Original Article

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Nano-emulsion Formulation of Lambda - Cyhalothrin Preparation Technique, Characterization, and Larvicidal Activity

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Abstract

Conventional pesticide formulations are addressed along with several issues connected to environmental pollution. Lipophilic properties and resistance solubility in the water phase led to low-effective pesticide utilization. In order to improve pesticide solubility and efficacy and reduce the dose and side effects, nanotechnology approaches are applied. The efforts and chances to develop nanoemulsions as preparation for agriculture chemicals have been the issues of heavy investigation. The lambda-cyhalothrin-loaded nano-delivery system was developed, using Tween 80 as a hydrophobic surfactant through a solvent evaporation method. The novel compound of lambda-cyhalothrin has unique chemical, physical, and biological properties. The recorded properties of lambda-cyhalothrin nano-emulsion showed spherical particles having an average size of 70.3 nm using transmission electron microscopy. Dynamic light scattering—Zetasizer—reached 77.51 nm and Zeta potential has a negative surface charge value of −17.8 mV. Lambda-cyhalothrin and lambda-cyhalothrin nano-emulsion showed a strong absorption band at different wavelengths. Fourier transform infrared spectroscopy analysis revealed the addition of new bonds in the lambda-cyhalothrin nano-emulsion compound. Larvicidal activity indicated a significant difference between the mortality means in the larvae of *Culex pipiens*. The produced lambda-cyhalothrin nano-emulsion has a new hydrophilic attendance and better efficacy for pest control resulting in promising nano-pesticide formulations to improve agricultural practice.

Keywords: Lambda-cyhalothrin, larvicidal activity, nano-emulsion, solvent evaporation method

Introduction

In the agricultural practice, one of the main challenges is the need to invent advanced methodols related to the pesticide's usage due to environmental contamination, bioaccumulation, and pest resistance enlargement, which requires a lowering in the number of pesticides used for crop and product storage and preservation. Nanotechnology is considered a perfect way to reach this target. Hence, nanopesticide formulations are presenting new techniques for the production and delivery of efficient pesticides, as well as new powerful ingredients (Hayles et al., 2017).

Pesticides may have an impaired effect on environmental biodiversity and produce hazards to non-specific species. The recent developments in nanotechnology carrier formulation for agriculture products produced high alteration and reduction to these impairments, such as nanopesticides, that became more effective, safe, and durable. In this regard, it is worth representing the risk impact, activity, and toxicity of nanopesticides in terrestrial and aquatic fauna (Oliveira et al., 2019).

Some studies supposed that nano-formulation in agriculture may be advanced to traditional products and huge expectations are placed on the usage of nano-techniques in the agriculture industry. However, no adequate trials have yet been conducted (Kah et al., 2018).

Lambda-cyhalothrin is a synthetic pyrethroid insecticide, which has been applied around the world to control the insects in agriculture and disease vector pests. The increased usage of lambda in conventional form may induce various impacts on the environment. Pesticides in nanoparticles form can provide a successful solution to a problem (Liu et al., 2008; Abouelkassem et al., 2016).

The colloidal dispersion formulations known as nanoemulsions are thermo-dynamically stable compounds, constructed from two immiscible liquids mixed uniformly with emulsifying compounds

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(surfactants and co-surfactants) to obtain a single phase. Several methods had been applied to produce nanoemulsions such as high-pressure homogenization, microfluidization, phase inversion, spontaneous emulsification, solvent evaporation, and hydrogel production. The utilization of the double emulsion solvent evaporation method helped in the formation of multiple emulsions (Qin et al., 2017, 2019).

Different techniques of characterization for nanoemulsions include the measurement of entrapment efficiency, particle size, polydispersity index, zeta potential, as well as characterization through differential scanning calorimetry, fourier-transform infrared spectroscopy, and transmission electron microscopy (Pan et al., 2015).

Earlier nanoemulsions were oil-in-water (O/W) type emulsions with average droplet diameter ranging from 50 to 1000 nm. Recently, nanoemulsions are broadly divided into three classifications, namely, O/W type (oil is dispersed in the aqueous phase), water-in-oil (W/O) type (water is dispersed in the oil phase), and bi-continuous type (microdomains of water and oil are inter-dispersed within the system). The alteration between the emulsion ingredients leads to transformation among these three types. Several emulsions are also category of nanoemulsions, where both O/W and W/O emulsions are found together in one compound structure. Due to stability, both hydrophilic and lipophilic surfactants are applied simultaneously (Hoa et al., 2012; Yan et al., 2019). The procedure of emulsification by melt shearing could resolve the issue of insoluble pesticides, lowering the formation expanse, and resolving the formulation methodology. The poor development of kinetics of the nanoparticles could be basically connected to the impact of hydropic surfactant which promotes the nano-compound to be more stable. Nano-compound has a unique particle size of less than 100 nm with the properties of a large particular surface zone, fast dissolution rate, and great power of penetration, and its features are principally addressed in the enhancement of solubility, wettability, and insecticidal impact (Elsharkawy, 2020; Mustafa & Hussein, 2020).

