:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Data curation. **Hattan S. Gattan:** Writing – original draft, Visualization, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Mohammed H. Alruhaili:** Writing – original draft, Validation, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Abeer Mousa Alkhaibari:** Writing – original draft, Visualization, Supervision, Methodology, Investigation, Funding acquisition, Data curation. **Abdelfattah Selim:** Writing – original draft, Validation, Investigation, Funding acquisition, Data

curation, Conceptualization. **Mohamed M. Baz:** Writing – original draft, Visualization, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Consent for publication

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Declaration of competing interest

The authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2025.147697.

Data availability

This article contains all of the data that was created or analyzed throughout the investigation.

References

[1] M.F. Ahmad, F.A. Ahmad, A.R.A. Alsayegh, M. Zeyaullah, A.M. AlShahrani, K. Muzammil, A.A. Saati, S. Wahab, E.Y. Elbendary, N. Kambal, M. H. Abdelrahman, S. Hussain, Pesticides impacts on human health and the environment with their mechanisms of action and possible countermeasures, Heliyon 10 (2024).
[2] M. Abd Elmohsen, A. Selim, A.E. Abd Elmoneim, Prevalence and molecular characterization of lumpy skin disease in cattle during period 2016-2017, Benha Vet. Med. J. 37 (1) (2019) 172–175.
[3] A.S. Hamdy, A. Selim, S.A. Shoulah, A.M.M. Ibrahim, Sero-surveillance infectious bovine rhinotracheitis in ruminants and assessment the associated risk factors, Benha Vet. Med. J. 42 (2) (2022) 160–163.
[4] M. Nuruzzaman, M.M. Rahman, Y. Liu, R. Naidu, Nanoencapsulation, Nano-guard for Pesticides: A New Window for Safe Application, J. Agric. Food Chem. 64 (7) (2016) 1447–1483.
[5] W.I. Ahmed, M. Elshaier, G. E.AA, Toward efficient and safe control strategy against cotton leaf worm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) applying onion and pepper extracts and their oils, Al-Azhar Bull. Sci. 12 (2021) 9–15.
[6] R. Emam, O.A.A. Zedan, Efficacy of certain insecticides and alternative agrochemicals in controlling aphids and their side effects on the predator *Coccinella undecimpunctata* in Eggplant fields, Al-Azhar J. Agric. Res. 48 (2) (2023) 163–169.
[7] T.A. Selim, S.H. Ragab, S.A. Riad, R.I. Eltaly, S.H. Mohammed, S.E. Sharawi, N. A. Alkenani, R. Almahallawi, H.S. Al-Rashidi, M.A.M. El-Tabakh, Abundance, diversity and distribution of mosquito species and molecular detection of its associated Hepatitis C virus in Sharkia Governorate, Egypt, Insects 16 (2025).
[8] P. Hemalatha, D. Elumalai, A. Janaki, Babu Muthu, K. Velu, K. Velayutham, P. K. Kaleena, Larvicidal activity of *Lantana camara* aculeata against three important mosquito species, J. Entomol. Zool. Stud. 3 (2015) 174–181.
[9] M. Sudo, D. Takahashi, D.A. Andow, Y. Suzuki, T. Yamanaka, Optimal management strategy of insecticide resistance under various insect life histories: heterogeneous timing of selection and interpatch dispersal, Evol. Appl. 11 (2017) 271–283.
[10] T.M. Clark, B.J. Flis, S.K. Remold, pH tolerances and regulatory abilities of freshwater and euryhaline Aedine mosquito larvae, J. Exp. Biol. 207 (13) (2004) 2297–2304.
[11] A.C. Ukubuiwe, C.C. Ojianwuna, I.K. Olayemi, F.O. Arimoro, C.C. Ukubuiwe, Quantifying the roles of water pH and hardness levels in development and biological fitness indices of *Culex quinquefasciatus* Say (Diptera: Culicidae), J. Basic Appl. Zool. 81 (2020) 1–10.
[12] L.C. Multini, R. Oliveira-Christe, A.R. Medeiros-Sousa, E. Evangelista, K.M. Barrio-Nuevo, L.F. Mucci, W. Ceretti-Junior, A.A. Camargo, A.B.B. Wilke, M.T. Marrelli, The influence of the pH and salinity of water in breeding sites on the occurrence and community composition of immature mosquitoes in the Green Belt of the city of São Paulo, Brazil, Insects 12 (9) (2021) 797.
