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Synthesis of eco-friendly lipid-magnetite nanocomposite encapsulated Poinciana extract as promising insecticide against *Culex pipiens*

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Mosquito-borne diseases represent a growing health challenge over time. Nanostructured lipid carriers (NLCs) are the second generation of solid lipid nanoparticles (SLNs), and they continue to attract significant interest as potential diagnostic and therapeutic tools in disease inhibition and insect control. Activated ingredients presented in the *Poinciana* leaves were extracted and GC–MS data indicated an increased abundance of terpenes, flavonoids, and phenolic substances. *Poinciana* extract was encapsulated to the vicinity of nanostructure lipid carrier, Po-NLC, and surface modified with magnetic nanoparticles, Po-NLC-MNPs. The synthesized nanoparticles depicted average particle size of 73.2 and 75.55 nm while zeta potential of (− 29.4) and (−4.44 mV) for Po-NLC and Po-NLC-MNPs, respectively. Transmission electron microscope and morphology determination showed regular, irregular spherical and oval shapes with diverse single particle size. X-rays diffraction pattern of the freely synthesized MNPs was compared to the decorated NLC and the results manifested that the NLC was successfully decorated with MNPs. The larvicidal activity of plant extract, *Poinciana* extract (Po), and their nanoparticle conjugates against 3rd instar larvae of *Culex pipiens* was evaluated at 50, 100, 200, 500, 1000, and 1500 ppm concentrations. Both high and low concentrations of Po-NLC-MNPs, indicated potential larval mortality than plant extracts (Po extract) itself. The mortality rate reached 100% for 3rd instar larvae. Based on their relative toxicity, (Po-NLC-MNPs) was the best at killing larvae, followed by Po-NLC. The synthesized nps were checked for their cytotoxic effect against wi38 cell line. The *in-vitro* cytotoxicity results indicated that there was no significant cytotoxicity and the nanocomposite barely caused weak changes in the tested cells. The synthesized nanoparticles have potential to create a new generation of eco-friendly, effective alternatives for controlling mosquito-borne diseases.

Keywords *Poinciana* extract, Nanostructured lipid carriers, *Culex pipiens*, Phytochemical analysis

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Mosquitoes are an extremely important insect because of their critical role as a vector in disease transmission[1]. They can transmit diseases such as dengue, malaria, filariasis, yellow fever, and Japanese encephalitis. Dengue viruses, which are transmitted by infected females of the Culicidae family, namely *Aedes aegypti* and *Aedes albopictus*, have become a major concern for international public health in recent years[2,3]. Gibbons identified *Ae. aegypti* as the primary vector of arboviral dengue virus infections in tropical and subtropical regions[4]. Every year, around 50–100 million individuals are infected worldwide: with nearly 2.5% of those infected dying[5].

The recent emergence of mosquito-borne viruses has raised serious concerns in many countries about the need to identify the mosquito species responsible for transmitting the pathogen. This situation has coincided with the emergence and evolution of several exotic and highly invasive mosquito species associated with global trade[6]. The most prominent of these is West Nile virus (WNV). Researchers discovered WNV in humans in Egypt in 1950 and identified it in the native mosquito species *Culex pipiens*, as well as in other regions such as southern Europe, the Middle East, and Asia[7,8]. Laboratory experiments have confirmed this observation, demonstrating the ability of *Cx. pipiens* to infect others and the isolation of the virus from the saliva of fully susceptible individuals.

Multiple and diverse mechanisms are used to control mosquitoes, in addition to several surveillance methods routinely used to control mosquito-borne hazards. Synthetic insecticides have been introduced over the years in programs to control medical insects and other pests, but although they are effective for a period of time, the insect is forced to adapt to the insecticide, and over time the insects acquire resistance to insecticidal products. In addition to the environmental risks resulting from excessive and repeated use of pesticides[9], phytochemicals are considered one of the most important alternatives to synthetic pesticides. They are known as secondary metabolites in plants, which in turn contain many chemical compounds that participate in many activities, including innate immunity, defense response signals, response to environmental stressors, and defense against pests and diseases, which Among them, the effects on mosquitoes include ovicidal, larvicidal, adulticidal, oviposition inhibitors, developmental toxicants, antifeedants, repellents, hatching inhibitors, and emergence inhibitors[10].

Many medicinal plant preparations have yet to be evaluated for their mosquito repellent properties or larvicidal activity. While developing new pesticides, more emphasis has recently been placed on insecticides of plant origin and those that do not harm the ecology. As a result, natural products are the best option because they cause less harm to the ecosystem and non-target species, are eco-friendly[11], biosourced, and safe to use. Furthermore, numerous extracts and chemicals from various plant groups have been studied as potential novel larvicides[12]. Studies have shown that saponin[13], steroids[14], isoflavonoids[15], essential oils[16], alkaloids[17], and tannins[18] are all effective against mosquito larvae.

Royal Poinciana, *Delonix regia,* is one of these plants more widely distributed in temperate and tropical regions, and its leaves have long been used in folk medicine, notably as an antimalarial, particularly in East Asia. A wide spectrum of bioactivities was reported for the *Delonix Regia* extract. The plant has demonstrated positive antioxidant and antimicrobial properties[19–21]. Additionally, it has been reported that the flower's acetone and methanol extracts have effective larvicidal properties.

Nowadays, many dipterans, mosquitoes, are becoming a significant public health issue in many countries[22,23]. The elevated usage of pesticides has led to dramatic situations where certain organisms have developed the resistance necessary to prevent the spread of dangerous diseases, such as *Cx. pipiens* mosquitoes, which are able to resist the action of insecticides and are liable to spread their infection[24]. The increasing resistance of mosquitoes against pesticides has many implications worldwide[25]. As a result, many methods have been used to control their rapid spread. According to their corresponding approaches, these methods are divided into chemical, physical, biological, and environmental. Biological and environmental approaches seemed to be receiving more attention due to their fewer side effects and high effectiveness. Due to the significant resistance to insecticides, the usage of efficient and eco-friendly insecticides seems to be the right way to control insects[26].

Magnetic nanoparticles are safe and eco-friendly materials because they can be used and reassembled again due to their magnetic properties[27,28]. Being easy to prepare and handle, whether through chemical or even physical methods, this opens the door wide for its development and modification to achieve maximum biological benefit and effectiveness. The need to use ferrites, especially magnetic nanoparticles, has been investigated by many researchers[29]. Researchers not only used free magnetic nanoparticles but also widely employed loaded magnetic nanoparticles, which offer dual benefits. It's possible to form the insecticide by mixing the magnetic nps with Volkameriana citrus (*Volkamer lemon*) essential oil[30]. Therefore, the combination of magnetic nps and Volkameriana citrus oil could potentially be used for insect control. For example, larvicides control *Ae. aegypti* (yellow fever mosquito) due to their simple preparation, non-toxicity, and high affinity.

Typical applications of magnetic nps as larvicides or insecticides involve maintaining the nps in aqueous media and then activating them in the region of the affected area. The most successful nps used for activation possess both high biocide properties and are simply controlled. These magnetic nps are called MNP@LDH, which have large magnetic moments and can be implemented for biocide action in the presence of alternating magnetic fields of relatively low frequencies[31]. The magnetic field induces particle movement, allowing scientists to collect substantial data on their structure, other sensory systems, and anatomical features. However, the most significant advantage is that nanoparticles are likely to have no side effects on the physiological or biochemical processes of plants[32]. The MNP@LDH is a good candidate for protecting plants because it has multitargeted biopesticide activity, fast larvicidal kinetics, and stays stable in solution for a long time.

Nanostructured lipid carriers (NLCs) are the second generation of solid lipid nanoparticles (SLNs) in the lipid NP family[33]. NLCs employ a binary blend of melted lipid materials, which consists of solid lipids and liquid lipids (oils), to eliminate limitations of pure SLNs such as drug loading capacity, drug expulsion during storage, undesired modifications, and offer various compositions to fulfill tailor-made physicochemical properties of NLCs[33]. It is well accepted that NLCs are scalable and commercially viable platforms to load drugs and modify

their characteristics like solubility and enhanced selective toxicity. The biodegradable and biocompatible nature of NLCs makes them safe for patients[34]. NLCs are used to selectively treat infected tissue and minimize harmful damage to the body. This technology will improve patients's lives by benefiting from lower drug doses, short healing time and lower treatment costs. NLCs assist in controlled drug release, exhibiting lower drug excipient toxicity and low allergic reactions[35]. As such, NLCs have attracted significant need for a wide range of clinical conditions. It is expected that biodegradable and biocompatible lipid nps including NLCs, could result in minimizing the typical side effects of agents such as chemotherapeutics and allowing for more effective treatment with lower drug dosages.

Nanopesticides are a fast-growing trend in insect control due to their environmental safety and biodegradability. One of the most popular lipid-based nanoparticles is nanostructured lipid carriers, which are composed of phospholipids and both solid and liquid lipids. These lipids are not only safe to use as components, but they are also integral parts of our nutritional regimen. They are biodegradable, biocompatible, and capable of encapsulating and releasing substantial quantities of active ingredients from both hydrophilic and hydrophobic drugs. This makes NLC a good candidate to encapsulate *Poinciana* extract. Nanoparticles possess unique and tunable magnetic properties at the nanoscale; heat typically affects magnetite nanoparticles due to their strong photocatalytic properties. Magnetic nanoparticles have the capability to store heat upon exposure to the sunlight, causing the insects to warm up in their vicinity, making irritant reflux and biochemical changes easier and making the chances of insect elimination easier[36].

Materials and methods

Chemicals

Butyl alcohol, stearic acid, lauric acid, oleic acid, tween 20, sodium glycocholate, ferric chloride (III) hexahydrate, ferrous sulfate (II) heptahydrate, and ammonium hydroxide 50% hydrolyzed were purchased from Alfa Aesar Germany (Fisher Scientific). Methyl alcohol from El-Gomhoria Company (Cairo, Egypt) and distilled water were prepared in our lab using two-stages high purity distillator. All solvents and chemicals were used as they are and without any purification.

Plant collection and extraction

Leaves of *Poinciana* (*Delonix regia* Bojer) were collected from different areas in Qalyubiya Governorate, Egypt, during August–September 2023. *Delonix regia* is considered among the most widespread ornamental plants. The Herbarium Flora and Phytotaxonomic Section classified the plant *Poinciana* under the number [11-09-2024], identifying the studied specimen by comparison with specimens preserved in the Botany Department, Faculty of Science, Cairo University, Egypt. The plant materials were air-dried in a shaded place for five days at the room temperature until all moisture was removed. The dried leaves were ground in a stainless-steel electric mixer and transferred into airtight containers to protect them from humidity. Exactly 50 g of ground leaves were placed in a clean and dry 500-mL conical flask. To the powder, 150 mL of methanol (absolute) was added, and stirring for 48 h was processed at room temperature. After incubation completion, the solid fibers and insoluble remains were removed by filtration using cellulose cotton. Followed by filtration using Whatman cellulose filter paper (0.45 mm) to remove all fine insoluble materials. The supernatant was re-concentrated using a rotary evaporator for 10 min (35 °C, under reduced pressure)[37]. The obtained past represented 100% extract preserved in dark glass bottles and kept under low temperature conditions (-5 °C). The aqueous extract was prepared using the same protocol with little difference in the re-concentration step; freeze dryer lyophilization was used instead of a rotary evaporator at -55 °C for 48 h. Semi-solid paste was removed and preserved in the same way as extraction with methanol.

Design of dual functionalized nanostructure lipid carrier encapsulated *Poinciana* extract and decorated with magnetic nanoparticles (Po-NLC-MNPs)

The incorporation of more than one nano system in one formulation may work synergistically to fight insects. Here, we attempted to create nano systems that impact the *Cx. pipiens* in various ways, utilizing the adaptability of nanotechnology to engineer or functionalize them for specific purposes. The aim of this study was to create a nano delivery system using two types of nanomaterials: *Poinciana* extract, nanostructured lipid carriers, and magnetic nanoparticles (Fig. 1). We also aimed to compare the effectiveness of these systems against 3rd instar *Cx. pipiens* larvae before and after mixing them in one nanoformulation, to present an effective insecticidal agent based on greener and safer components.