This study intends to construct a safe, green, and eco-friendly pesticide preparation. This nanopesticide formulation has the properties of raising the rate of absorption and presenting in an aqueous dosage form dissolved in water. In addition, nanoformulation is applied to increase efficiency while decreasing total dose and hazard impacts. The methodology utilized to achieve this goal is a simple, inexpensive technique. Thus, lambda-cyhalothrin (LC) nano-formulation could be suggested as an effective pesticide preparation for crops safety and preservation of ecosystems.

Methods

Chemicals

λ-cyhalothrin technical grade. The compound [3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-cyano (3 phenoxy phenyl) methyl cyclopropanecarboxylate], with 97.8% purity (CAS number 91465-08-6) was purchased from Kafr El Zayat Pesticides and Chemicals Co., Egypt. Tween 80 and methanol are pure analytical grade chemicals purchased from Sigma–Aldrich.

Preparation of Lambda-Cyhalothrin Nano - Emulsion

Lambda-cyhalothrin nano-emulsions (LCNs) were successfully prepared by solvent evaporation method according to Knieke et al. (2014) and Pan et al. (2015) with some modifications. The preparation followed the key steps: (1) 100 mg of LC was dispersed in methanol. (2) One milliliter of a hydrophilic surfactant Tween 80 in the concentration of 3% was dispersed in deionized water and added dropwise to LC solution. (3) The mixture was emulsified in a high shearing machine (NANOJ H10, ATS, Shanghai, China) at 1500 rpm for 5 minutes. (4) Subsequently, the emulsion was cooled to ambient temperature by stirring at 600 rpm on a magnetic stirrer. Then, the solution was sonicated for 10 minutes and filtered with a 200 nm nano-filter. Finally, LCN was obtained.

Lambda-Cyhalothrin Nano-emulsion Characterization

Transmission Electron Microscopy

The transmission electron microscopy (TEM) images were conducted in the unit of Electron Microscopy of Assuit University and the morphology of the nano-LC was identified(TEM, HT7700, Hitachi Ltd., Tokyo, Japan) with 80 kV accelerating voltage. A solution of 25 µg/mL was prepared upon a carbon-coated copper grid and was dried at room temperature for TEM imaging.

Particle Size and Polydispersity Index Assay

The deionized water was added to samples before the assay. The mean particle size, 90% diameter percentile (D90), and particle size and polydispersity index (PDI) of the nanoparticles were measured by dynamic light scattering (DLS) at 25°C, using the Zetasizer Nano ZS90, Malvern, UK at Nanotechnology Unit, Faculty of Pharmacy, Alazhar University, Assiut branch. Particle size and polydispersity index less than .3 indicated a minimal narrow size distribution and good dispersion. The results were recorded in triplicate.

The Zeta potential of the nano-emulsion was documented by the electrophoretic mobility procedure in the Zetasizer device in which