[13] R.S. Kookana, A.B.A. Boxall, P.T. Reeves, R. Ashauer, S. Beulke, Q. Chaudhry, G. Cornelis, T.F. Fernandes, J. Gan, M. Kah, I. Lynch, J.L. Ranville, C.J. Sinclair, D. J. Spurgeon, K. Tiede, P.J. van den Brink, Nanopesticides: guiding principles for regulatory evaluation of environmental risks, J. Agric. Food Chem. 62 (19) (2014) 4227–4240.
[14] J.L. de Oliveira, E.V.R. Campos, M. Bakshi, P.C. Abhilash, L.F. Fraceto, Application of nanotechnology for the encapsulation of botanical insecticides for sustainable agriculture: prospects and promises, Biotechnol. Adv. 32 (8) (2014) 1550–1561.
[15] M.S.E.-d. Salem, A.Y. Mahfouz, R.M. Fathy, The antibacterial and antihemolytic activities assessment of zinc oxide nanoparticles synthesized using plant extracts and gamma irradiation against the uro-pathogenic multidrug resistant *Proteus vulgaris*, BioMetals 34 (2020) 175–196.
[16] A.E. Asbahani, K. Miladi, W. Badri, M. Sala, E.H.A. Addi, H. Casabianca, A. E. Mousadik, D. Hartmann, A. Jilale, F.N.R. Renaud, A. Elaissari, Essential oils: from extraction to encapsulation, Int. J. Pharm. 483 (1–2) (2015) 220–243.
[17] C. Cimino, O.M. Maurel, T. Musumeci, A. Bonaccorso, F. Drago, E.B. Souto, R. Pignatello, C. Carbone, Essential oils: pharmaceutical applications and encapsulation strategies into lipid-based delivery systems, Pharmaceutics 13 (2021).
[18] M. Hamidi, A. Azadi, P. Rafiei, Hydrogel nanoparticles in drug delivery, Adv. Drug Deliv. Rev. 60 (15) (2008) 1638–1649.
[19] H. Jonassen, A.L. Kjøniksen, M. Hiorth, Effects of ionic strength on the size and compactness of chitosan nanoparticles, Colloid Polym. Sci. 290 (2012) 919–929.
[20] J. Choubey, A.K. Bajpai, Investigation on magnetically controlled delivery of doxorubicin from superparamagnetic nanocarriers of gelatin crosslinked with genipin, J. Mater. Sci. Mater. Med. 21 (2010) 1573–1586.
[21] L. Keawchaoon, R. Yoksan, Preparation, characterization and in vitro release study of carvacrol-loaded chitosan nanoparticles, Colloids Surf. B: Biointerfaces 84 (1) (2011), 163–71.
[22] N. RabindraK, S.S. Patil, D.A. Navathar, Chiotsan Nanoparticles Loaded with Thiocolchicoside, 2012.
[23] P. Ganesan, D. Narayanasamy, Lipid nanoparticles: Different preparation techniques, characterization, hurdles, and strategies for the production of solid lipid nanoparticles and nanostructured lipid carriers for oral drug delivery, Sustain. Chem. Pharm. 6 (2017) 37–56.
[24] E. Gomaa, H.A. Fathi, N.G. Eissa, M. Elsabahy, Methods for preparation of nanostructured lipid carriers, Methods 199 (2022) 3–8.
[25] I.T. Radwan, N. Bagato, M.S. Ebaid, M.M. Hegazy, M.A. Farghali, A. Selim, H. S. Gattan, M.H. Alruhaili, M.M. Baz, A.M. Alkhaibari, Synthesis of eco-friendly lipid-magnetite nanocomposite encapsulated *Poinciana* extract as promising insecticide against *Culex pipiens*, Sci. Rep. 14 (1) (2024) 1–21.
[26] I.T. Radwan, N. Bagato, M.M. Hegazy, M.M. Baz, H.S. Gattan, M.H. Alruhaili, A. M. Mashlawi, A.M. Alkhaibari, S.M. Alasmari, A. Selim, Synthesis of eco-friendly nanostructured lipid carriers decorated with magnetic nanoparticle encapsulated *Sesbania sesban* extract against vector borne *Culex pipiens* (Diptera: Culicidae) and *Musca domestica* (Diptera: Muscidae) as green insecticides, Entomol. Res. 55 (3) (2025) e70029.