Synthesis of nanostructure lipid carrier encapsulated *Poinciana* extract and decorated with magnetic nanoparticles

In a clean and dry beaker, a lipid mixture of 450 mg of stearic acid, 450 mg of lauric acid, and 600 mg of oleic acid was mixed and heated up to 70 °C until all solid components transformed into a molten solution. Exactly 200 mg of *Poinciana* extract was dissolved in 5 ml (butanol/chloroform, 3:1) and added to the molten lipid solution, stirring and processing for a while until all solvent evaporated. In another clean beaker, an aqueous solution of 0.05 g of sodium glycocholate dissolved in 5 ml of distilled water was prepared and heated up to the same temperature of 70 °C. The aqueous solution was poured into the lipid mixture, and, using a spatula, the mixture was agitated for 5 min. Followed by the addition of 5 ml of distilled water containing 5 g of tween 20 and the mixture shaked using mechanical high shearing force for two minutes. Addition of 5 ml of ice-cold water was added, and sonication was processed for 10 min. The resulting emulsion was centrifuged to remove non-encapsulated extract, and the supernatant was removed to calculate the drug loading capacities, while the precipitate spread in 30 distilled waters with 3 g of tween 20 and the mixture dispersed using an ultrasonic probe

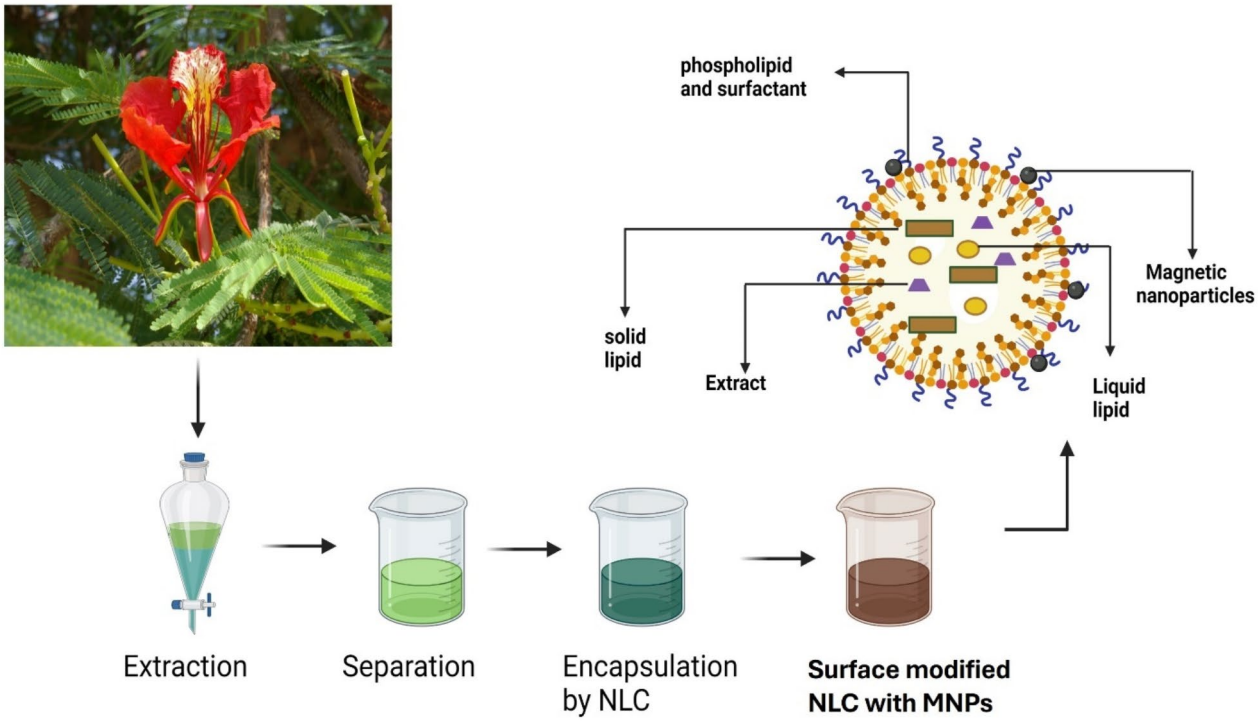


Fig. 1. Design of dual functionalized nanostructure lipid carrier encapsulated *Poinciana* extract and decorated with magnetic nanoparticles.

(the same as step 1) for 10 min to produce (Po-NLC) nanoparticles. Lyophilization at reduced temperature was applied for two days to produce Po-NLC as semisolid.

As for the synthesis of Po-NLC decorated with magnetic nanoparticles that is prepared as follows, a stock solution of ferric chloride hexahydrate ($FeCl_3.6H_2O$, 5×10^{-4} M) and ferrous sulfate heptahydrate ($FeSO_4.7H_2O$, 5×10^{-4} M), each one dissolved in 40 mL distilled water. The synthesized (Po-NLC) nanoparticles were placed in a clean beaker and dispersed very well before the addition of 7.5 mL of each iron di and trivalent salt, Fe(II) / Fe(III), and stirring continued for 10 min. The precipitating agent, NH_4OH (5% v/v), was added drop by drop with vigorous stirring (on hot). The solution tends to be darker due to the formation of magnetic nanoparticles when all the quantity of the precipitating agent is finished (5 mL). The dispersion was transferred into a 50-mL Falcone tube to be centrifuged for 10 min at 6000 rpm under reduced temperature (cooling centrifuge). After filtration and intensive washing with distilled water to confirm all the non-reacting iron salts and other impurities were removed, the slurry was collected and lyophilized to obtain a semi-solid past of Po-NLC-MNPs (Fig. 2). A free magnetic nanoparticle was synthesized using the same protocol to compare the XRD pattern with the decorated one to make sure that the decorated nps have the same structure as free magnetite. High magnetite enriched Po-NLC, Po-NLC-MNPs, was prepared by using 550 mg (0.55 g) of the crud Po-NLC and low magnetite enriched Po-NLC, Po-NLC-MNPs (L), was prepared by using 3600 mg (3.6 g) of the crud Po-NLC, followed by well sonication before the addition of the mixed iron salts.

Particle size (DLS) and zeta potential (ZP)

To grantee the quality of the produced nps, the hydrodynamic radius and the polydispersity index were measured using the dynamic light scattering (DLS) at the angle of 173° and at room temperature. The DLS and PDI were measured three times for the best result. Zeta potential (ZP) is measured by using the frequency of the scattered light shifting because of the particle charge at the scattering angle 12°. Measuring ZP is essential to inform about the stability of nps. ZP describing the electrochemical equilibrium between particles and liquids, such as in the case of nps[38]. The NP radius, ZP, and PDI are measured at the Egyptian Petroleum Research Institute (EPRI) using the Zeta sizer Nano series (HT), Nano ZS, and Malvern Instruments (UK). 5–10 mg was taken from each nanoformulation liquid, disseminated, and sonicated in 10 ml of dist. water (a sonication bath method). Then, the sample was placed in a quartz cell to be analyzed.

Surface morphology and topography by transmission *electron* microscope (TEM)

Transmission electron microscopy (TEM) is a versatile tool describing the interior form of NLCs to insure not only the regularity of nps but confirm particle size. Thus, indicate that aggregations are the essential characteristics of nps. The morphology of NLCs is measured by using the high-resolution transmission electron microscope (HR-TEM, JSM-7100F) in the Egyptian petroleum research institute (EPRI, Cairo). Images were recorded by

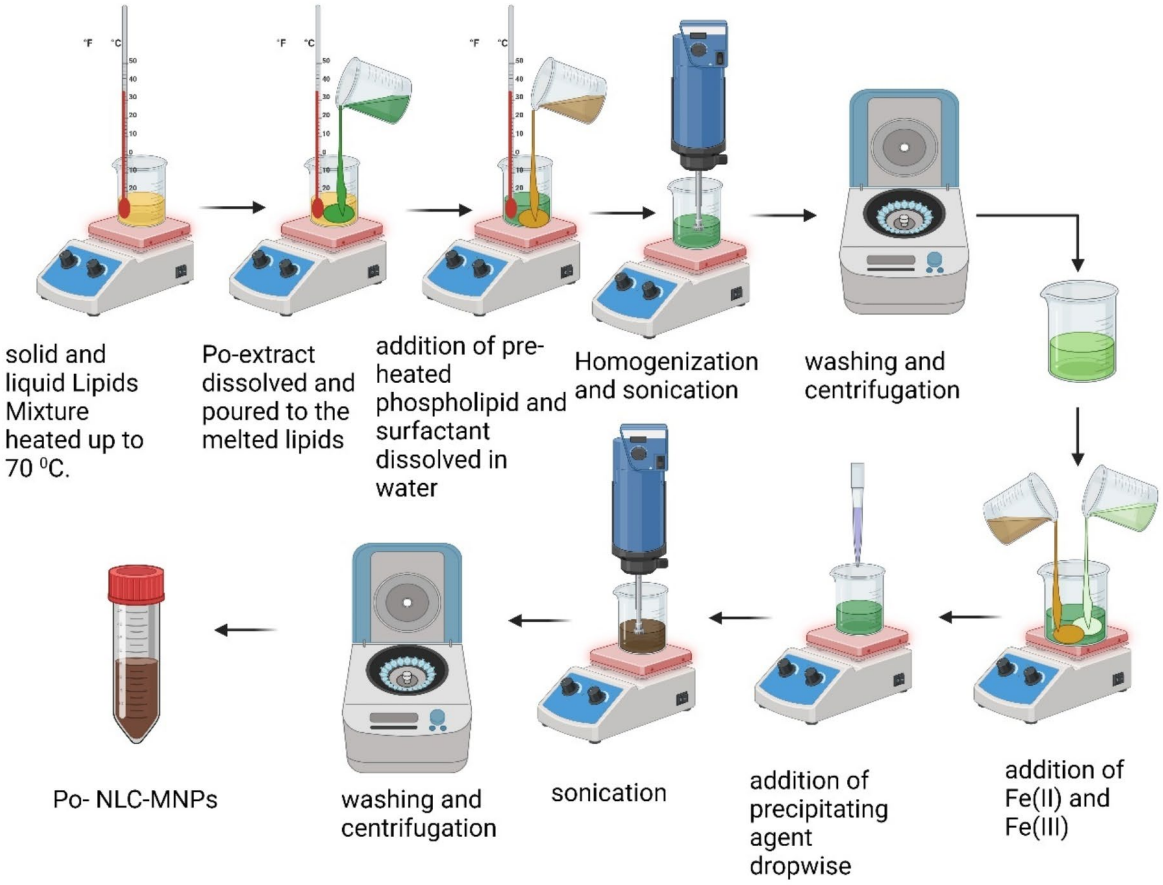


Fig. 2. Schematic illustration of the synthetic routes of both Po-NLC and Po-NLC-MNPs nanoparticles.

JOEL JEM-2100F HR-TEM at 200 kV FE (field emission) analytical electron microscope. 1 µl of NLCs is diluted with double distal water (1:200) and then placed on a carbon-coated grid (200 mesh) for 2 min. Two drops of 2% (w/w) phosphotungastic acid (PTA) are added to the grid for 10 s, and the excess liquid was absorbed by the filter paper. Subsequently, the grid was transferred to TEM, high-resolution images were recorded, and the morphology of NLCs was further studied.

X-ray diffraction (XRD)

Power X-ray diffraction (PXED) patterns of the powdered magnetic nanoparticles (MNPs) and powdered nanostructure lipid carriers decorated with magnetic nanoparticles (Po-NLC-MNPs) were investigated using X, pert PRO P analytical with Cu Kα radiation ($\lambda = 1.5406$ Å). The diffraction pattern was measured at 2θ of (4–80) with a scanning rate of 2.4°/min.

Fourier transform infra-red

For Fourier transform infrared spectrum, Thermo Scientific, Nicolet™ iS50 FTIR Spectrometer was used, and the sample was placed directly without KBr disc the wave number screening from 400 to 4000 cm^{-1}.

Vibrating sample magnetometer (VSM)

The magnetic measurement was conducted using vibrating sample magnetometer (VSM, Lake Shore, 7410 model) at room temperature.

X-ray dispersive spectrometer (EDS)

The identification and quantification of the elements presented on the sample were measured by Field Emission Electron Microscope (JOEL, JEM-2100F) equipped with X-ray dispersive spectrometer (EDS).

