

RESEARCH PAPER

Lavender as a Green Biosynthetic Agent for Zinc Oxide Nanoparticles: A Novel Environmental Strategy to Manage Culex Mosquitoes in Their Immature Stage

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ABSTRACT

This study assessed the larvicidal effectiveness of zinc oxide nanoparticles (ZnO-NPs) synthesized using lavender (*Lavandula angustifolia* Mill.) extract, alongside the plant's alcoholic and aqueous extracts, against various life stages of *Culex pipiens* mosquitoes. ZnO-NPs showed the highest efficacy, with mortality rates of 33.87% (eggs), 60.32–45.72% (first and second instars), 37.28% and 27.11% (third and fourth instars), and 11.72% (pupae). Nanoparticle characteristics were confirmed using XRD, UV-Vis, EDS, and FESEM analyses, demonstrating their appropriate size and uniform distribution. The alcoholic extract was moderately effective, with mortality ranging from 51.04% to 15.99% across larval stages, and 8.5% in pupae. The aqueous extract was least effective, with mortality rates not exceeding 25% in early stages and dropping to 3% in pupae. Egg mortality did not exceed 12% with either extract. Mortality increased with higher concentrations, while larval sensitivity declined in later stages. DPPH antioxidant assays showed rising inhibition rates with increasing concentrations, peaking at 83.1% at 1 mg/ml. Overall, ZnO-NPs synthesized from lavender demonstrate potential as eco-friendly alternatives to chemical pesticides, supporting sustainable mosquito control strategies.

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INTRODUCTION

Controlling Mosquito-Borne Disease Vectors: The Role of Lavender and Modern Technologies Vector-borne diseases, particularly those caused by mosquitoes, are among the most significant global health challenges, due to their high morbidity and mortality rates among populations. In this context, the World Health Organization (2016) emphasizes the importance of implementing effective and

sustainable vector control programs to reduce the spread of these diseases [1,2]. Several strategies have already been implemented, including physical, chemical, and biological methods [3].

Within chemical methods, synthetic compounds with insecticidal properties are used, and *Culex pipiens* mosquitoes are among the species most targeted by these pesticides [4]. This species belongs to the Culicidae family and is considered

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one of the most important vectors of St. Louis encephalitis virus in North America, as well as West Nile virus in Europe, and is widely distributed globally [5].

Due to the environmental and health risks associated with synthetic pesticides, calls are growing for the development of “green” or biopesticides that are environmentally friendly and harmless to non-target organisms [6]. Among the promising natural alternatives, several active compounds have been studied, including monoterpenes, which are secondary phenolic compounds derived from plants, including lavender. In this study, lavender (*Lavandula* spp.) was chosen for its effective biological and chemical properties in repelling insects, especially mosquitoes. Several previous studies have shown that a class of volatile essential oils found in lavender, particularly linalool and linalyl acetate, are effective insect repellents [7].

Lavender oil is a potential natural alternative to traditional chemical pesticides such as DEET, which can cause negative side effects with prolonged use, because it is generally safe for human use and does not irritate the skin, Lavender oil’s potential for indoor and outdoor use is enhanced by its pleasant scent, which contrasts with the strong, unpleasant odors of many insect repellents. It also has antibacterial, antifungal, and anti-insect properties [8].

The perennial lavender shrub (*Lavandula angustifolia*) belongs to the Lamiaceae family and is a Mediterranean plant widely cultivated in Egypt for medicinal, aromatic, and culinary purposes. The essential oil extracted from it has multiple properties, including antiviral properties. Fungi, bacteria, and insects [9]. Numerous studies indicate lavender’s anti-inflammatory, antibacterial, and antiviral effects, as well as its anticancer properties and its role in alleviating mood disorders [10].

In a recent study [11], the insecticidal effect of three plant species from the Lamiaceae family, including lavender and mint (*Mentha piperita* L.), was evaluated against *Culex pipiens* mosquitoes. The essential oils of these plants demonstrated high efficacy, recording a 100% mortality rate for immature mosquito stages at LC₅₀ concentrations of 0.81% and 0.91%, respectively.

In addition to its high efficacy, lavender oil has insect repellent properties, making it a promising

option for personal prevention or indirect control techniques. It is also considered environmentally safe, thanks to its low toxicity to humans and non-target organisms, and the lower likelihood of insects developing resistance to it compared to traditional chemical pesticides [12].

Based on these properties, lavender is one of the most promising plant alternatives for the biological control of insect pests and disease vectors.