[27] M.E. Salem, A.H. Elwahy, H.M. Hassaneen, A.M. Selim, H. Hashem, N. Bagato, I. T. Radwan, Design, synthesis, and in-silco ADME prediction of some novel bis (1, 3, 4-thiadiazoles) encapsulated lipid-chitosan nano capsule decorative with magnetic nanoparticles and their potential anti-helicobacter pylori activity, Int. J. Biol. Macromol. 296 (2025) 139746.
[28] O. Gartziandia, E. Herran, J.L. Pedraz, E. Carro, M. Igartua, R.M. Hernandez, Chitosan coated nanostructured lipid carriers for brain delivery of proteins by intranasal administration, Colloids Surf. B: Biointerfaces 134 (2015) 304–313.
[29] I.T. Radwan, I.M. El-Sherbiny, A.M. Selim, N.H. Metwally, Design, synthesis of some novel coumarins and their nanoformulations into lipid-chitosan nanocapsule as unique antimicrobial agents, Sci. Rep. 14 (1) (2024) 30598.
[30] M. Dawoud, Chitosan coated solid lipid nanoparticles as promising carriers for docetaxel, Journal of Drug Delivery Science and Technology 62 (2021) 102409.
[31] N.E. Elkholy, A.A. Sultan, S.E. Abu-Risha, G.M. El Maghraby, Chitosan coated lipid carriers as nanoplatform for repurposed anti-breast cancer activity of niclosamide, Journal of Drug Delivery Science and Technology 93 (2024) 105414.
[32] I.T. Radwan, I.M. El-Sherbiny, N.H. Metwally, Synergistic and potential antifungal properties of tailored, one pot multicomponent monoterpenes co-delivered with fluconazole encapsulated nanostructure lipid carrier, Sci. Rep. 14 (1) (2024) 14382.
[33] A. Espinel-Ingroff, B. Arthington-Skaggs, N. Iqbal, D. Ellis, M. Pfaller, S. Messer, M. Rinaldi, A. Fothergill, D. Gibbs, A. Wang, Multicenter evaluation of a new disk agar diffusion method for susceptibility testing of filamentous fungi with voriconazole, posaconazole, itraconazole, amphotericin B, and caspofungin, J. Clin. Microbiol. 45 (6) (2007) 1811–1820.
[34] A. Espinel-Ingroff, E. Canton, A. Fothergill, M. Ghannoum, E. Johnson, R. Jones, L. Ostrosky-Zeichner, W. Schell, D. Gibbs, A. Wang, Quality control guidelines for amphotericin B, itraconazole, posaconazole, and voriconazole disk diffusion susceptibility tests with nonsupplemented Mueller-Hinton agar (CLSI M51-A document) for nondermatophyte filamentous fungi, J. Clin. Microbiol. 49 (7) (2011) 2568–2571.
[35] N. Ganot, S. Meker, L. Reytman, A. Tzubery, E.Y. Tshuva, Anticancer metal complexes: synthesis and cytotoxicity evaluation by the MTT assay, Journal of visualized experiments: JoVE 81 (2013) 50767.
[36] M.M. Baz, Strategies for Mosquito Control, Benha University, Faculty of Science, 2013.
[37] A.A. Haggag, M.A. Mahmoud, A.S. Bream, M.S. Amer, Family Variation of Aquatic Insects and Water Properties to Assess Freshwater Quality in El-Mansouriya Stream, Egypt, African Entomology 26 (2018) 162–173.
[38] A. Khursheed, M.A. Rather, V. Jain, A.R. Wani, S. Rasool, R. Nazir, N.A. Malik, S. A. Majid, Plant based natural products as potential ecofriendly and safer biopesticides: A comprehensive overview of their advantages over conventional pesticides, limitations and regulatory aspects, Microb. Pathog. 173 (2022) 105854.

[39] T.H. Truong, K.P. Alcantara, B.P.I. Bulatao, F.N. Sorasitthiyanukarn, C. Muangnoi, N. Nalinratana, O. Vajragupta, P. Rojsitthisak, P. Rojsitthisak, Chitosan-coated nanostructured lipid carriers for transdermal delivery of tetrahydrocurcumin for breast cancer therapy, Carbohydr. Polym. 288 (2022) 119401.