The encapsulation efficacy (EE) and drug loading capacity (DL)

The encapsulation efficiency (entrapment) of *poinciana* extract is measured by using the in-direct ultrafiltration centrifugation method[39–41]. One ml of freshly prepared Po-NLC or Po-NLC-MNPs was placed in vivspin 20 centrifugal concentrator tube (MWCO 5 k Da), then centrifuged at 8000 rpm for 10 min at temperature of 4 °C. The obtained supernatant, which contained free or non-encapsulated poinciana extract, was separated by decantation and dissolved in ethanol by probe sonication (Scientz, ultrasonic homogenizer-HD, Ningbo

Scientz Biotechnology Co., Ltd., China). The concentration of the non-encapsulated drug was measured using UV spectrophotometer (Genway spectrophotometer 6305, Japan), ethanol as blank at 450 nm using 1 cm glass cuvette at room temperature and subsequently, the non-encapsulated drug (free drug) calculated from this equation. 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:

$$\text{Entrapped drug} = \text{Total drug incorporated} - \text{free drug (supernatant)}$$

The calculation of the entrapment efficiency (EE) and the drug loading capacity (DL):

$$EE\% = \frac{Amount of the Entrapped Drug}{Amount of Total Drug Added} x100$$

$$DL\% = \frac{Weight of the Entrapped Drug}{total weight of the nanoparticles} x100$$

In-vitro drug release

The in vitro drug release study of the encapsulated Po-NLC and Po-NLC-MNPs nanoconjugates were done using dialysis bag method[40,42]. About 10 cm of Dialysis tube was impregnated in 20 ml of phosphate buffer pH 7.4 and preserved overnight prior performing the release study. Accurately, 5 ml of Po-NLC or Po-NLC-MNPs nanoformulation was poured into, tied at one end, dialysis bag (MWCO 12 K Da, Sigma Aldrich) and the other side must be tied before fully immersed in a 250 ml beaker containing 100 ml of PBS (pH 7.4). About 1 ml tween 20 and continuously stirred using battery based magnetic stirrer(IKA® C-MAG, HS4 Digital, India) at 150 rpm and placed in an incubator at 37 °C. One milliliter of sample withdrawn at different time intervals (0–72 h) from the release medium, and another 1 ml of fresh phosphate buffer added to the release media to compensate what is lacking. The withdrawn volume diluted with 1 ml ethanol (1:1) for complete solubility. After samples collection and by using the same procedures in drug loading capacity, the concentration of poinciana extract released determined. For best and reliable results, all experiments were done in triplicates.

In-vitro cytotoxicity assessment

The cytotoxicity assessment of the synthesized nanoparticles of Po-NLC and Po-NLC-MNPs 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 the 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 completed 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 incubation, cells were subsequently treated with several doses of the prepared dilutions. The treated plates were re-incubated for 48 h at 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 at 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).

Mosquito larvicidal assay

Rearing of Culex pipiens

In the insectary section of the Medical and Molecular Entomology Section, Faculty of Science, Benha University, Egypt, *Cx. pipiens* larvae were raised for many generations at 27 ± 2 °C and 75–80% RH over a photoperiod of 14:10 h (light/dark)[12].

Larvicidal activity

The *Delonix regia* extracts and their nanoparticles were tested to evaluate their larvicidal efficacy against 3rd instar larvae of *Cx. pipiens*. Six concentrations were prepared for plant extracts and nanoparticles (50, 100, 200, 500, 1000, and 1500 ppm). Twenty mosquito larvae were used for each replicate, and three replicates (60 larvae) were used for each concentration. Mortalities were recorded at 24, and 48 h after the initial exposure and post-treatments (PT).

The efficacy of Poinciana nanoformulation against non-target predators

The larvicidal activity of *Poinciana* extract and their nanoformulations was evaluated under laboratory conditions (27 ± 2 °C, 75–80% RH, 14:10 h light/dark). The efficacy of *Poinciana* extracts against selected common predators caught using a nylon fishing net (43 × 51 × 76 cm) and stander mosquito dipping (400 ml with log hand) in different mosquito larval habitats such as *Gambusia affinis*, *Stratiomys sp.*, *Cybister tripunctatus*, and *Sphaerodema urinator* was investigated (Fig. 3). The predators were collected from Kafr Saad village and placed in plastic bags half filled with water from the field to the laboratory. Insect predators were identified and classified by Dr. Yasser El-Sayed at the Entomology Department, Faculty of Science, Benha University, Egypt.

GC/MS analysis

For the GC/MS analyses, Thermo Scientific Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary columns, 0.1 mm, 0.251 mm, and 30 m thick, were used. It was done using an electronic ionizer with

Fig. 3. Efficacy of *Poinciana* extract and their nanoformulations against non-target predators.

70 eV ionization energy. As a carrier gas, helium gas was used (flow rate: 1 ml/min). The MS transmission line and injector were both set to 280 °C. The oven was preheated to 35 °C, then increased to 150 °C at a rate of 7 °C per min, 270 °C at a rate of 5 °C per minute (pause for two minutes), and lastly 310 °C at a rate of 3.5 °C per minute (continued for 10 min). A relative peak area was employed to explore the quantification of all components discovered. The chemicals were at least partially identified by comparing the retention times and mass spectra of the chemicals to those of NIST and Willy Library data from the GC/MS instrument. Identification was done using the aggregate spectrum of user-generated reference libraries. To evaluate peak homogeneity, single-ion chromatographic reconstructions were performed. To verify GC retention times, co-chromatographic analysis of reference substances was used whenever possible[43].

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 $P < 0.05$.

Results

Drug delivery system

Particle size and stability (zeta potential)

The particle size and polydispersity index of the synthesized nanoparticles according to the dynamic light scattering were 73.25 nm, 0.139, 75.5 nm, and 0.335 for the synthesized *Poinciana* extract encapsulated nanostructure lipid carrier (Po-NLC) (Fig. 4a,b), *Poinciana* extract encapsulated nanostructure lipid carrier and decorated magnetic nanoparticles (Po-NLC-MNPs), respectively. As well as the stability test of zeta potential revealed (−29.44 mV) and (−4.44 mV) for Po-NLC and PO-NLC-MNPs, respectively (Fig. 5a,b).

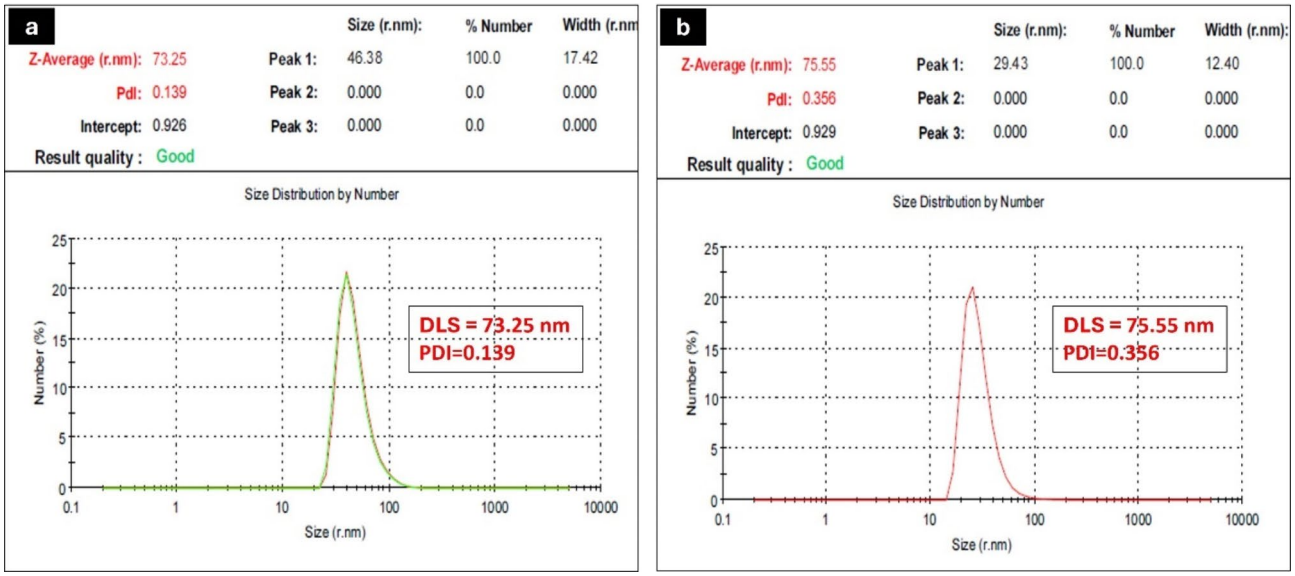


Fig. 4. Average particle size and polydispersity index of the synthesized nanoparticles: (**a**) *Poinciana* extract encapsulated nanostructure lipid carrier (Po-NLC), (**b**) *Poinciana* extract encapsulated nanostructure lipid carrier and decorated magnetic nanoparticles (Po-NLC-MNPs).

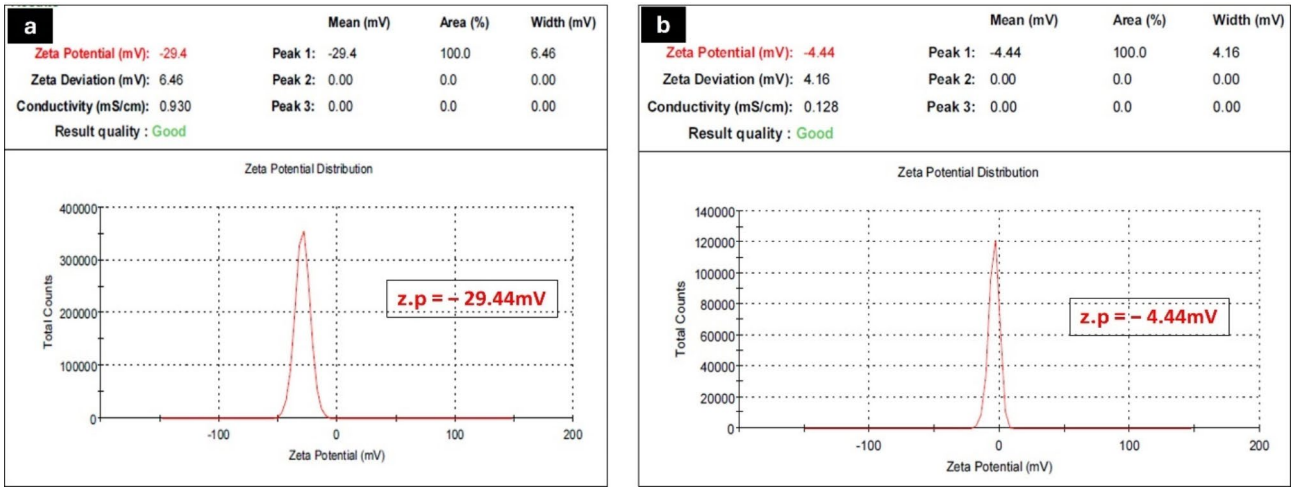


Fig. 5. Zeta potential of the synthesized nanoparticles: (**a**) *Poinciana* extract encapsulated nanostructure lipid carrier (Po-NLC), (**b**) *Poinciana* extract encapsulated nanostructure lipid carrier and decorated magnetic nanoparticles (Po-NLC-MNPs).

Transmission electron microscope and internal morphology

According to Fig. 6a,b, transmission electron microscope visualization of the synthesized Po-NLC-MNPs showed that different types of regular and irregular spherical shapes with dimensions varied from small to large particle sizes less than 100, but Po-NLC manifested spherical and size-enlarged nps extended to be between 300 and 500 nm. Figure 7a. Magnetic nanoparticles decorated the surface of the NLC, and the selected area diffraction presented in Fig. 7b showed some type of crystallinity.

X-ray diffraction (XRD)

The peaks observed at approximately 18.52°, 30.25°, 35.60°, 43.34°, 53.60°, 57.12°, and 62.86° in both samples correspond to the spinel structure of magnetite (Fig. 8).

Fourier transform infra-red (FT-IR)

The FT-IR spectrum (Fig. 9) of the free MNPS and Po-NLC-MNPs showed strong absorption peak at very low wave number of 580 cm^{-1} and some other common peaks at 3290 cm^{-1} and 1625 cm^{-1}.