Nanotechnology has witnessed remarkable development in various fields, such as industry, medicine, and consumer goods. Its importance in combating insect pests has increased due to its effective biological effects and high targeting precision [13]. In this context, a study has shown that green nanoemulsions extracted from the lavender plant (*Lavandula officinalis*) are significantly more effective in eliminating *Culex quinquefasciatus* mosquitoes than metallic nanoparticles such as silver or biodegradable particles such as chitosan. Mammalian toxicity evaluations have also confirmed the safety of these compounds, enhancing their potential for use as safe and sustainable methods for disease vector management [14].

Among the various types of nanoparticles, zinc oxide nanoparticles (ZnONPs) have emerged as a promising option due to their high bioactivity and selective toxicity towards mosquito larvae, along with their ease of preparation using environmentally friendly methods. ZnONPs possess unique physical and chemical properties that enable them to damage cell membranes and produce reactive oxygen species (ROS) within cells, leading to larval death through multiple mechanisms that insects find difficult to develop resistance to. These properties make nanotechnology a more effective and safer method compared to traditional insecticides [15].

The researchers [15] synthesized ZnONPs for the first time using *Lavandula angustifolia* leaf extract in an environmentally sustainable manner. The particles exhibited distinct crystalline properties and a truncated octahedral shape, with an average size of approximately 74.58 nm. The study demonstrated the high efficacy of these particles against *Aedes albopictus* mosquito larvae, with 100% mortality within 24 hours at a concentration of 160 mg/L, confirming their potential for effective and safe disease vector

control.

MATERIALS AND METHODS

Conditions for Rearing Mosquito Colonies (*Cx. pipiens*):

Multiple generations of *Culex pipiens* mosquitoes were reared in the Advanced Insect Ecology Laboratory at the College of Science for Women, University of Babylon. Immature stages (eggs, larvae, and pupae) were collected from sewage sites in Babylon Governorate using a long scoop and transferred to glass tanks filled with tap water inside the rearing room. Larval food was distributed at a rate of 1 g per tank of mouse feed consisting of corn, wheat, rice, and protein (1:1:1:0.25).

Pupae were transferred to plastic containers within wooden cages, and adults were provided with different feeding sources. Males were given sugar solutions and females were given de-feathered pigeon blood three days after emergence. Water containers were placed inside the cages to encourage egg laying, and the egg boats were then transferred to new containers to continue the life cycle.

The process continued until the third generation, where samples of larvae and adults were collected for diagnosis based on taxonomic keys and confirmed at the Natural History Museum of the University of Baghdad as *Culex pipiens*. Experiments were carried out under controlled laboratory conditions ($28\pm 2^{\circ}\text{C}$, relative humidity $43\pm 2\%$, lighting for 12 hours) according to [16].

Preparation of a Plant Extract (Aqueous and Alcoholic)

Lavandula angustifolia leaves and flowers were shade-dried after being sterilized and thoroughly washed, then ground into a fine powder. 10 grams of the powder was taken and added to 200 ml of distilled water (or methanol for the alcoholic extract) in a glass flask. The mixture was placed in an electric shaker for 24 hours at room temperature, covered with aluminum foil.

After extraction, the mixture was filtered using Whatman No. 1 filter paper under vacuum and then centrifuged at 3,000 rpm for 10 minutes. The resulting extract was concentrated using a rotary evaporator at 40°C for 5–10 minutes under reduced pressure to obtain a dry extract. It was



Fig. 1. Mosquito breeding cage.

then stored at 4°C until use [17].

Evaluation of the Biological Activity of Aqueous and Alcoholic Extracts of Lavender

3 grams of the dry crude aqueous extract were dissolved in 100 ml of distilled water to prepare a 3% base (stock) solution, equivalent to 30 mg/ml. Diluted concentrations of 25, 20, 15, and 10 mg/ml were prepared from this solution. For the control treatment, only distilled water was used.

For the alcoholic extract, 3 grams of the crude extract were dissolved in 3 ml of methanol, then the volume was brought to 100 ml using distilled water to prepare a 3% (30 mg/ml) stock solution. Diluted concentrations of 25, 20, 15, and 10 mg/ml were prepared from this solution. In the control treatment, 1.5 ml of methanol alcohol was used and the volume was completed to 100 ml with distilled water.

Preparation and Synthesis of Zinc Oxide Nanoparticles (ZnONPs) Using a Plant Extract