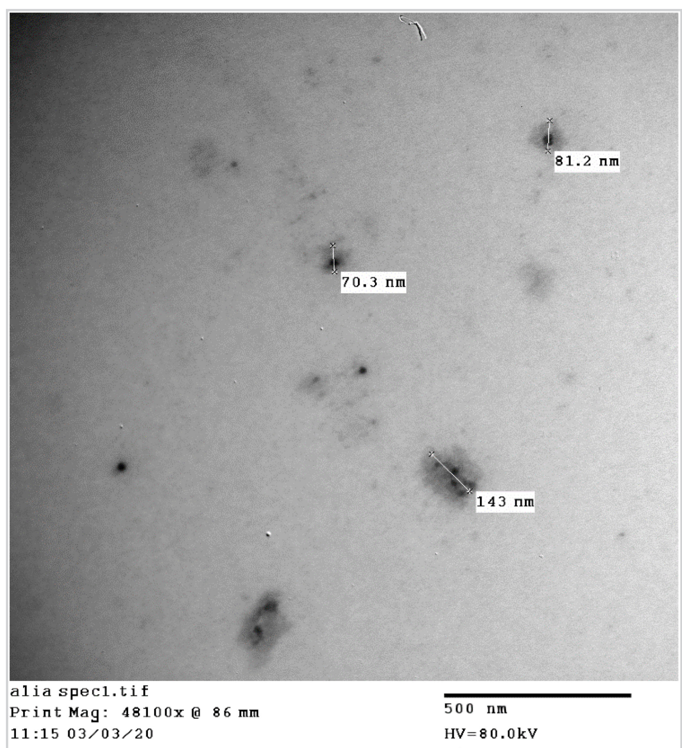

Figure 1
TEM Image of LCN Revealed an Average Size of 70.3 nm in Diameter.
TEM = Transmmision Electron Microscopy; LCN = Lambda-Cyhalothrin Nano-formulation.

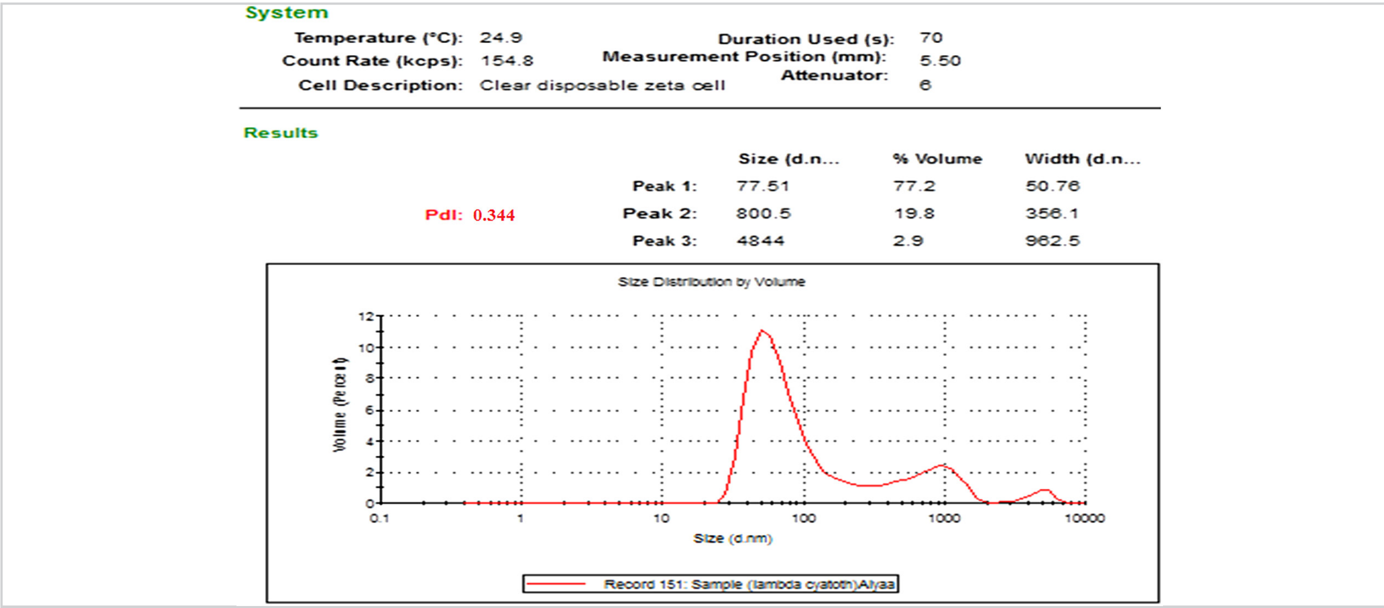

Figure 2
The Average Dynamic Nano-size of LCN by DLS-Zetasizer and PDI.

LCN = Lambda-Cyhalothrin Nano-formulation; DLS = Dynamic Light Scattering; PDI = Particle Size and Polydispersity Index.

the samples were diluted four-fold with milli-Q water and values were represented in millivolts (mV) obtained from two cycles with an average of 20 scans.

UV-Visible Absorption Spectroscopy

The analyzed spectra were reported on a Perklin–Elmer lambda 40 spectrophotometer using a 1 cm matched quartz cell over a wavelength range of 200–1000 nm. Aliquots (3 mL) of the suspension were measured to determine the surface plasmon resonance absorption maxima with distilled water as a reference.