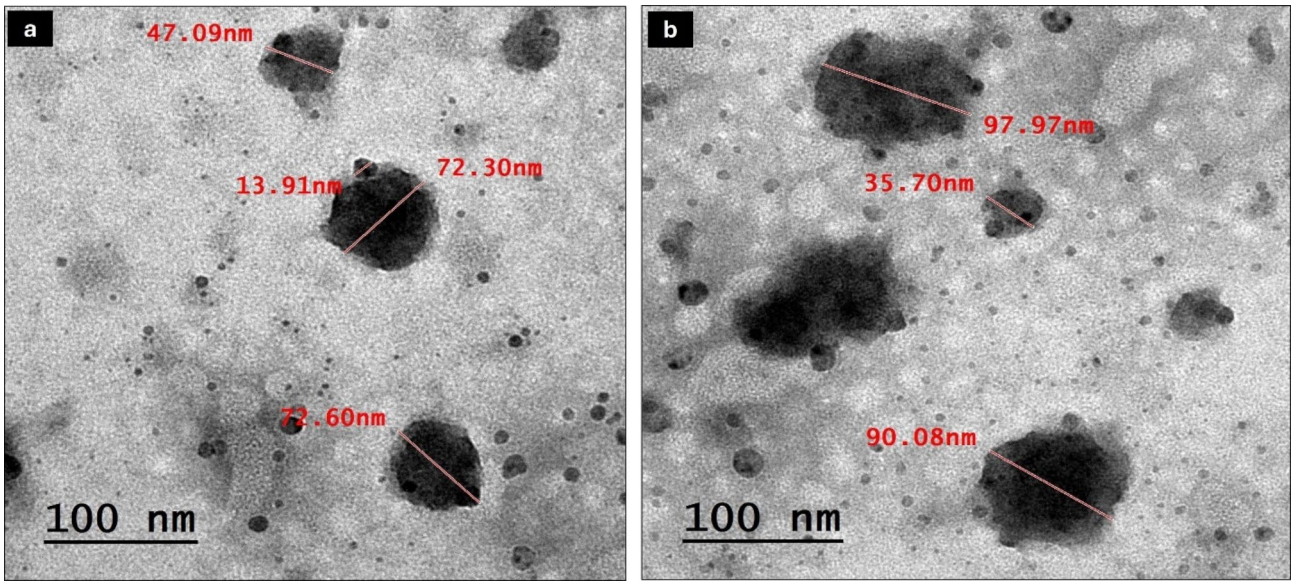


Fig. 6. Internal particle morphology by Transmission electron microscope of different fields (**a**, **b**) of the examined Po-NLC-MNPs nanoparticles.

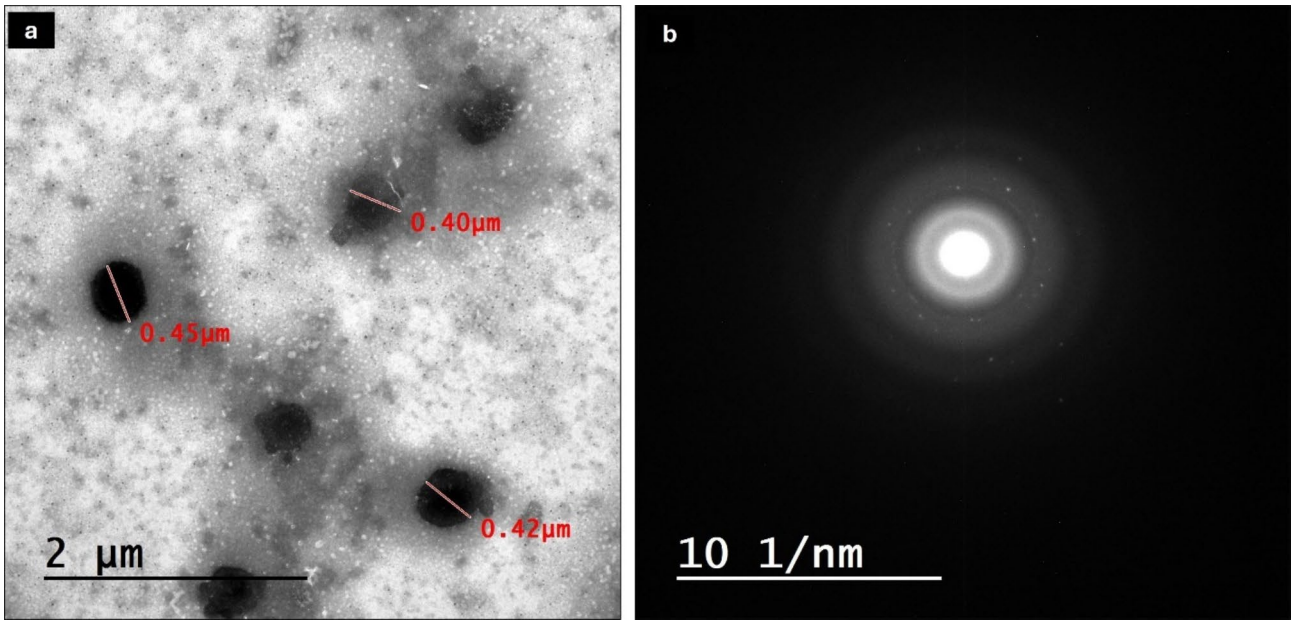


Fig. 7. Internal particle morphology by Transmission electron microscope of different fields of the examined Po-NLC nanoparticles: (**a**) represents regular spherical shape particles of Po-NLC, (**b**) selected area electron diffraction of Po-NLC-MNPs.

Vibrating sample magnetometer (VSM)
The magnetism of the synthesized samples was 35 emu/g, 32 emu/g, 0.28 emu/g and zero for MNPs free, Po-NLC-MNPs, PO-NLC-MNPs (L) and Po-NLC, respectively according to (Fig. 10).

X-ray dispersive spectrometer (EDS)
The EDS analysis for free MNPs and Po-NLC-MNPs were determined. According to (Figs. 11 and 12), the elemental analysis confirmed the existence of C, O and Fe with weight 3.22, 0, 2.62, 0, 66.42% respective to the free MNPs while the Po-NLC-MNPs presented weight percents of 33.67, 0.02, 11.57, 0.03, and 54.71% attributable to C, N, O, S, and Fe elements.

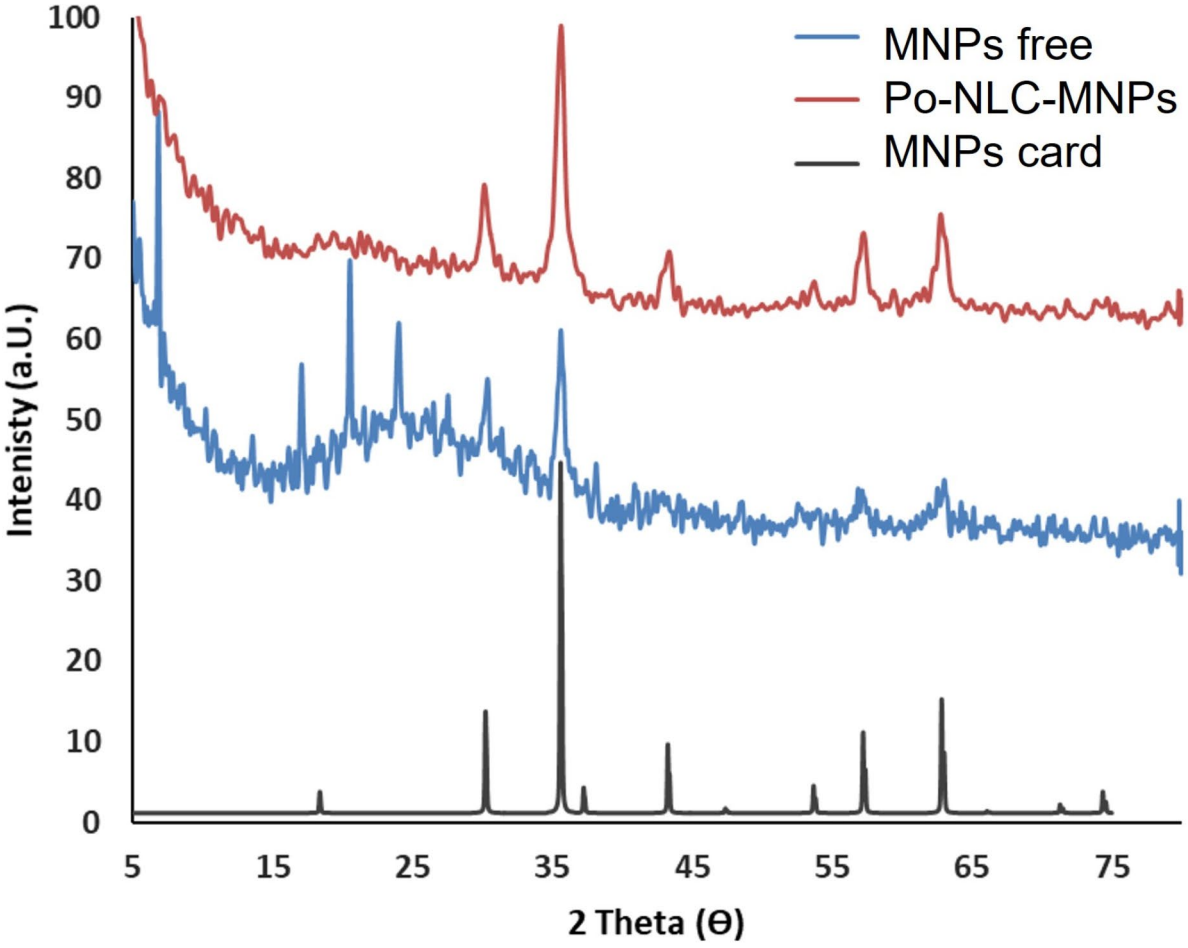


Fig. 8. XRD-pattern of both free MNPs and Po-NLC-MNPs.

The encapsulation efficacy (EE) and drug loading capacity (DL)
The entrapment efficiency of the *poinciana* extract in the synthesized Po-NLC and Po-NLC-MNPs were found to be 78.23% and 69.97%, while the drug loading capacity of the Po-NLC and Po-NLC-MNPs revealed 31.6% and 23.45%, respectively.

In-vitro drug release
The drug release study was conducted, and the results listed in Table 1. Based on the profile in (Fig. 13) the release of *Poinciana* extract from Po-NLC was faster than those in Po-NLMNPs.

In vitro assessment of drug cytotoxicity

The cytotoxicity assessment was done using normal human lung fibroblast WI38, and optical densities, cell viability, and IC_{50} values are presented in Table 2. That assessment showed IC_{50} of 151.23 and 183.75 µg/mL for Po-NLC and Po-NLC-MNPs, respectively.

Mosquito larvicidal activity

This study indicated the larvicidal effects of *Poinciana* extracts (Po) and their nanoparticles were evaluated against the 3rd larvae, *Cx. pipiens*, suggesting an insecticidal activity against *Cx. pipiens*. The insecticidal results showed that the highest larval mortalities were observed after post-treatments (PT) with nanoparticles rather than their corresponding plant extracts, whereas low to medium results were furnished by Free-NLC.

This study specified that the complete (100 MO%) larvicidal effects of PO extracts and their nanoparticles were recorded at the highest concentration of 1500 ppm, 24 h PT, except for Free-NLC and Free-MNPs, where the mortality was 30 and 35%, respectively. At 1000 ppm, the MO% of Po, Po-NLC, and Po-NLC-MNPs were 98, 100, and 100%, respectively (Table 3), with LC_{50} (50%, median lethal concentration) = 183.19, 110.19, and 100.55 ppm, respectively, for methanol extracts (Table 4), whereas those of aqueous extracts were 82, 97, and 95% (MO%), respectively, with LC_{50} values = 287.66, 192.68, and 177.78 ppm. The negative control (99 ml of distilled water with 1 ml of Tween 80) was not toxic to all larvae with zero mortality, while the positive control (temephos) had significant effects on the mosquito larvae (91 MO%).