Zinc oxide nanoparticles (ZnO-NPs) were prepared using the green method using the aqueous extract of *Lavandula angustifolia* leaves as a natural reducing agent and stabilizer, according to the study by [18]. A 0.05 mol/L solution of zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) was prepared, and 20 ml of 5 mg/ml lavender extract was gradually added to 50 ml of zinc nitrate solution under continuous stirring at 60–80°C. A color change was observed in the solution, indicating the initiation of ZnO nanoparticle formation. The pH of the mixture was adjusted to 10 using 1M NaOH to promote precipitation. After the reaction was completed, the resulting precipitate was collected by centrifugation at 8,000 rpm for 15 min and then washed several times with distilled water and ethanol to remove impurities. The resulting mixture was dried in an oven at 60 °C for 12 h to obtain pure ZnO-NPs powder, which was then stored in a tightly sealed dark glass container until use. Particle formation was confirmed using analytical techniques such as UV–Vis spectroscopy, XRD, and SEM to determine the size, shape, and crystal structure. The concentration of the extract added to the reaction mixture was calculated by dividing the total amount of active ingredient ($5 \text{ mg/mL} \times 20 \text{ mL} = 100 \text{ mg}$) by the total volume (70 mL), yielding a final concentration of 1.43 mg/ml. This concentration served as a control solution to evaluate the effect of the extract alone without

reaction with salts.

Characterization of nanoparticles

Nano-scale characterization is a pivotal step in the field of nanotechnology, as it involves the precise and comparative analysis of the structural, physical, and chemical properties of nanoparticles. This aspect is increasingly important when designing nano-delivery systems based on natural extracts, such as lavender (*Lavandula angustifolia*), to enhance their therapeutic efficacy and biological safety. The primary goal of nanoscale characterization is to verify the efficacy of nanoparticle formulations, carefully assessing a range of biological parameters such as geometry, size distribution, surface density, encapsulation stability, and structural equilibrium [19]. These factors are critical to ensuring the efficacy and safety of the final products when used biologically. To achieve comprehensive and accurate characterization, a range of advanced analytical techniques are employed, most notably:

Dynamic Light Scattering (DLS): This technique is used to determine the average particle size and polydispersity index (PDI). Size homogeneity (low PDI) is essential for ensuring stability and consistent biological activity. The ideal size range (10–200 nm) promotes cellular uptake and efficient interaction [20].

Fourier Transform Infrared Spectroscopy (FTIR): This technique helps detect functional groups and chemical bonds, enabling us to confirm the success of encapsulation and identify potential interactions between particles and extracts, enhancing formulation stability and compatibility [21].

Scanning Electron Microscopy (SEM): This technique provides high-resolution images to analyze the external appearance of particles, determining their shape and surface texture, including detecting any structural defects that may affect the integrity of the formulation.

Transmission electron microscopy (TEM): This technique allows for the examination of the internal structure of particles at nanoscale resolution, helping to assess the distribution of components and the effectiveness of nanoencapsulation systems [22].

X-ray diffraction (XRD): This technique is used to determine the crystalline or amorphous nature of particles and provides important insights into the structural changes resulting from encapsulation

processes, helping to improve methodology [23].

Antioxidant properties

The test known as 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to assess the antioxidant activity of the lavender plant extract and green zinc oxide nanoparticles that were manufactured. In short, 3 ml of the bio-synthesized green zinc oxide nanoparticles and plant extract in ethanol at varying concentrations (0.12, 0.5, 0.5, and 1 mg/mL) were mixed with 1 ml of 0.1 mM DPPH solution. Using the dilution method, these concentrations were made [24].

As a standard, ascorbic acid was employed. The control DPPH was measured without sample. After a thorough shake, the mixture was allowed to sit at room temperature for half an hour. The absorption was measured at a wavelength of 517 nm using a spectrophotometer (UV-VIS Milton Roy, USA). The experiment was carried out three times. The IC50 value was calculated using Log dose inhibition curve. The percentage of DPPH scavenging effect was calculated using the Eq. 1 [25,26]:

$$A_0 - A_1 / A_0 * 100 \quad (1)$$

where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of test.

Study of the effect of alcoholic, aqueous extracts and synthetic zinc nanoparticles of *Lavandula angustifolia* on the biological performance of *Cx. pipiens* mosquitoes (Non-cumulative mortality).

The effect on egg mortality

After obtaining one- to two-day-old egg boats (containing 110–180 eggs/boat) from the reared colony in rearing cages, they were transferred to plastic containers containing 60 ml of alcoholic and aqueous extracts of *Lavandula angustifolia*, and zinc nanoparticles synthesized using lavender extract, each separately, with three replicates for each concentration (one boat/replicate). A 3% methanol solution diluted to 100 ml with distilled water served as the control for the alcohol, and de-chlorinated water served as the aqueous extract. The control treatment for the nanoparticle experiment was aqueous thyme extract at the

same synthesis concentration. Post-hatching egg mortality rates were calculated and corrected according to the Abbott (1925) equation (Eq. 2) [27].

The effect on larval stage mortality

Larvae of different instars were exposed to the studied concentration levels of each extract separately. Ten first-instar larvae were taken from each replicate (three replicates for each concentration) and placed in plastic containers containing 60 ml of extracts at concentrations of 10, 15, 20, and 25 mg/ml, with 0.1 g of feed added to each replicate. Mortality was recorded after 24 hours, and the same steps were repeated for the second, third, and fourth instars separately. The control group was used as mentioned in the previous paragraph, and values were corrected using the Abbott equation (1925) [27].