Fourier-Transform Infrared Spectroscopy

Fourier-transform infrared spectroscopy (FTIR) was used for detecting the molecule distribution. Solutions of LCN and conventional LC were loaded on the cuvettes of infrared spectrometers (Gasco FT-IR Japan). Spectra were measured in transmission mode with a

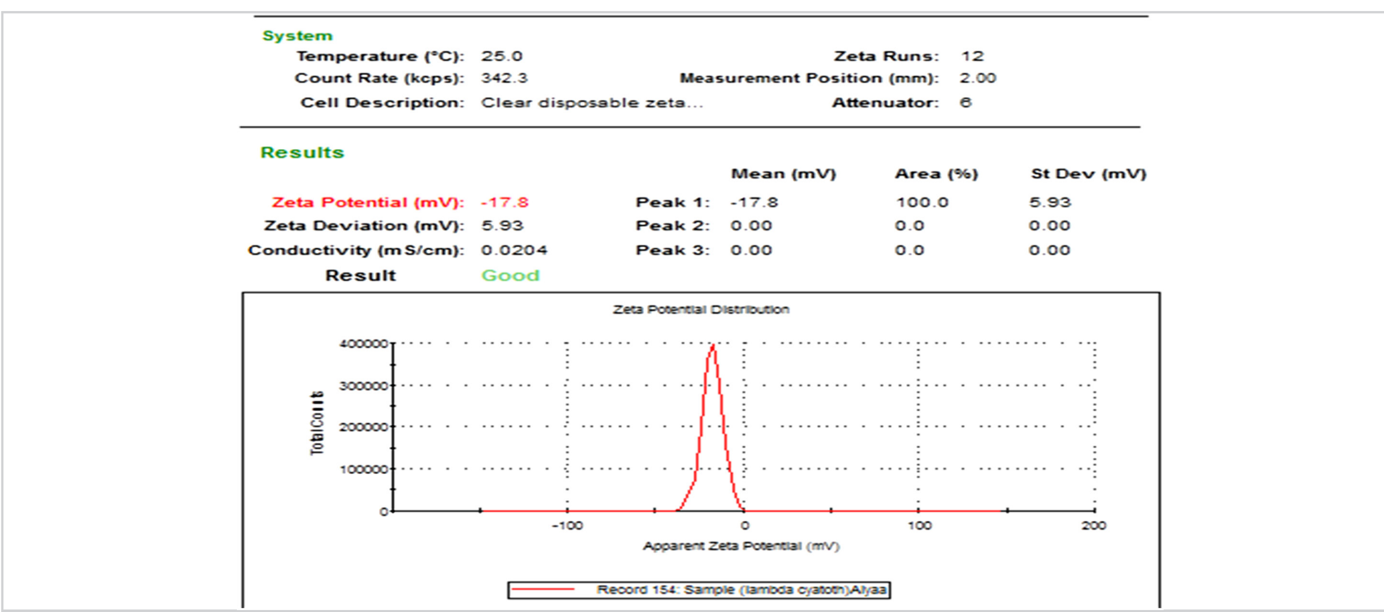

Figure 3
Zeta Potential of LCN Showed a Negative Surface Charge Value.

LCN = Lambda-Cyhalothrin Nano-formulation.