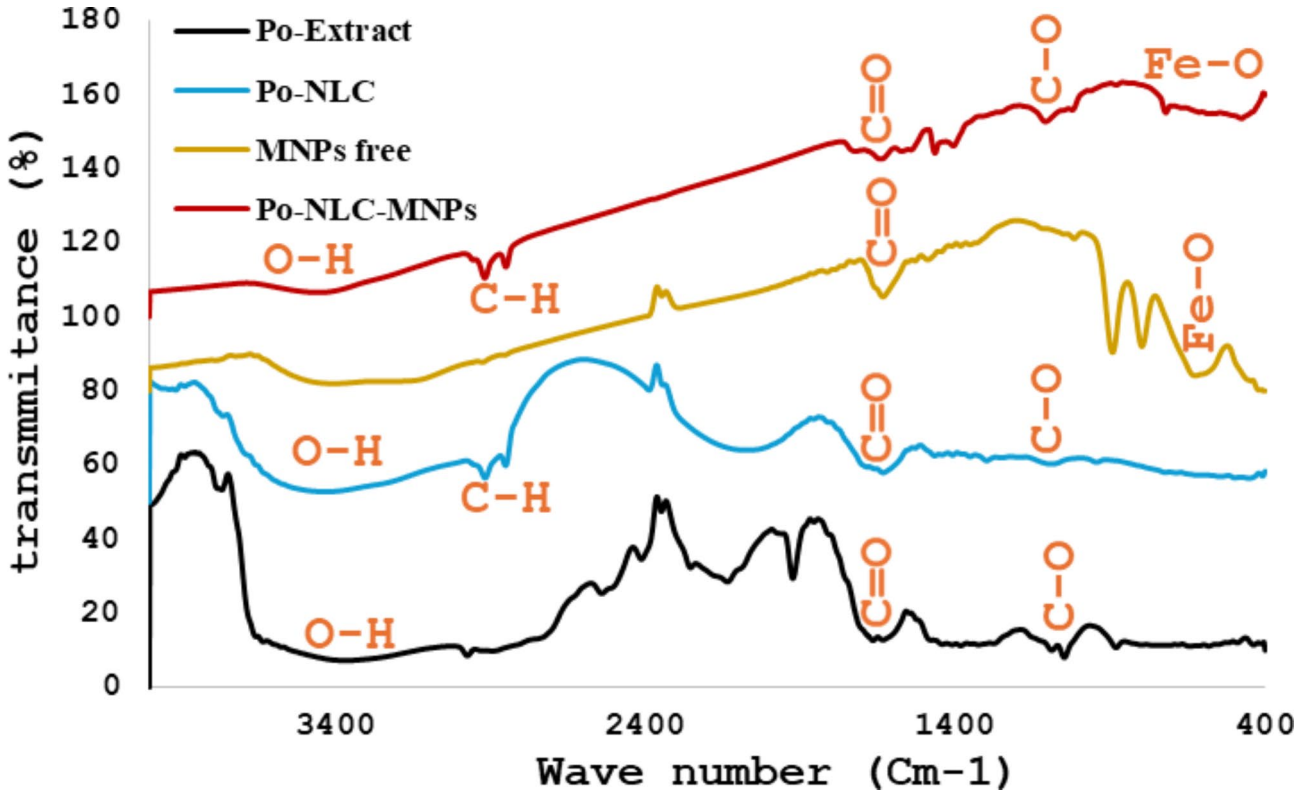


Fig. 9. Fourier Transform infra-red of free magnetic, Po-NLC and Po-NLC-MNPs.

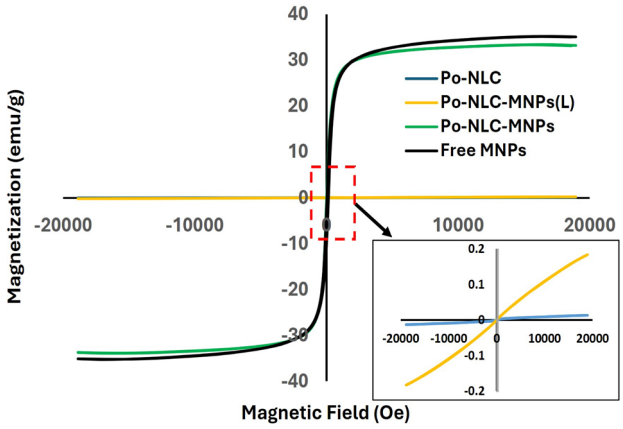
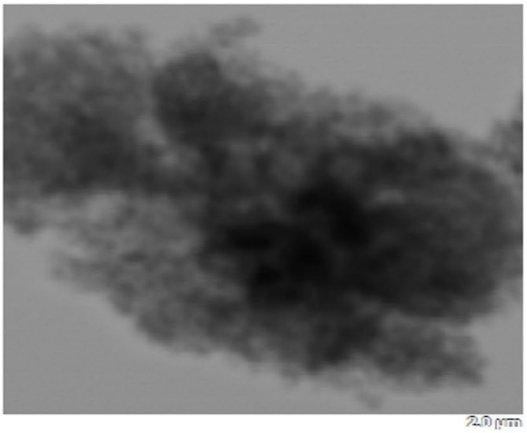


Fig. 10. Vibrating Sample Magnetometer of free magnetic, Po-NLC and Po-NLC-MNPs.

The highest larval mortality was recorded after 48 h of PT, where the mortality reached 100% in Po methanolic extract, Po-NLC, and Po-NLC-MNPs, and 96, 100, and 100% in aqueous extracts, respectively, at 1000 ppm (Table 5). In terms of lethal concentrations, LC_{50} (50%, median lethal concentration) for *Poinciana* NLC decorated MNPs (Po-NLC-MNPs) appeared to be most effective against *Cx. pipiens* larvae (LC_{50} = 70.10 ppm), followed by *Poinciana* NLC nano (LC_{50} = 77.18 ppm) and *Poinciana* extract (LC_{50} = 119.70 ppm) (Table 6).

The efficacy of plant materials against non-target predators

The efficacy of the plant extract was evaluated against several predators as nontarget insects, including *G. affinis, C. tripunctatus, S. urinator*, and *Stratiomys sp.* after being treated with LC_{50}. No significant difference was observed between the mean predation rates of plant materials (P = 0.515, df = 15, F = 0.954). The predation rate of the mosquitofish, *G. affinis* increased 79% for *Poinciana* extract compared to 75.67, 74.33, 78.33, and 76.67% for Po-NLC, Po-NLC-MNPs, Free-NLC, and Free-MNPs, respectively. Also beetle, *C. tripunctatus* and *Stratiomys sp.* increased 62.67% and 45.0% for *Poinciana* extract only, compared to 60.0, 57.33, 60.0, 63.67% and



MNPs free		
element	weight %	atom %
C	3.22	13.02
N	0	0
O	2.62	7.95
S	0	0
Fe	66.42	57.81

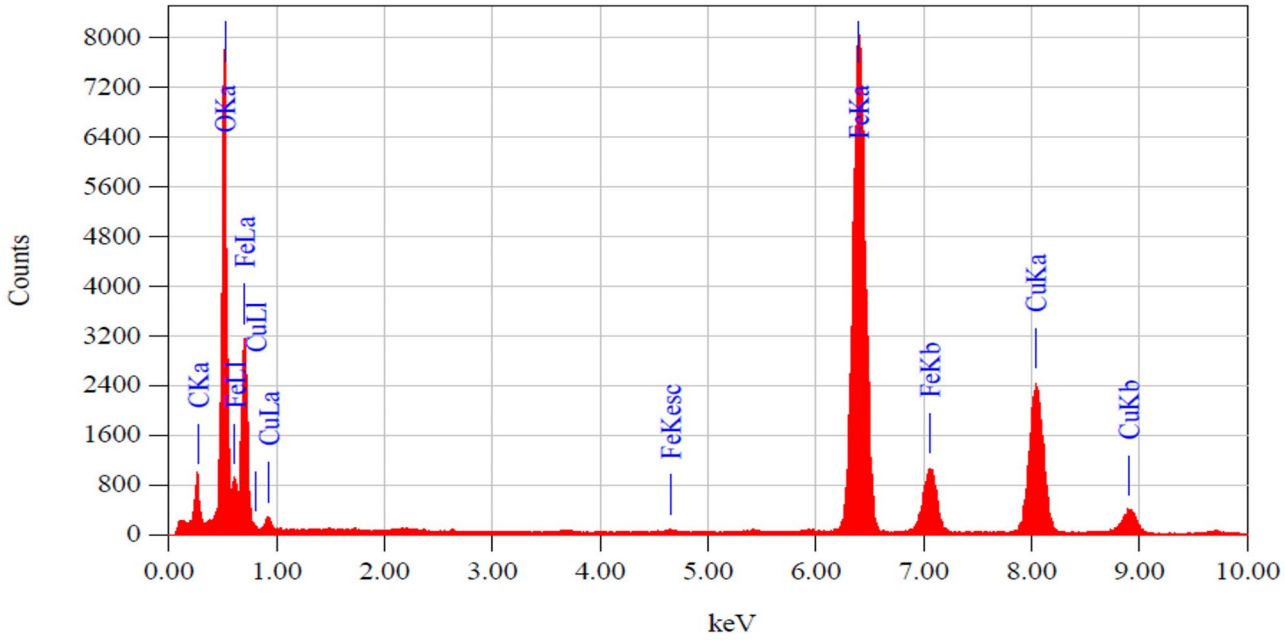


Fig. 11. EDS and elemental analysis of the fre MNPs.

42.0, 43.0, 42.0, 44.0% for Po-NLC, Po-NLC-MNPs, Free- NLC, and Free- MNPs, respectively. In contrast, the predation rate in the absence of treatments exceeded 25% for *S. urinator* (Table 7).

Metabolomic analysis of four plant extracts

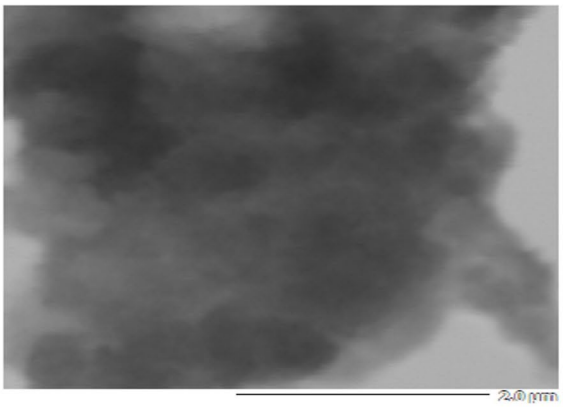
GC–MS data analysis

The plant extracts were subjected to metabolomics analysis, and a comparison was made between the methanol and aqueous extracts using GC–MS analysis. The results of our study's GC–MS analysis led to the identification of various compounds such as terpenes, fatty acids, esters, ketone, alkane, steroids, aliphatic amines, and phenols in the leaves of *Poinciana* using two different solvents (methanol and aqueous). The methanol leaf extract of *Poinciana* had 19 compounds (Table 8), while the aqueous leaf extract had 6 compounds (Table 9). The methanol extract of *Poinciana* had a lot of α-amyrin (25.25%), lupeol (18.0%), and 9-Octadecylphenanthrene (13.35%). The aqueous extract had Cis-13-eicosenoic acid (53.18%), 11-Octadecenoic acid, methyl ester (18.02%), and Methyl 9-cis,11-trans-octadecadienoate (12.44%).

Discussion

Plants are living entities that engage in the production of a diverse array of compounds, commonly referred to as secondary metabolites. Secondary metabolites like alkaloids, carbohydrates, flavonoids, saponins, tannins, and terpenoids are responsible for the pharmacological effects of medicinal plants. Many different groups of bioactive compounds that originate from or are present in botanical insecticides have the potential to harm pests or animals that consume or encounter them[44].