[40] K. Krambeck, V. Silva, R. Silva, C. Fernandes, F. Cagide, F. Borges, D. Santos, F. Otero-Espinar, J.M.S. Lobo, M.H. Amaral, Design and characterization of Nanostructured lipid carriers (NLC) and Nanostructured lipid carrier-based hydrogels containing Passiflora edulis seeds oil, Int. J. Pharm. 600 (2021) 120444.
[41] N. Aibani, R. Rai, P. Patel, G. Cuddihy, E.K. Wasan, Chitosan nanoparticles at the biological interface: implications for drug delivery, Pharmaceutics 13 (10) (2021) 1686.
[42] M.L. Del Prado-Audelo, I.H. Caballero-Florán, J. Sharifi-Rad, N. Mendoza-Muñoz, M. González-Torres, Z. Urbán-Morlán, B. Florán, H. Cortes, G. Leyva-Gómez, Chitosan-decorated nanoparticles for drug delivery, Journal of Drug Delivery Science and Technology 59 (2020) 101896.
[43] L. Peng, X. Mei, J. He, J. Xu, W. Zhang, R. Liang, M. Wei, D.G. Evans, X. Duan, Monolayer nanosheets with an extremely high drug loading toward controlled delivery and cancer theranostics, Adv. Mater. 30 (16) (2018) 1707389.
[44] C. Liu, L. Lin, Z. Huang, Q. Wu, J. Jiang, L. Lv, X. Yu, G. Quan, G. Li, C. Wu, Novel inhalable ciprofloxacin dry powders for bronchiectasis therapy: mannitol–silk fibroin binary microparticles with high-payload and improved aerosolized properties, AAPS PharmSciTech 20 (2019) 1–11.
[45] Y. Pérez-Pacheco, B. Tylkowski, R. García-Valls, Chitosan Micro/Nanocapsules in Action: Linking Design, Production, and Therapeutic Application, Molecules 30 (2) (2025) 252.
[46] J. Kurczewska, Chitosan-based nanoparticles with optimized parameters for targeted delivery of a specific anticancer drug—a comprehensive review, Pharmaceutics 15 (2) (2023) 503.
[47] H. Zhang, Z. Gu, W. Li, L. Guo, L. Wang, L. Guo, S. Ma, B. Han, J. Chang, pH-sensitive O-carboxymethyl chitosan/sodium alginate nanohydrogel for enhanced oral delivery of insulin, Int. J. Biol. Macromol. 223 (2022) 433–445.
[48] J. Wu, X. Wang, H. Li, M. Qu, W. Sun, X. Yan, Z. Zhao, B. Li, A hollow chitosan-coated PLGA microsphere to enhance drug delivery and anticancer efficiency, Journal of Drug Delivery Science and Technology 73 (2022) 103482.
[49] R. Biswas, S. Mondal, M.A. Ansari, T. Sarkar, I.P. Condiuc, G. Trifas, L.I. Atanase, Chitosan and its derivatives as nanocarriers for drug delivery, Molecules 30 (6) (2025) 1297.
[50] S. Wang, Y. Jing, Effects of a chitosan coating layer on the surface properties and barrier properties of kraft paper, Bioresour 11 (1) (2016) 1868–1881.
[51] S. Chouhan, K. Sharma, S. Guleria, Antimicrobial activity of some essential oils—present status and future perspectives, Medicines 4 (3) (2017) 58.
[52] D. Cox-Georgian, N. Ramadoss, C. Dona, C. Basu, Therapeutic and Medicinal Uses of Terpenes, Medicinal Plants (2019) 333–359.
[53] A. Guarnieri, M. Triunfo, C. Scieuzo, D. Ianniciello, E. Tafi, T. Hahn, S. Zibek, R. Salvia, A. De Bonis, P. Falabella, Antimicrobial properties of chitosan from different developmental stages of the bioconverter insect Hermetia illucens, Sci. Rep. 12 (2022).
[54] Z. Ma, A. Garrido-Maestu, K.C. Jeong, Application, mode of action, and in vivo activity of chitosan and its micro- and nanoparticles as antimicrobial agents: A review, Carbohydr. Polym. 176 (2017) 257–265.
[55] A.A. Pereira Filho, G.K.A. Pêssoa, L.F. Yamaguchi, M.A. Stanton, A.M. Serravite, R. H.M. Pereira, W.S. Neves, M.J. Kato, Larvicidal activity of essential oils from *Piper* species against strains of *Aedes aegypti* (Diptera: Culicidae) resistant to pyrethroids, Front. Plant Sci. 12 (2021).