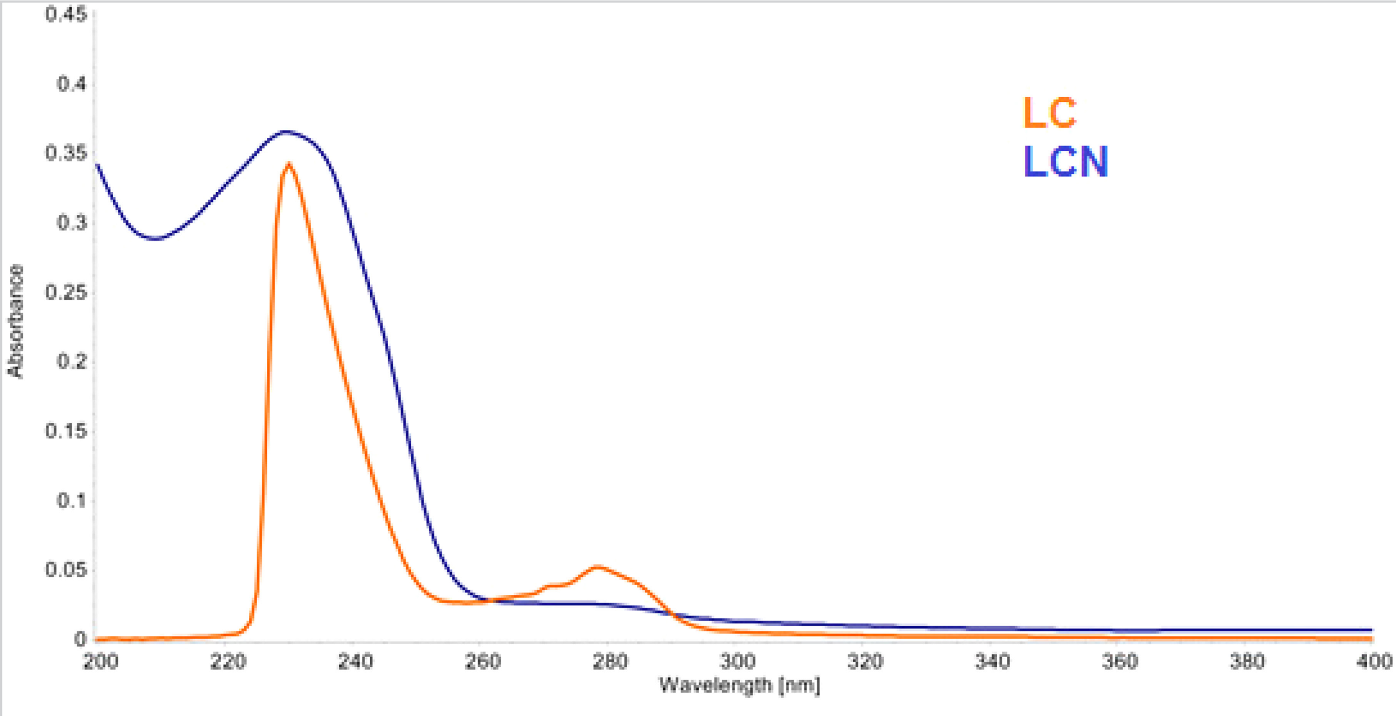

Figure 4
Ultraviolet-visible spectral analysis of LC and LCN.

LC = Lambda-Cyhalothrin; LCN = Lambda-Cyhalothrin Nano-formulation.

resolution of 4 cm-1. The scanning was processed from 400 to 4000 cm^{-1} scans per sample on a Perkin–Elmer Spectrum RX1 apparatus. The data were represented in infrared transmittance percentage.

Larvicidal Activity Bioassays of LC and LCN Against the Target Insect Pests

Larvae of field strain were collected from the faculty of science, zoology department, Entomological Research Laboratory, Assiut University, Egypt. The larvae were recognized as *Culex pipien* strains. The larvae were acclimatized under suitable temperature and humidity for a period of 24 hours. The larvae were fed with glucose and yeast mixture. Twenty larvae of *C. pipiens* were placed in a 250 mL sterile beaker containing 200 mL of water (WHO, 2005). Conventional and nano-LC formulations were added to larvae in two separated beakers. The control larvae were also used. The larvae were kept at room temperature. The

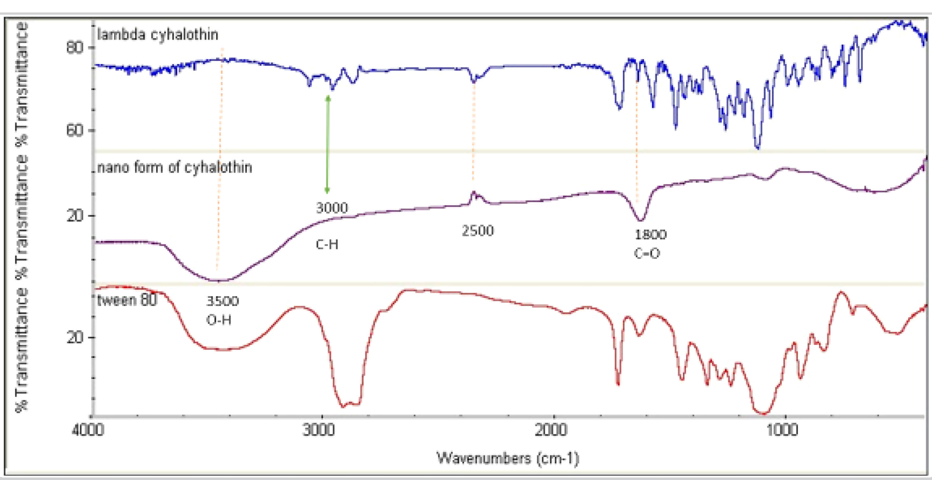

Figure 5
FTIR spectral analysis of LC and LCN.

LC = Lambda-Cyhalothrin; LCN = Lambda-Cyhalothrin Nano-formulation; FTIR = Fourier-Transform Infrared Spectroscopy.

Table 1.
Larvicidal Activity (Number of Dead Larvae) After 24,48, and 72 Hours of Exposure to Nano and Conventional Lambda-Cyhalothrin Pyrethroid.