The effect on pupa stage mortality.

Ten newly metamorphosed pupae from each replicate, three replicates for each concentration and extract type, were placed in containers containing 60 ml of the concentrations used in the experiment. After 24 hours of treatment, mortality was monitored, and corrected mortality rates were calculated using the Abbott equation (Abbott, 1925) [27].

The effect of alcoholic, aqueous extracts and ZnONPs from *Lavandula angustifolia* on cumulative mortality, immature development duration, and adult productivity of *Culex pipiens* mosquitoes

To evaluate the cumulative effect of alcoholic and aqueous extracts and ZnONPs prepared from *Lavandula angustifolia* on immature stages of *Culex pipiens* mosquitoes, eggs were treated separately with the specified concentrations of each extract, according to the approved egg treatment protocol. After hatching, first-instar larvae were transferred to containers containing 100 ml of the extract at a rate of 20 larvae/replicate, with three replicates for each concentration. The growth of larvae and pupae was tracked until adulthood, with daily mortality recorded, and any morphological abnormalities were classified under microscopic examination. Distilled water was used

$$\text{Corrected mortality rate\%} = \frac{\text{Treatment mortality rate} - \text{Mortality rate in control treatment}}{100 - \text{Mortality rate in control treatment}} * 100 \quad (2)$$

to compensate for evaporation losses [28]. The duration of insect development from egg to adult was also calculated. Adults emerging from each treatment were transferred to separate cages for mating and feeding on pigeon blood. Males were given a 10% sugar solution, and a water container was placed to encourage females to lay eggs. The number of egg boats and hatchability were recorded for each treatment independently.

Statistical Analysis

The experiments were conducted according to the global model of experiments and using a completely randomized design. Mortality rates were corrected using the Abbott equation, while

adjusted ratios were converted to angular values for use in statistical analysis. The least significant difference (LSD) test was performed to determine the significance of differences between treatments, and the Duncan test was used to compare means at a probability level of less than 0.05. Data were analyzed using SPSS version 26 [29].

RESULTS AND DISCUSSION

Characterization of biosynthesized ZnO NPs(Physical characterization)

UV-visible Spectroscopy

Lavender (*Lavandula angustifolia*) extract is used in the synthesis of zinc oxide nanoparticles

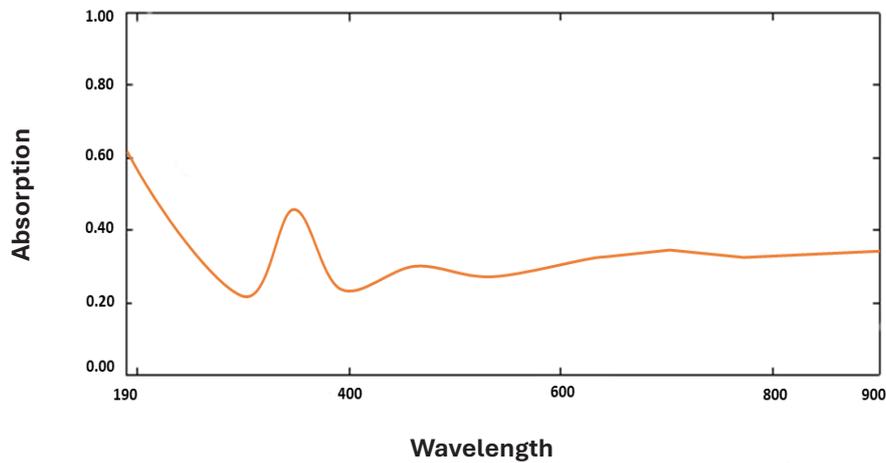


Fig. 2. UV-visible spectroscopy analysis of ZnO NPs synthesis by *Lavandula angustifolia*.