Before the discovery of chemical pesticides, researchers tried many plant treatments as effective mosquito control methods[45]. Furthermore, the community's favorable reaction to phytochemicals and their expanding



Po-NLC-MNPs		
element	weight %	atom %
C	33.67	62.17
N	0.02	0.04
O	11.57	16.04
S	0.03	0.02
Fe	54.71	21.73

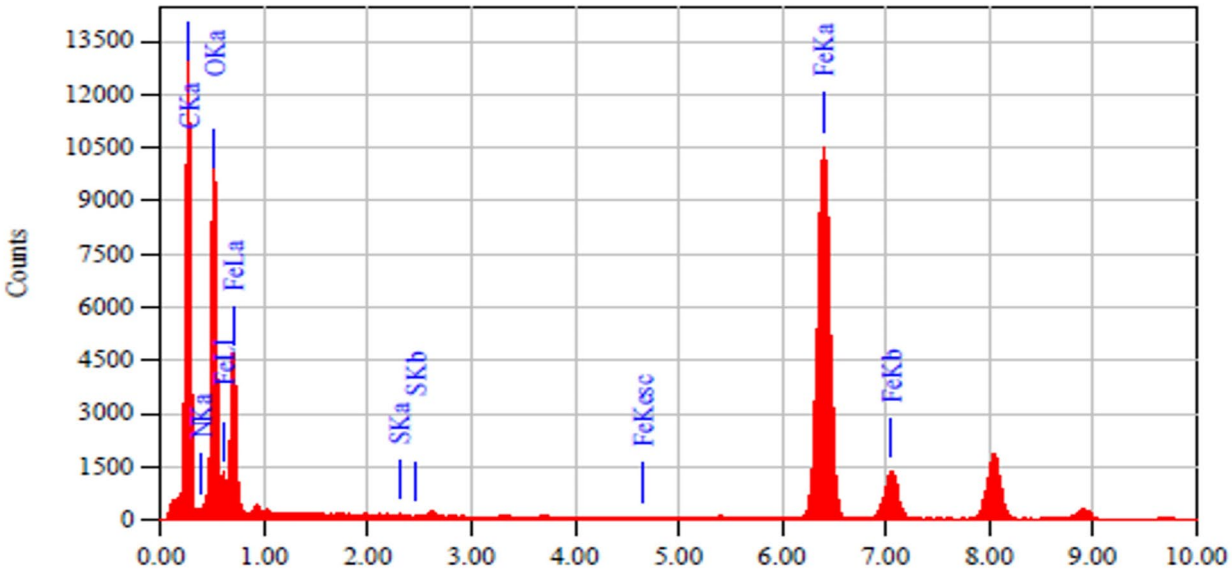


Fig. 12. EDS and elemental analysis of the Po-NLC-MNPs.

Time (h)	Conc (µg/ml) of *Poinciana* extract from Po-NLC	Conc (µg/ml) of *Poinciana* extract from PO-NLC-MNPs
0	0	0
2	24	12
4	33	19
6	39	29
8	49	36
10	44	32
12	48	20
14	39	23
16	35	17
18	34	14

Table 1. *Poinciana* extract release from Po-NLC and Po-NLC-MNPs.

trend, together with their environmentally benign nature, open up new avenues for the study and development of plant-based insecticides. Numerous other plant groups, such as the Fabaceae family, have demonstrated harmful characteristics towards various mosquito species[46,47].

This study examined the organic compounds in the methanol and water extracts of *Poinciana*. The results indicated that the methanol extract exhibited a greater variety of organic compounds, particularly a higher concentration of terpene compounds. Conversely, the aqueous extract predominantly contained fatty acid compounds, with terpenes being the most prevalent. The extraction yield and phytochemical content were impacted by the polarity of the extracting solvents[48]. The utilization of distinct solvents led to diverse extraction

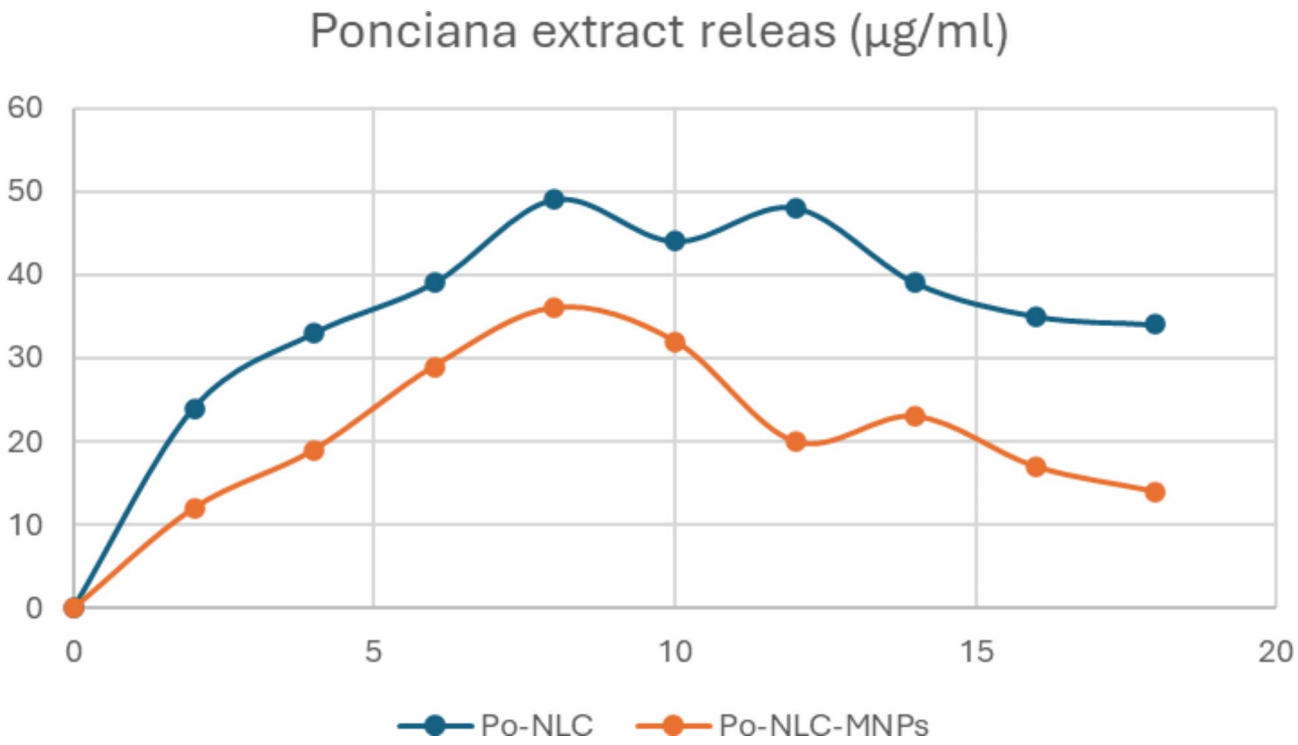


Fig. 13. *Poinciana* extract release from Po-NLC and Po-NLC-MNPs.

I.D	Conc	R1(O.D)	R2(O.D)	R3(O.D)	Mean O.D	SE	viability %	Toxicity %	IC_{50}
Wi38	-	0.579	0.566	0.571	0.572	0.003786	100	0	151.23 ± 0.55
Po-NLC	1000	0.031	0.036	0.03	0.032333	0.001856	5.652681	94.34732	
	500	0.059	0.057	0.055	0.057	0.001155	9.965035	90.03497	
	250	0.129	0.139	0.132	0.133333	0.002963	23.31002	76.68998	
	125	0.367	0.369	0.373	0.369667	0.001764	64.62704	35.37296	
	62.5	0.505	0.519	0.501	0.508333	0.005457	88.86946	11.13054	
	31.25	0.553	0.566	0.562	0.560333	0.003844	97.96037	2.039627	
Po-NLC-MNPs	1000	0.039	0.029	0.033	0.033667	0.002906	5.885781	94.11422	183.75 ± 0.81
	500	0.042	0.047	0.049	0.046	0.002082	8.041958	91.95804	
	250	0.204	0.21	0.195	0.203	0.004359	35.48951	64.51049	
	125	0.388	0.396	0.391	0.391667	0.002333	68.47319	31.52681	
	62.5	0.495	0.51	0.501	0.502	0.004359	87.76224	12.23776	
	31.25	0.54	0.551	0.566	0.552333	0.007535	96.56177	3.438228	

Table 2. *In-vitro* cytotoxicity assessment of Po-NLC and Po-NLC-MNPs against wi38.

yields. Differences in solvent polarity can explain the change in the amount of bioactive chemicals in the extract. This is because plant materials include significant levels of polar molecules that are soluble in solvents with high polarity. Another study showed that the acetone extract had more alkanes, flavonoids, terpenes, ketones, and phenols than the water-based extract[49].

The plant extracts have many biologically active compounds that are antimicrobial and antiparasitic. GC–MS revealed the presence of antimalarial bioactive compounds in the *Poinciana* plant, including myo-inositol, 4-C-methyl-, and Neophytadiene. Our findings align with prior research that endorses methanol as the optimal solvent for extracting more potent chemicals from different medicinal plants[50]. Earlier research has also shown that methanol and acetone are better at getting polar phytocompounds like phenolics out of the leaves of *L. camara* because it is more polar[51]. Our findings align with prior research that endorses methanol as the optimal solvent for extracting more potent chemicals from different medicinal plants[50]. Furthermore, the results showed that the methanol extracts from plant materials were more harmful to mosquito larvae of the species *Cx. pipiens* than the water-based extracts. We ranked the leaf extracts in terms of their toxicity to mosquito larvae as follows: Bosly[52] aligns with our ranking of acetone, methanol, aqueous, and hexane. Plant extracts' impact on mosquito species is dependent on the solvent used to extract the phytochemicals responsible for the observed reactions[53].

Solvent	System	Concentration (ppm)						
		0	50	100	200	500	1000	1500
Water	*Poinciana* extract	0 ± 0^{aG}	9 ± 1.87^{bF}	18 ± 1.22^{cE}	38 ± 2.55^{cD}	60 ± 2.24^{cC}	82 ± 2.55^{cB}	100 ± 0.00^{aA}
	Po-NLC	0 ± 0^{aG}	15 ± 1.58^{aF}	25 ± 1.58^{bE}	49 ± 4.30^{bD}	70 ± 3.54^{bC}	97 ± 2.00^{aB}	100 ± 0.00^{aA}
	Po-NLC-MNPs	0 ± 0^{aG}	16 ± 1.87^{aF}	29 ± 1.87^{aE}	54 ± 2.92^{aD}	72 ± 2.55^{aC}	95 ± 2.24^{bB}	100 ± 0.00^{aA}
	Free- NLC	0 ± 0^{aG}	1 ± 1.00^{cF}	3 ± 1.22^{dE}	7 ± 1.22^{dD}	12 ± 2.55^{dC}	20 ± 5.24^{dB}	30 ± 4.18^{bA}
	Free- MNPs	0 ± 0^{aG}	2 ± 1.22^{cF}	4 ± 1.87^{dE}	9 ± 1.00^{dD}	15 ± 0.00^{dC}	23 ± 1.22^{dB}	35 ± 2.74^{bA}
Methanol	*Poinciana* extract	0 ± 0^{aG}	14 ± 1.87^{cF}	25 ± 3.16^{cE}	52 ± 2.00^{cD}	80 ± 3.16^{bC}	98 ± 2.00^{bB}	100 ± 0.00^{aA}
	Po-NLC	0 ± 0^{aE}	20 ± 1.58^{bD}	42 ± 2.00^{bC}	66 ± 3.67^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	Po-NLC-MNPs	0 ± 0^{aF}	23 ± 1.22^{aE}	49 ± 4.3^{aD}	70 ± 3.54^{aC}	99 ± 1.00^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	Free- NLC	0 ± 0^{aG}	1 ± 1.00^{cF}	3 ± 1.22^{dE}	7 ± 1.22^{dD}	12 ± 2.55^{cC}	20 ± 5.24^{cB}	30 ± 4.18^{bA}
	Free- MNPs	0 ± 0^{aG}	2 ± 1.22^{cF}	4 ± 1.87^{dE}	9 ± 1.00^{dD}	15 ± 0.00^{dC}	23 ± 1.22^{dB}	35 ± 2.74^{bA}
	Temphos (1 mg/L)	91%	-	-	-	-	-	-

Table 3. The larvicidal effects of *Poinciana* extracts and their nanoformulation against *Culex pipiens*, 24 h post-treatment. a, b & c: There is no significant difference ($P > 0.05$) between any two means for each plant, 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.