Exposure		**Nano L-Cyhalothrin**				**L-Cyhalothrin**			
24 h	**Conc. (µg)**	**.1**	**.05**	**.01**	**.001**	**.1**	**.05**	**.01**	**.001**
	First trial	19	12	10	6	10	7	5	1
	Second trial	15	10	6	4	11	8	4	1
	Third trial	17	11	8	5	10	8	4	1
Mean		17	11	8	5	10.3	7.6	4.3	1
48 h	First trial	20	19	15	10	12	10	9	4
	Second trial	16	15	12	8	15	13	7	2
	Third trial	17		14	9	13	11	8	3
Mean		17.6	15	13.6	9	13.3	11.3	8	3
72 h	First trial	20	20	17	12	19	19	11	8
	Second trial	18	18	17	10	19	18	16	6
	Third trial	19	19	16	11	18	19	15	7
Mean		19	19	16.6	11	18	18	14	7

larvicidal effects of both formulations were tested by obtaining the mortality rate after 24, 48, and 72 hours of the exposure time. Dead larvae were recognized when they were immobile. The test was done in three replicates. The mortality data in both treatments with LC and LCN were obtained after 24, 48, and 72 hours of exposure periods. The dead larvae were recognized when they were stunted and failed to reach the surface water (Macêdo et al., 1997).

Statistical Analysis

The means of data values of larval mortality and standard error were calculated for each concentration of LCN and conventional cyhalothrin. The Student's *t*-test was applied to investigate the significance between the different concentrations of LC and LCN and the mortality rate after 24, 48, and 72 hours of exposure. Results were considered to be statistically significant at $p < .05$.

Results

TEM Analysis

The TEM image revealed that the LCN morphology is nearly spherical and has an average size of 70.3 nm in diameter (Figure 1).

Dynamic Light Scattering for Zeta Potential and Particle Size

The average dynamic nano-size by DLS. Zetasizer reached 77.51 nm diameter. The prepared LCN showed a good stability condition of nano-solution as the poly-disparity index (pdi) is lower than .5 (Figure 2). Zeta potential of LCN showed a negative surface charge value (−17. 8 mV) which was sufficiently high to avoid LCN aggregation. This value represents a stable and dispersed suspension of LCN and there is no tendency to form aggregates in a short period of time (Figure 3).

UV-Visible Spectral Analysis

The statement of the composition of conventional lambda and its nanoemulsion formulation of strong absorption band centered at 216 and 245 nm, respectively (Figure 4).

FTIR Spectral Analysis

In the spectrum of the free LC, two peaks appeared at 1800 cm^{-1} and 2500 cm^{-1} due to the stretching vibration of the benzene skeleton and represented the stretching vibration of the C=O in ester groups, which were regarded as the characteristic peaks of LC. In the spectra of LCN, the peak at 3500 cm^{-1} was attributed to the stretching vibration of the O–H group associated with hydrogen bonds. Meanwhile, the peak at 3000 cm^{-1} represented the deformation vibration of the C–H alkyl group and substituted in benzene structure indicating that the LC has changed and the new form is obtained in LCN (Figure 5).

The Larvicidal Activity Bioassay

The larvicidal activity of conventional lambda and its nanoemulsion formulation was investigated against the susceptible mosquito larvae (*Culex pipiens*) during 24, 48, and 72 hours of exposure (Table 1 and Figure 6a–c). The statistical analysis of the data of LC indicated high significant differences between the mortality means. There were also highly significant differences between the means of mortality rates caused by conventional lambda and lambda nanoparticles; hence, the exposure periods exhibited high significant differences between 24,48, and 72 hours.

Discussion, Conclusion and Recommendation

Nanoemulsions have been addressed with great concern for its characteristic features in several sectors, such as long-term kinetic stability (Pan et al., 2014), transparent or translucent appearance (Fryd & Mason, 2012; Yu & Huang, 2012), and gravitational sedimentation or creaming than conventional emulsions. In this study, nanoparticles of LC are formulated by emulsification solvent evaporation technique similar to Bhakay et al. (2016), Knieke et al. (2014), Pan et al. (2015), and Wang et al. (2019a), with some novel alterations.