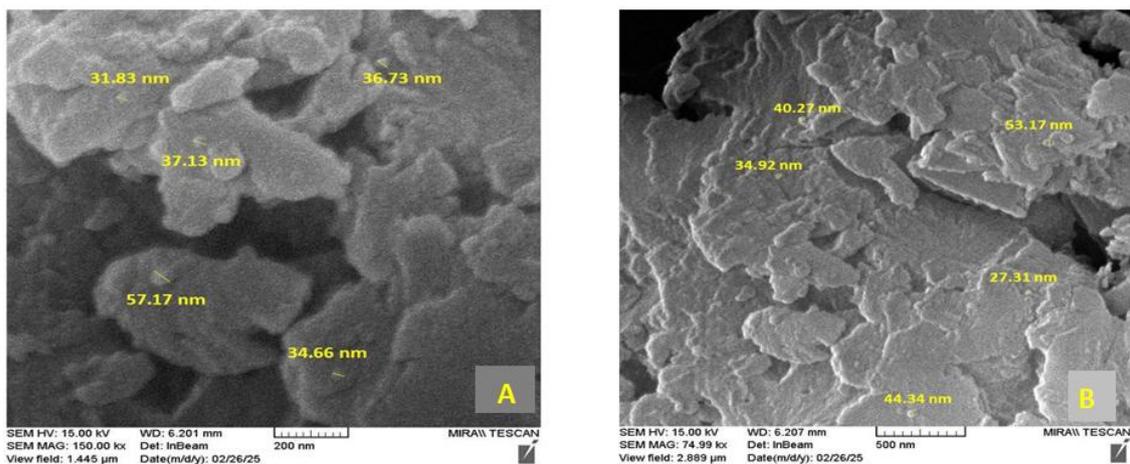


Fig. 3. Field-emission scanning electron microscope (FESEM) images of bio-zinc oxide nanoparticles extracted from lavender. The particles appear spherical and well-distributed, with an average size of 57 nm. A: at 200 nm, B: at 500 nm.



using the green preparation method, due to its content of flavonoids and potent phenolic compounds.

UV-Vis spectra of the lavender extract and the resulting nanoparticles revealed a clear absorption peak at approximately 365 nm. This is primarily due to the presence of phenolic compounds, especially flavonoids, in lavender leaves, which act as reducing and stabilizing agents for the nanoparticles during the reaction Fig. 2 [30]. This result also indicates the efficient and effective formation of zinc oxide nanoparticles, with an absorption spectrum consistent with the

nanostructure and influenced by the composition of the plant extract. The absorption pattern at 365 nm is also evidence of the richness of the lavender extract in antioxidants and its role in conferring stability and purity to the produced particles [31].

Field Emission Scanning Electron Microscopy (FESEM) analysis

Field-emission scanning electron microscopy (FESEM) was used in this study to analyze the surface structure and determine the nanoscale dimensions of zinc oxide nanoparticles prepared using aqueous extracts of lavender, as shown in

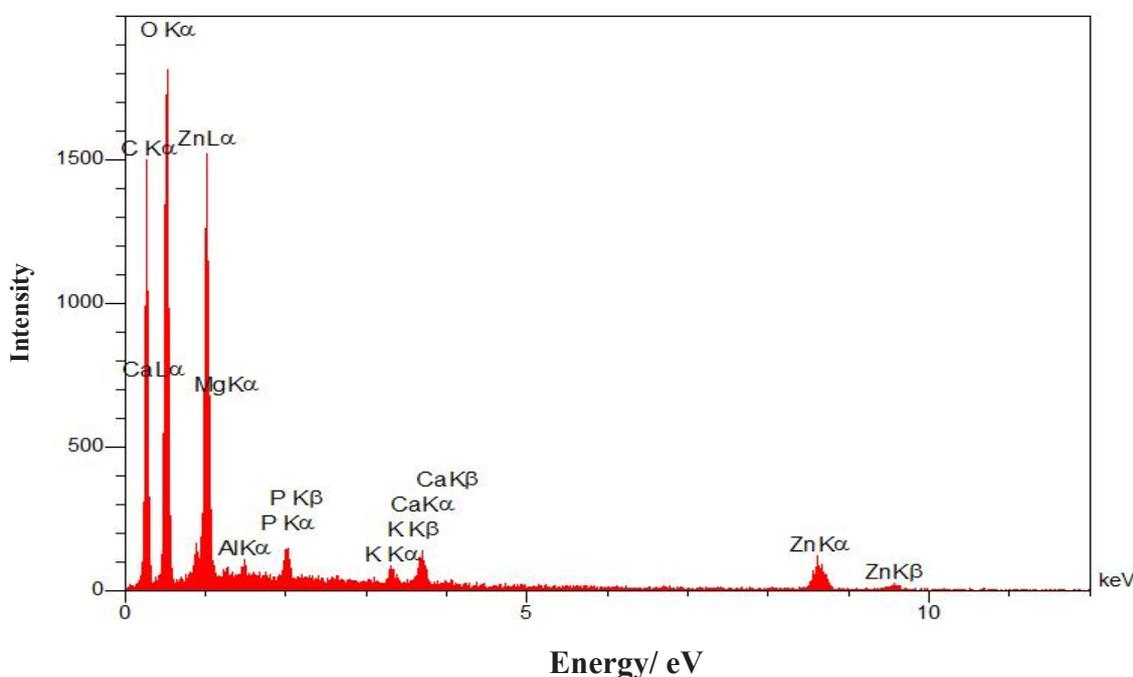


Fig. 4. EDS analysis of ZnO NPs synthesized by *Lavandula angustifolia* extract.