[56] S. Javed, B. Mangla, A. Salawi, M.H. Sultan, Y. Almoshari, W. Ahsan, Essential oils as dermocosmetic agents, their mechanism of action and nanolipidic formulations for maximized skincare, Cosmetics 11 (6) (2024) 210.
[57] W. Wu, Y. Yang, Y. Feng, X. Ren, Y. Li, W. Li, J. Huang, L. Kong, X. Chen, Z. Lin, X. Hou, L. Zhang, Y. Chen, Z. Sheng, W.D. Hong, Study of the repellent activity of 60 essential oils and their main constituents against *Aedes albopictus*, and nano-formulation development, Insects 13 (2022).
[58] M.A. Tabari, M.R. Youssefi, A. Esfandiari, G. Benelli, Toxicity of β-citronellol, geraniol and linalool from Pelargonium roseum essential oil against the West Nile and filariasis vector Culex pipiens (Diptera: Culicidae), Res. Vet. Sci. 114 (2017) 36–40.
[59] D. Matiadis, P.G. Liggri, E. Kritsi, N. Tzioumaki, P. Zoumpoulakis, D. P. Papachristos, G. Balatsos, M. Sagnou, A. Michaelakis, Curcumin derivatives as potential mosquito larvicidal agents against two mosquito vectors, *Culex pipiens* and *Aedes albopictus*, Int. J. Mol. Sci. 22 (2021).
[60] E. Scalerandi, G. Flores, M. Palacio, M.T. Defagó, M.C. Carpinella, G. Valladares, A. Bertoni, S.M. Palacios, Understanding synergistic toxicity of terpenes as insecticides: contribution of metabolic detoxification in *Musca domestica*, Front. Plant Sci. 9 (2018).
[61] F. Xue, Z.-c. Zhu, Z. Wei, X. Peng, Y. Wang, T. Li, G. Ma, Y. Wu, L. He, K. Qian, The preparation of prochloraz pH-responsive nanocapsules by the Pickering emulsion polymerization method and the study of their performance, RSC Adv. 10 (2020) 4598–4606.
[62] A.G. Khalifa, W.A. Moselhy, H.M. Mohammed, T.M. Nabil, M.N. Shaban, S. M. Aboelhadid, K.H. Abdou, Toxicological evaluations of chitosan and silica nanoparticles loaded with deltamethrin with improved efficiency against *Culex pipiens* larvae, Int. J. Environ. Sci. Technol. 19 (2022) 11809–11828.
[63] M.M. Baz, A. Selim, I.T. Radwan, A.M. Alkhaibari, H.F. Khater, Larvicidal and adulticidal effects of some Egyptian oils against *Culex pipiens*, Sci. Rep. 12 (1) (2022) 4406.
[64] M.M. Baz, A.M. Selim, I.T. Radwan, H.F. Khater, Plant oils in the fight against the West Nile vector, *Culex pipiens*, Int. J. Trop. Insect Sci. 42 (3) (2022) 2373–2380.
[65] M.M. Baz, A.M. Selim, I.T. Radwan, A.M. Alkhaibari, H.S. Gattan, M.H. Alruhaili, S. M. Alasmari, M.E. Gad, Evaluating larvicidal, ovicidal and growth inhibiting activity of five medicinal plant extracts on *Culex pipiens* (Diptera: Culicidae), the West Nile virus vector, Sci. Rep. 14 (1) (2024) 19660.
[66] N.A.R. Ghazawy, I.T. Radwan, H.S. Gattan, M.H. Alruhaili, M.M. Baz, E. A. AbdelFattah, A.M. Mashlawi, A. Selim, Asafetida plant extract as potential antioxidant, antimicrobial, and odor retardant insecticidal agent against Culex pipiens, Sci. Rep. 15 (1) (2025) 27076.
[67] M.B. Isman, Bioinsecticides based on plant essential oils: a short overview, Z. Naturforsch. C 75 (2020) 179–182.
[68] A.S. Lemus de la Cruz, J. Barrera-Cortés, L.P. Lina-García, A.C. Ramos-Valdivia, R. E.R. Santillan, Nanoemulsified formulation of *Cedrela odorata* essential oil and its larvicidal effect against *Spodoptera frugiperda* (J.E. Smith), Molecules 27 (2022).