Nanoemulsions were recognized as formulations constructed from nano-scaled oil or water droplets (exactly beyond the range of 20–200 nm) that spread in an opposite phase as the

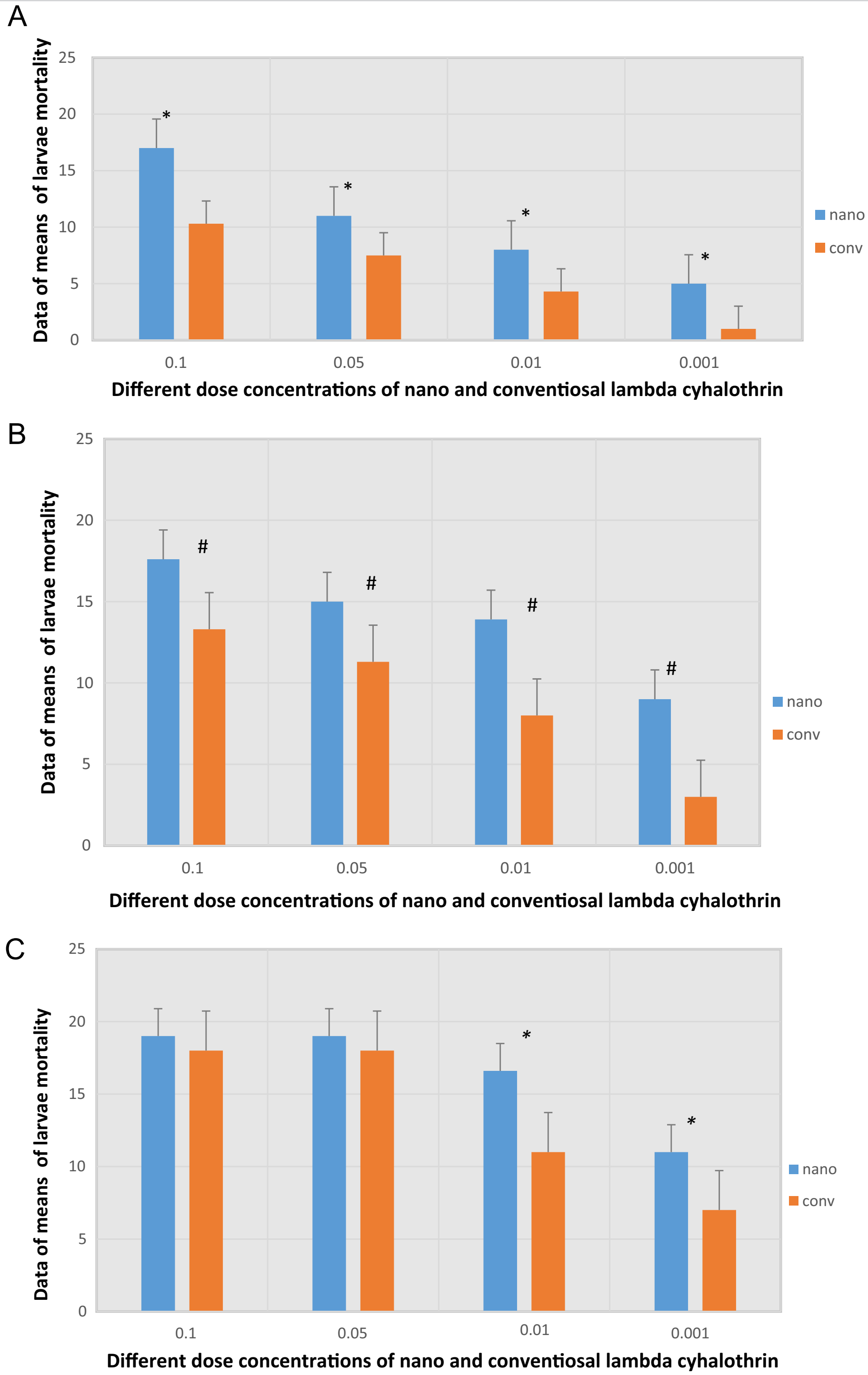

Figure 6
Larvicidal Effect LC annd LCN on Mosquito Larvae (Culex pipeins).
LC = Lambda-Cyhalothrin; LCN = Lambda-Cyhalothrin Nano-formulation.

surfactants lining the oil/water interface (Du et al., 2016). In our work, the hydrophilic surfactant Tween 80 in 3% concentration was applied and dispersed in deionized water. The pesticide b-cypermethrin has been contributed to the system water/poly(oxyethylene) nonionic surfactant/methyl decanoate (Wang et al., 2007) and this emulsion can be a typical form of a water-insoluble pesticide delivery system. Furthermore, another b-cypermethrin nanoemulsion preparation has been conducted by utilizing methyl laurate as oil phase and alkyl polyglycoside (APG) and polyoxyethylene 3-lauryl ether (C12E3) as mixed surfactants (Du et al., 2016; Zhao et al., 2017).