Solvent	System	LC_{50} (Low-Up.)	LC_{90} (Low-Up.)	LC_{95} (Low-Up.)	Slope ± SE	X^2 (sign.)
Water	*Poinciana* extract	287.66 (193.62–421.41)	1270.69 (939.22–2505.94)	1936.09 (1427.07–4277.50)	1.986 ± 0.132	13.620 (0.008)
	Po-NLC	192.68 (129.27–286.05)	796.70 (592.48–1533.36)	1184.44 (886.07–2540.87)	2.109 ± 0.140	14.685 (0.005)
	Po-NLC-MNPs	177.78 (127.30–249.23)	792.00 (587.20–1376.26)	1202.05 (875.75–2310.39)	2.005 ± 0.137	10.047 (0.039)
	Free- NLC	4910.90 (2860.54–12419.29)	67631.94 (22658.00–478020.48)	142224.95 (40505.59–1353123.18)	1.125 ± 0.165	0.788 (0.940)
	Free- MNPs	4011.02 (2437.99–9094.04)	61046.09 (20537.41–348289.78)	122080.25 (36677.27–958567.22)	1.083 ± 0.150	1.040 (0.903)
Methanol	*Poinciana* extract	183.19 (159.58–209.26)	688.12 (570.32–866.30)	1001.36 (802.01–1322.21)	2.229 ± 0.150	4.986 (0.288)
	Po-NLC	110.91 (85.29–149.78)	331.88 (260.85–538.65)	448.28 (348.14–796.40)	2.782 ± 0.218	9.669 (0.046)
	Po-NLC-MNPs	100.55 (90.35–117.46)	319.27 (268.40–398.50)	439.31 (356.99–576.78)	2.620 ± 0.212	5.874 (0.208)
	Free- NLC	4910.90 (2860.54–12419.29)	67631.94 (22658.00–478020.48)	142224.95 (40505.59–1353123.18)	1.125 ± 0.165	0.788 (0.940)
	Free- MNPs	4011.02 (2437.99–9094.04)	61046.09 (20537.41–348289.78)	122080.25 (36677.27–958567.22)	1.083 ± 0.150	1.040 (0.903)

Table 4. Lethal concentrations (ppm) of *Poinciana* extracts and their nanoformulation against *Culex pipiens*, 24 h post-treatment.

Solvent	System	Concentration (ppm)						
		0	50	100	200	500	1000	1500
Water	*Poinciana* extract	0 ± 0^{aG}	13 ± 1.22^{cF}	28 ± 3.39^{cE}	56 ± 1.87^{cD}	78 ± 4.90^{cC}	96 ± 2.45^{bB}	100 ± 0.00^{aA}
	Po-NLC	0 ± 0^{aF}	19 ± 1.00^{bE}	32 ± 2.55^{bD}	60 ± 3.54^{bC}	85 ± 2.24^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	Po-NLC-MNPs	0 ± 0^{aF}	21 ± 1.00^{aE}	36 ± 2.45^{aD}	64 ± 2.92^{aC}	89 ± 3.32^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	Free- NLC	0 ± 0^{aG}	2 ± 1.22^{dF}	6 ± 1.00^{dE}	13 ± 2.00^{dD}	20 ± 2.24^{dC}	30 ± 5.24^{dB}	44 ± 1.87^{bA}
	Free- MNPs	0 ± 0^{aG}	3 ± 1.22^{dF}	9 ± 1.00^{dE}	18 ± 2.55^{dD}	26 ± 3.67^{dC}	35 ± 3.16^{cB}	48 ± 3.74^{bA}
Methanol	*Poinciana* extract	0 ± 0^{aF}	20 ± 2.24^{cE}	42 ± 2.55^{cD}	70 ± 2.24^{cC}	94 ± 1.87^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	Po-NLC	0 ± 0^{aE}	31 ± 2.45^{bD}	58 ± 2.55^{bC}	85 ± 1.58^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	Po-NLC-MNPs	0 ± 0^{aE}	34 ± 2.45^{aD}	61 ± 4.00^{aC}	88 ± 2.55^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	Free- NLC	0 ± 0^{aG}	2 ± 1.22^{dF}	6 ± 1.00^{dE}	13 ± 2.00^{dD}	20 ± 2.24^{cC}	30 ± 5.24^{B}	44 ± 1.87^{A}
	Free- MNPs	0 ± 0^{aG}	3 ± 1.22^{dF}	9 ± 1.00^{dE}	18 ± 2.55^{dD}	26 ± 3.67^{dC}	35 ± 3.16^{cB}	48 ± 3.74^{bA}
	Temphos (1 mg/L)	100%	-	-	-	-	-	-

Table 5. The larvicidal effects of *Poinciana* extracts and their nanoformulation against *Culex pipiens*, 48 h post-treatment. a, b & c: There is no significant difference ($P > 0.05$) between any two means for each plant, 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.

Solvent	System	LC_{50} (Low-Up.)	LC_{90} (Low-Up.)	LC_{95} (Low-Up.)	Slope ± SE	X^2 (sign.)
Water	*Poinciana* extract	179.00 (155.85–203.98)	702.92 (591.83–862.82)	1035.87 (846.50–1326.83)	2.157 ± 0.133	3.192 (0.526)
	Po-NLC	145.52 (126.52–166.14)	527.82 (439.74–660.98)	760.50 (612.42–999.32)	2.290 ± 0.161	7.675 (0.104)
	Po-NLC-MNPs	130.54 (113.27–149.12)	466.23 (388.64–584.38)	668.84 (538.60–880.85)	2.318 ± 0.168	5.146 (0.272)
	Free- NLC	2366.34 (1632.77–4112.35)	30538.21 (13,534.04–111770.90)	63,054.58 (24443.11–287435.57)	1.153 ± 0.139	1.774 (0.777)
	Free- MNPs	1863.41 (1313.58–3091.21)	27623.42 (12789.41–91570.91)	60092.43 (22128.78–246182.60)	1.080 ± 0.126	2.131 (0.711)
Methanol	*Poinciana* extract	119.70 (103.66–136.96)	419.74 (347.08–534.52)	599.02 (477.18–805.62)	2.352 ± 0.184	3.556 (0.469)
	Po-NLC	77.18 (68.93–90.84)	233.97 (197.23–293.43)	317.30 (257.92–421.49)	2.745 ± 0.256	2.212 (0.696)
	Po-NLC-MNPs	70.10 (63.56–84.44)	214.61 (181.09–269.45)	290.10 (235.88–386.74)	2.775 ± 0.270	2.093 (0.718)
	Free- NLC	2366.34 (1632.77–4112.35)	30538.21 (13534.04–111770.90)	63054.58 (24443.11–287435.57)	1.153 ± 0.139	1.774 (0.777)
	Free- MNPs	1863.41 (1313.58–3091.21)	27623.42 (12789.41–91570.91)	60092.43 (22128.78–246182.60)	1.080 ± 0.126	2.131 (0.711)

Table 6. Lethal concentrations (ppm) of *Poinciana* extracts and their nanoformulation against *Culex pipiens*, 48 h post-treatment.

Plant materials	*G. affinis*	*C. tripunctatus*	*S. urinator*	*Stratiomys sp.*
Poinciana extract	79.00 ± 2.08^{aA}	62.67 ± 2.33^{abB}	24.67 ± 3.71^{bcD}	45.00 ± 2.65^{aC}
Po-NLC	$75.67 \pm 0.33b^{cA}$	60.00 ± 2.89^{bcB}	21.67 ± 0.67^{dD}	42.00 ± 1.53^{bC}
Po-NLC-MNPs	74.33 ± 1.76^{cA}	57.33 ± 3.93^{cB}	28.33 ± 0.88^{aD}	43.67 ± 1.33^{abC}
Free- NLC	78.33 ± 1.20^{abA}	$60.00 \pm 2.65b^{cB}$	22.00 ± 0.58^{cdD}	42.00 ± 1.53^{bC}
Free- MNPs	76.67 ± 0.88^{abcA}	63.67 ± 0.88^{aB}	$23.33 \pm 0.33b^{cdD}$	44.00 ± 1.53^{abC}
Control	78.67 ± 0.67^{aA}	61.67 ± 3.48^{abB}	25.00 ± 0.58^{bD}	43.00 ± 1.53^{abC}

Table 7. The mean number (± SE) of *Culex pipiens* larvae consumed by some predators treated with *Poinciana* extracts and their nanoformulation under laboratory conditions. 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 & C: There is no significant difference ($P > 0.05$) between any two means, within the same row have the same superscript letter.

The ethanolic extracts from *Poinciana* bark and *Carica papaya* leaf were analyzed qualitatively and found to contain flavonoids, alkaloids, triterpenoids, steroids, tannins, and glycosides, among other phytochemicals[54]. The GC–MS test showed that the leaves of *Poinciana* contained a number of chemicals, such as phenols, terpenes, fatty acids, esters, ketones, alkanes, steroids, aliphatic amines, and esters. We achieved this identification by using two distinct solvents, namely methanol and water.

Certain plant compounds, such as elemene, caryophyllene, and copaene, can interfere with various processes or pathways, including the nervous system of insects, potentially leading to neurotoxic effects. This could lead to disruptions in nerve function, affecting the insects' ability to move, feed, or reproduce[55], interfere with feeding deterrence[56], interfere with chitin synthesis, a crucial component of the insect exoskeleton, and disrupt its formation, which can lead to developmental abnormalities[57], inhibit cholinesterase activity[58], disrupt cell membranes, leading to cell leakage and eventual cell death[59], or act as a repellent, influencing the behavior of insects and deterring. Myo-inositol and 4-C-methyl have demonstrated anti-alopecic, anti-cirrhotic, anti-neuropathic, anti-cancer, cholesterolytic, lipotropic, and deterrent properties[60].

As a result of the continuous increase in drug resistance in its crud form, the role of nanotechnology raises as a gateway that helps humanity to get out of the epicenter of the drug resistance, whether those used to treat humans or used to eliminate what is hostile to or attacks humans from microorganisms such as microbes and other large invaders such as insects. A nanostructured lipid carrier decorated with magnetic nanoparticles was designed to encapsulate Poinciana extract for many purposes, including protecting the low-stable activated ingredients from degradation. The introduction of the active ingredients in nanosize will make more effective and fast-acting mortalities.

The principle of Brownian motion is best describing the motility of colloidal solutions, with lighter particles moving faster and their speed directly correlated with their size. The particle size measurements of the synthesized nanostructure-based particles showed a narrow size distribution, with an average size of less than 100 nm. The nanostructure lipid carrier (Po-NLC) and its decorated form, Po-NLC-MNPs, hold a lot of the same-sized particles but have different polydispersity indices (PDIs). Such a value indicates the homogeneity of the particle size, or, based on the mean, the variation in the particle size. An increase in the PDI value indicates a more heterogeneous solution[61]. he synthesized Po-NLC-MNPs exhibit a PDI value that is twice that of Po-NLC itself. This could be attributed to the inability to incorporate some smaller-sized magnetic nanoparticles onto the NLCs' surface, or to the disengagement of the MNPs from the surface during the sonication process prior to DLS measurements, yet they remain within a good range of heterogenicity.

Zeta potential, an electrokinetic potential in colloidal dispersions, is defined as the electric potential at the slipping plane by the mean. This interface, which separates mobile fluid from fluid that remains attached to

No	RT	Area %	Compound Name	M. F	M.W
Cyclohexane (2.63)					
1	22.29	2.63	Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$	194
Faty acids and esters (32.51)					
2	13.10	2.02	1,2,3-propanetriol, triacetate	$C_9H_{14}O_6$	218
3	25.29	1.37	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
4	26.96	3.79	hexadecanoic acid	$C_{16}H_{32}O_2$	256
5	29.66	2.69	phytol	$C_{20}H_{40}O$	296
6	30.19	2.57	9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester, (z,z,z)- (Linolenoylglycerol)	$C_{21}H_{36}O_4$	352
7	30.66	1.40	octadecanoic acid	$C_{18}H_{36}O_2$	284
8	36.33	0.35	hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568
9	37.89	3.62	ç-sitosterol	$C_{29}H_{50}O$	414
10	39.19	1.35	ethyl iso-allocholate	$C_{26}H_{44}O_5$	436
11	43.04	13.35	9-Octadecylphenanthrene	$C_{32}H_{46}$	430
Acylaminosugars (2.04)					
12	39.52	2.04	17-ethyl[2.2.2](1,3,5)benzeno(3,3',3")triphenylmethanophane	$C_{32}H_{30}$	414
Terpene (Monoterpene and Sesquiterpene) (55.1)					
13	24.43	1.49	Neophytadiene	$C_{20}H_{38}$	278
14	38.90	25.25	α-amyrin	$C_{30}H_{50}O$	426
15	39.82	10.36	squalene	$C_{30}H_{50}$	410
16	39.94	18.00	lupeol	$C_{30}H_{50}O$	426
Glycoside (3.27)					
17	41.05	2.71	4 h-1-benzopyran-4-one, 2-(3,4-dihydroxyphenyl)-6,8-di-á-d-glucopyranosyl-5,7-dihydroxy-	$C_{27}H_{30}O_{16}$	610
18	42.08	0.56	9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á,5z,7e)-	$C_{27}H_{44}O_3$	416
Steroid (4.45)					
19	41.36	4.45	pregn-4-ene-3,20-dione, 16,17-epoxy-, (16à)-	$C_{21}H_{28}O_3$	328

Table 8. The major chemical constituents of *Poinciana* methanol extracts detected by GC-MS.