Table 1. Relative distribution of elements in zinc oxide nanoparticles synthesized from lavender extract

Element	(Wt %)	A%
C	30.27	46.15
O	37.28	42.67
Mg	0.98	0.74
Al	1.03	0.70
P	2.04	1.21
K	1.00	0.47
Ca	2.17	0.99
Zn	25.23	7.07
	100.00	100.00

Fig. 3. The microscopic images obtained using this technique showed that the particles were predominantly spherical in shape, with diameters ranging from 24 to 75 nm. Dense, multilayered structures were also observed within the sample, which may be attributed to the role of the active chemical compounds in the lavender extract, which are believed to act as reducing and stabilizing agents during the biosynthesis process. However, their ability to stabilize the crystalline structure of the particles appears to have been insufficient, resulting in some interparticle agglomeration. These results are consistent with previous studies, which documented that zinc oxide nanoparticles produced from plant extracts exhibit similar morphological characteristics [32]. study also supported these observations, showing that secondary plant compounds, particularly polyphenols and flavonoids, effectively contribute to the formation and stabilization of nanoparticles by interacting with their surface during formation [33].

Energy dispersive X-Ray spectroscopy (EDS) analysis

The composition of zinc oxide nanoparticles prepared using lavender extract was analyzed

using energy-dispersive spectroscopy (EDS) to monitor the optical absorption peaks and identify their constituent elements. The results showed that the weight percentages of the elements were: 37.28% oxygen, 25.23% zinc, 30.27% carbon, 2.04% phosphorus, 2.17% calcium, and 1.00% potassium, as shown in Fig. 4 and Table 1. The presence of carbon and trace elements such as phosphorus, calcium, and potassium is attributed to the residues of organic compounds and metal ions present in the lavender extract, indicating their role in the green synthesis process. These results are consistent with previous studies, where EDS spectra of ZnO nanoparticles prepared using plant extracts showed dominant peaks of zinc and oxygen, along with minor elements resulting from the components of the plant extract [34-38].

Antioxidant activity

The results of the DPPH assay to evaluate antioxidant activity showed that the percentage of free radical inhibition increased significantly with increasing sample concentration, reaching 40.868% at 0.12 mg/ml, 57.091% at 0.25 mg/ml, and 72.418% at 0.5 mg/ml, peaking at 83.068% at 1 mg/ml Fig. 5. This ascending pattern indicates that antioxidant activity is dose-dependent,

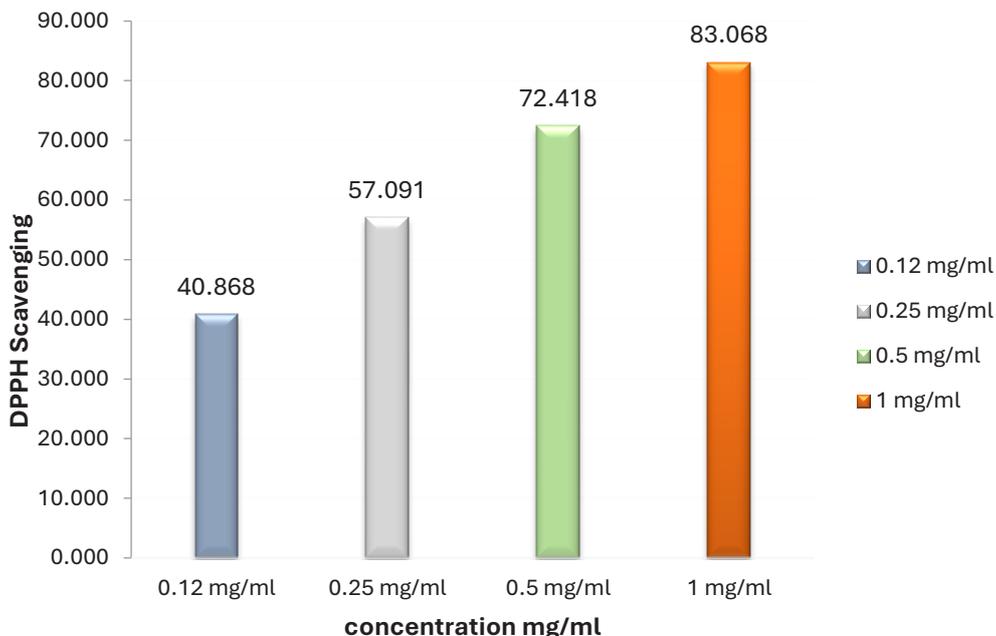


Fig. 5. Antioxidant activity of ZnO NPs, *Lavandula angustifolia* extract and Ascorbic acid.

indicating the presence of active compounds, such as phenols and flavonoids, capable of donating electrons or hydrogen atoms to inhibit DPPH free radicals. This inhibition is attributed to the ability of antioxidant compounds to convert the purple DPPH radical to its colorless or yellow reduced form, which is measured spectrophotometrically as an indicator of efficacy. These results are consistent with previous literature that confirmed

the effectiveness of plant extracts in DPPH tests due to their containing biologically active compounds [39,40].