The parameters of the nanosuspension formulation, composition, and preparation were precisely researched using the particle size and PDI as determining indices. Our findings of particle size by DLS-Zetasizer revealed that an average dynamic nano-size reached 77.51 nm diameter less than 100 nm. And also, the TEM image recorded that the morphology of LCN is nearly spherical with an average size of 70.3 nm in diameter. These findings were in constant with similar techniques conducted by Pan et al. (2015), who reported that among 12 surfactants, 4 surfactants (SDS, MRES, Tween 80, and PEGNPE) decreased the mean particle size of the nano-preparation by less than 200 nm. The Zeta potential of LC nanosuspension declared a negative surface charge value (−17. 8 mV) which was sufficiently high to eradicate LCN aggregation. This value indicates a stable and well-dispersed suspension of LCN and there is no tendency to form aggregates in a short period of time. The high PDI value represented the poor disparity in water and PDI values less than 0.3 introduced a narrow size distribution (Ahuja et al., 2015). The pesticide particles were well dispersed via electrostatic repulsion (Zhang et al., 2015). Non-ionic surfactants, such as Tween 80, could inhibit the aggregation of particles by adsorbing into the nanoparticles through the hydrophobic section, which decreases the van Edward attraction between particles (Wang et al., 2019), and interactions between hydrophobic areas of LCN and Tween 80 were substantial for nanoparticles (Cui et al., 2015; Pan et al., 2015; Young et al., 1996).

The strong absorption bands centered at 216 and 245 nm are recorded as the composition of conventional lambda and its nano-emulsion formulation on ultraviolet-visible spectral analysis. This clearly suggested the formation of LCN nanoparticles embedded in the investigated matrix. For the broadening observation, and according to the literature, the broad and variable peaks are attributed to the formation of new particles (Abouelkassem et al., 2016). The recorded two different wavelengths, which suggested the presence of new compound, have new chemical properties that are reflected as another peak at different spectrums of wavelength.

The results of FTIR analysis showed the addition of new functional groups such as O-H and also substitution of another groups such as C-H. These structural changes revealed that LCN possesses new bonds formed by the conversion of the conventional LC to nano-lambda – cyhalothrin formulation. These results are resampled to the previous work conducted by Qin et al. (2019).

The data interpretation of larvicidal effect of LC and LCN declared high significant differences between the obtained mortality means of exposed larvae. There were also high significant differences between the means of mortality rates at variable exposure periods. Hence, the periods of exposure exhibited high significant differences between 24, 48, and 72 hours. These results are in agreement with the view of Abouelkassem et al. (2016), Bhan et al. (2014), Patil et al. (2012). A similar study by Desheesh et al. (2019) declared that LC-encapsulated nanoparticles loaded with polyethylene glycol are released slowly and are persistently very efficient against mosquito larvae for up to 72 hours than the conventional form. These findings suggested that LCN had an efficient larvicidal effect better than the conventional compound. The reduction of the particle size enlarges the zone area of the particles surface, which give the power to the active ingredients of pesticides to penetrate the biological tissues. Furthermore, nanoparticles added penetration power and enhanced the absorption and accumulation of the pesticide in the tissues (Ahmed et al. 2019; Wang et al., 2019b).

There are many factors that affected the nanoformulation compound composition such as the used solvent, surfactant type, time and temperature of stirring, or shearing machine. All these factors induce new properties for the new nano-compound. Each study applied specific factors, and consequently, unique parameters were obtained for its nano product. In this study, we used melt and solvent evaporation method and successfully provided a new compound of nano-LC pesticide with novel and unique nano-properties. Our methodology depends on simple and inexpensive techniques. Various procedures concerned with particle size were researched. These parameters addressed the polymer concentration in the organic phase, tween 80 concentration in the aqueous phase, and volume ratio of oil and water phases. After various trials, we obtained new LCN compound with promising, effective insecticide properties.

Ethics Committee Approval: This study is approved according to the scientific guidelines provided by our instructional community of Assiut university, faculty of veterinary medicine ethics committee on 22nd December 2021, approval no : 745. No animals or humans were included in this study.

Informed Consent: Written informed consent was obtained from all participants who participated in this study. The corresponding author on behalf of all authors has permission to publish this article.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – E.E.E.; Design – E.E.E., El-N.M., D.Y.; Supervision – G.S.Z., El-N.M.; Resources – A.A.B., G.S.Z.; Data Collection and/or Processing – A.A.B.; Analysis and/or Interpretation – E.E.E.; Literature Search – A.A.B.; Writing Manuscript – El-N.M.

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