No	RT	Area %	Compound Name	M. F	M.W
Faty acids and esters					
1	25.68	1.05	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
2	28.69	12.44	Methyl 9-cis,11-trans-octadecadienoate	$C_{19}H_34O_2$	294
3	28.87	18.02	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296
4	29.43	6.71	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298
5	42.74	8.60	13-Eicosenoic acid	$C_{20}H_{38}O_2$	310
6	44.56	53.18	Cis-13-eicosenoic acid	$C_{20}H_{38}O_2$	310
		100			

Table 9. The major chemical constituents of *Poinciana* aqueous extracts detected by GC-MS.

the surface, is caused by the net electrical charge within the region bounded by the slipping plane. Always zeta potential expressed the stability of the colloidal system; the further the charge is away from zero, positive or negative, the more stable the system becomes[62]. The nanoparticles of Po-NLC showed a densely negatively charged zeta potential of -29.44 mV, while the value dropped to -4.44 mV for the synthesized Po-NLC-MNPs. Such slipping to a more elctropositive value confirms the decoration of the magnetic nps, as it is well known that the MNPs have electropositive zeta potential[63]. Despite having a very small negative charge, Po-NLC-MNPs remain within the accepted range of stability.

The X-ray diffraction (XRD) patterns for the free magnetite nanoparticles (MNPs Free) and the magnetite-loaded nanostructured lipid carriers (Po-NLC-MNPs) reveal that the crystalline structure of magnetite (Fe_3O_4) is preserved in both samples, as evidenced by the characteristic peaks. The peaks observed at 2θ of 18.52°, 30.25°, 35.60°, 43.34°, 53.60°, 57.12°, and 62.86° in both samples correspond to the spinel structure of magnetite. Notably, the peaks in the Po-NLC-MNPs sample are sharper and more intense, indicating enhanced crystallinity compared to the free nanoparticles. This improvement suggests that the lipid matrix provides a protective effect, reducing surface defects and aggregation, thereby preserving the crystalline integrity of the magnetite. The presence of characteristic peaks in both samples confirms the successful incorporation of magnetite into the NLC. This structure retention is crucial for applications relying on magnetic properties, such as pest control, where enhanced crystallinity may lead to better performance and stability.

The description of particle size and morphology was determined by transmission electron microscopy. The particle morphology of both Po-NLC and Po-NLC-MNPs has regular, irregular, oval, and spherical shapes. Po-NLC exhibited greater regularity than Po-NLC-MNPs due to the MNPs' decoration on the NLC's surface. The single-particle size measurements showed that there was size variation from smaller in range of 40–80 nm in addition to other sizes exceeding 100 nm in both nanoparticles. The decoration of MNPs at the surface of the NLC was obviously shown at different fields in addition to some other unloaded debris of MNPs with unnoticeable increase in the particle size of the Po-NLC-MNP nanoparticles. The selected-area electron diffraction of Po-NLC-MNPs (SAED) is one of the most common techniques for acquiring two-dimensional electron diffraction patterns. A SAED pattern can be used to identify the crystal structure. Our data clearly showed the spot diffraction pattern, confirming the crystallinity resulting from the incorporation of magnetic nps, as confirmed by numerous previous studies[64].

The FTIR spectra of *Poinciana* extract Po, Po-NLC, free MNPs, and Po-NLC-MNPs revealed distinct functional groups, confirming interactions between the components and consequently the succession of the incorporation processes. In the *Poinciana* extract Po, characteristic peaks are observed at around 3300 cm^{-1} (O–H stretching from phenols), 1630 cm^{-1} (C = O stretching from carbonyl groups in flavonoids and proteins), and 1050 cm^{-1} (C-O stretching from polysaccharides), indicating the presence of some bioactive phytochemical compounds. For the Po-NLC sample, slight shifts in these peaks such as a shift of the O–H stretch to around 3280 cm^{-1}—suggest interaction between the *Poinciana* extract components and the lipid nanocarrier, likely through hydrogen bonding. Additionally, the peaks at 2848 cm^{-1} and 2915 cm^{-1} in Po-NLC correspond to the symmetric and asymmetric C-H stretching vibrations of alkyl chains in the lipid matrix, indicating successful lipid incorporation within the nanocarrier structure. The free magnetite exhibited a distinct Fe–O stretching vibration around 580 cm^{-1}, characteristic of magnetite. Similarly, Po-NLC-MNPs sample showed less intense and more broadened peak attributable to the Fe–O. Moreover, there is a noticeable shift in the O–H and C = O peaks (e.g., O–H at 3290 cm^{-1} and C = O at 1625 cm^{-1}), indicating successful incorporation the magnetite at the NLC surface. These spectral changes confirm the formation of the Po-NLC-MNPs composite, highlighting interactions between the magnetite, and the lipid matrix,

The magnetization curves of MNPs free, Po-NLC, and Po-NLC-MNPs nanoparticles reveal distinct magnetic behaviors, especially, the free MNPs sample showed typical superparamagnetic behavior with a high saturation magnetization (Ms) around 35 emu/g, reflecting the strong magnetic response of pure magnetite nanoparticles. In contrast, the sample of Po-NLC-MNPs, displayed a significantly reduced in Ms value (≈0.20 emu/g) due to the fatty nature of the NLC[65].

High concentration of magnetic nanoparticle encapsulation was accomplished with the addition of small amount of the NLC to produce (Po-NLC-MNPs). According to the results presented in Fig. 9 the magnetization of the Po-NLC-MNPs nanoparticle revealed mild Ms reduction to (Ms≈32 emu/g) superparamagnetic nps. *Nunzio Denora*[66] and co-workers reported the synthesis of solid lipid particles encapsulated with sorafenib and magnetic nps. The saturation magnetization was found to be less than 40 emu/g which is consistent with the results revealed by this study.

Energy X-ray spectroscopy is a convenient method for identifying the elements with weight and atomic percentage presented in a sample. EDX analysis of free MNPs showed spectrum due to the existence of the preliminary of Fe, and O as major components. Also, the EDX results for the Po-NLC-MNPs showed an increase in the percentage of both carbon and oxygen and a decrease in the percentage of iron with a noticeable presence of both sulfur and nitrogen. Such results confirm the following: First, the encapsulation of the *poinciana* extract within the NLC structure second, the decrease in the balance of the iron element confirms the decoration or immobilization of the magnetic nps at the surface of the NLC and consequently the succession of the loading processes.

The encapsulation efficiencies of the *poinciana* extract in the synthesized Po-NLC and Po-NLC-MNPs were found to be 78.23% and 69.97%, respectively. As a result of the fact that the *poinciana* extract contains multiple hydrophilic compounds as well as many essential oils, making the NLC a fertile environment for loading the *poinciana* extract, which enhanced the loading efficiency in the case of Po-NLC. The decreased loading efficiency of Po-NLC-MNPs with the extract can be explained by the increased sonication process that followed the decoration process by MNPs, which in turn led to the explosion of some capsules containing the *poinciana* extract instead of being decorated by the magnetic materials and ended up in the non-encapsulated solution. Loading capacity expressed the amount of drug per unit weight of the nanoparticles, indicating the percentages of mass of the nanoparticles that is due to the encapsulated drug. Drug loading capacity could be calculated by the amount of entrapped drug divided by the total nanoparticle weight. The DL of Po-NLC was 31.6% meanwhile Po-NLC-MNPs was 23.45%, respectively.

The main purpose of using nano delivery systems, especially lipid based nanocarrier, is to guarantee maximum utility of every small dose with lowest drawbacks. Drug loading capacity, control how much the carrier could load the target molecule. Also, drug release is a crucial parameter that indicating how much drug will release at time. The release profile of poinciana extract from Po-NLC and PO-NLC-MNPs using phosphate buffer saline (PBS) as releasing media at a fixed pH (7.5). It was found that the Poinciana extract releases from the Po-NLC more easily than those in PO-NLC-MNPs, confirming the extract's slow release to a slower time from Po-NLC-MNPs. The explanation may be because the magnetic materials on the surface represented an additional barrier that the extraction had to cross first, which takes a longer time extended to the first 24 h followed by relative constancy and then a noticeable decrease, especially in the case of Po-NlC. Such slow release in the case of Po-NLC-MNPs is best described as the Poinciana extract released sustainably.

The cytotoxic effect of the synthesized Po-NLC and Po-NLC-MNPs was studied against the Wi38 cell line. Cell viability, cytotoxicity, and IC_{50} calculations confirmed the low cytotoxic effect of both NLC nanoparticles, even though Po-NLC showed a lower IC_{50} value of 151.23 µg/mL. This results in agreement with the outcomes

reported by Rodenak-Kladniew, et al.[67]. which discuss the synthesis of NLC incorporated with 1,8-cineole (monoterpenes), and no toxicities were distinguished.

The use of botanicals as larvicides, including plant extracts and essential oils, is a promising area of research[68,69]. This study evaluated the larvicidal effects of Po and its nanoparticles on *Cx. pipiens* third larvae. All formulations, including nanoparticles, methanol, and aqueous plant extracts, showed the highest larval mortality rates, but at low concentrations, the Po-NLC and Po-NLC-MNP nanoparticles outperformed Po in killing mosquito larvae. Also, our data reveal that Free-NLC has low toxicity on mosquito larvae.

Our data were consistent with the work of Radwan, et al.[23] which clearly showed that jasmine and peppermint nanoparticles were better than essential oils against 2nd and 4th instar larvae of *Cx. pipiens* at 2000 ppm. The findings of the present work regarding the high insecticidal activity shown by Po-NLC and Po-NLC-MNP nanoparticles agreed with several studies reporting similar insecticidal efficacy of nanoparticles in killing mosquito larvae over plant extract or essential oils alone[70]. Like our results, the nanoparticles of *Mentha piperita* exhibited high toxicity ($LC_{50} = 3879.5 \pm 16.2$ µl/L) against the cotton aphid, *Aphis gossypii*[71]. A study revealed that Jasminum nanoformulation possesses potent anti-acaricidal properties against the two-spotted spider mite, *Tetranychus urticae*, with 68.50% mortality and a reduction rate of 49.03%[72].

Similar to our findings, Hikal, et al.[73] conducted a study to evaluate the efficacy of essential oils and their nanoparticles. The outcomes by Hikal's work showed that the larvicidal activity of honeysuckle ($LC_{50} = 88.30$ ppm) and patchouli ($LC_{50} = 93.05$ ppm) nanoparticles was much higher than that of honeysuckle ($LC_{50} = 247.72$ ppm) and patchouli ($LC_{50} = 276.29$ ppm) oils. Nanoparticles of *Poinciana* (*Delonix regia*) extracts could offer a promising method for controlling mosquito larvae.

These Po extract and their conjugate nanoparticles are excellent pesticides due to their chemical stability, low cost, water dispersibility, target action, and low ecological toxicity.

Conclusion

Recently, the demand for novel and eco-friendly control tools to combat vector outbreaks has become essential. Nanotechnology has the potential to broaden the range of existing insecticidal effects. Enhancing the physical, chemical, and biological properties of essentials, phytocompounds, and other materials improves them. Using lower concentrations and lowering the insecticide concentration results in increased pesticide effectiveness and reduce environmental side effects. The results of this study showed that *Poinciana* and their lipid nanoformulations could kill 3rd *Cx. pipiens* larvae insects. Interestingly, the nanoparticles of Po-NLC and Po-NLC-MNPs showed promising insecticidal activities. The synthesized nps exhibited a high loading capacity, significantly enhancing Po nanoparticles' ability to kill larvae. These nanoparticles are excellent pesticides because of their chemical stability, low cost, water dispersibility, target action, and low ecological toxicity. We could conclude that nanopesticides have the potential to create a new generation of eco-friendly, effective alternatives for controlling mosquito-borne diseases. We recommend conducting field applications and ecotoxicological studies.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Conceptualization, methodology, investigation, data curation, formal analysis, resources, writing-original draft preparation, M.M.B., I.T.R, A.S., M.M.H., M.S.E., M.H.A., N.B., M.A.F., H.S.G.; editing and writing-review, M.M.B., I.T.R, A.S., M.M.H., M.S.E., A.M.A., M.H.A., N.B., M.A.F., H.S.G.; project administration, A.S.; funding achievement, M.M.B., M.S.E.,I.T.R, A.S., A.M.A., M.M.H., M.H.A., N.B., M.A.F., H.S.G. All authors have read and approved the published version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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