Non-cumulative toxicity study of both thyme extracts and ZnO nanoparticles on immature larval stages of Culex pipiens mosquitoes

This study included an evaluation of the non-cumulative insecticidal effects of both aqueous

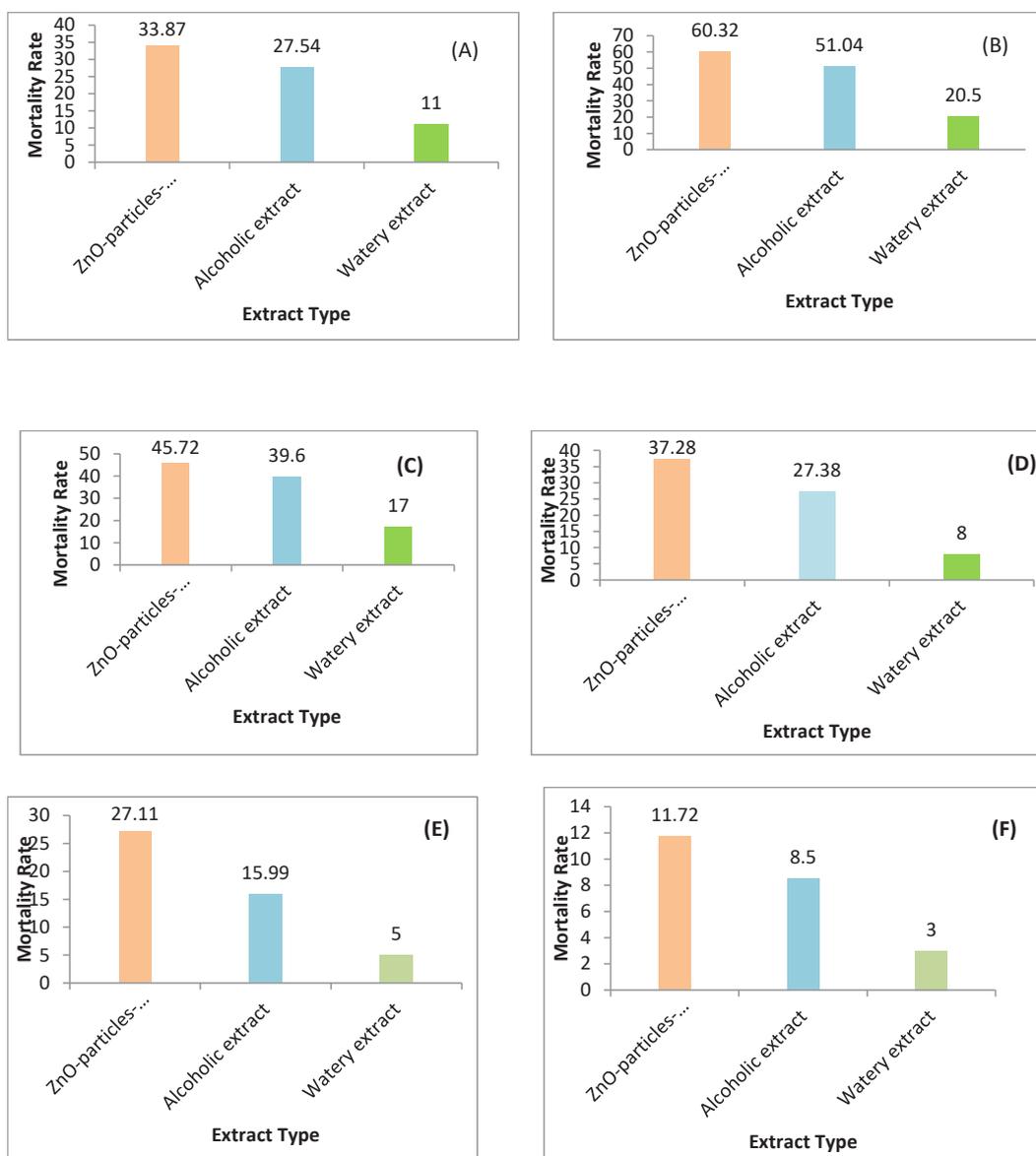


Fig. 6. Effect of the extract type of *Lavandula angustifolia* on the growth stages of *Culex pipiens* mosquitoes. a) Eggs (b) 1st larva stage (c) 2nd larva stage (d) 3rd larva stage (e) 4th larva stage (f) Pupa stage.

and alcoholic extracts of lavender, in addition to zinc oxide nanoparticles (ZnO-NPs) prepared using the aqueous extract, on immature stages of *Culex pipiens* mosquitoes (eggs, larvae, and pupae). The study aimed to evaluate the biological efficacy of these compounds as promising natural options within the framework of disease vector control strategies. The results, as shown in Fig. 6 and Table 2, showed differences in mortality rates between the different treatments, with ZnO nanoparticles significantly superior in terms of biological efficiency.

this study demonstrated a clear superiority of zinc oxide nanoparticles (ZnO-NPs) over alcoholic and aqueous extracts of *Lavandula angustifolia* in controlling the immature stages of

Culex pipiens mosquitoes. The egg mortality rate was approximately 33.87% with the ZnO-NPs, compared to 27.54% with the alcoholic extract and 11% with the aqueous extract. This is attributed to the ability of the nanoparticles to penetrate the egg envelope and induce oxidative stress that affects embryonic development [41]. In the first and second instar larvae, mortality rates increased to 60.32% and 45.72% with zinc oxide nanoparticles, compared to 51.04% and 39.6% with the alcoholic extract, and 20.5% and 17% with the aqueous extract. This is attributed to zinc accumulation in the mid-gut, elevated H₂O₂ concentrations, inhibition of antioxidant enzymes such as ALP and GPX, and decreased SOD activation. This leads to functional tissue damage and a growth reduction

Table 2. Effect the plant and extract type on mortality rate for all growth stages

plant	Extract	Mortality rate					
		Egg	The first larval stage	The second larval stage	The third larval stage	The forth larval stage	Pupa stage
Mean±S.D							
<i>Lavandula angustifolia</i>	Alcoholic	27.5±3.2 b	51.0±3.7 c	39.6±7.1 c	27.4±4.1 c	15.9±1.4 c	8.5±2.0 b
	Watery	11.0±2.2 a	20.5±1.6 a	17.0±2.1 a	8.0±1.9 a	5.0±0.8 a	3.0±0.1 a

Means within a column followed by different letters indicate statistically significant differences at P < 0.05.

Table 3. Effect the plant type and concentration of *Lavandula angustifolia* on mortality rate of *Culex pipiens* mosquitoes

Extract	Con.	Mortality rate					
		Egg stage	First larval stage	Second larval stage	Third larval stage	Forth larval stage	Pupa stage
Mean±S.D							
Alcoholic	0	5.0±1.0 b	2.5±0.1 a	2.5±0.1 a	2.5±0.1 a	2.5±0.1 a	5.0±1.0 b
	10	15.0±4.0 c	47.5±8.2 e	32.5±7.9 e	10.0±2.0 c	5.0±1.0 b	5.0±1.0 b
	15	25.0±2.1 e	47.5±8.2 e	35.0±4.7 e	22.5±5.4 e	15.0±4.0 c	5.6±1.1 b
	20	40.0±6.6 f	77.7±11.8 f	55.5±6.7 f	44.4±5.1 f	25.0±2.1 d	7.5±1.1 b
	25	52.7±10.3 g	80.0±10.4 f	72.5±8.4 g	57.5±12.4 g	32.5±7.9 f	19.4±2.3 c
Watery	0	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
	10	5.0±1.0 b	10.0±2.0 b	5.0±1.0 b	5.0±1.0 b	2.5±0.1 a	0.0 a
	15	12.5±3.0 c	15.0±4.0 c	17.5±2.4 c	5.0±1.0 b	5.0±1.0 b	2.5±0.1 a
	20	15.0±4.0 c	30.0±2.7 d	25.0±2.1 d	12.5±3.0 c	5.0±1.0 b	5.0±1.0 b
	25	22.5±5.4 d	47.5±8.2 e	37.5±4.4 e	17.5±2.4 de	12.5±3.0 c	7.5±1.1 b

Means within a column followed by different letters indicate statistically significant differences at P < 0.05.

of approximately 50% despite abstention from treatment during the recovery period [41]. Third and fourth instar larvae showed mortality rates of 37% and 15.99% with the nanoparticles, compared to 27.38% and 15.99% with the alcoholic extract, and 8% and 5% with the aqueous extract. This is explained by increased cuticle thickness and the development of self-defense mechanisms at this stage. However, zinc oxide nanoparticles (ZnO-NPs) remained the most effective due to their chronic accumulation in the mid-gut and continuous tissue oxidation [42]. At the pupal stage, mortality rates decreased to 11.72% for ZnO-NPs, 8.5% for the alcoholic extract, and 3% for the aqueous extract. This is due to physiological and morphological changes that confer relative resistance to the pupae, although the nanoparticles still exert a significant cumulative effect [41,43]. Recent findings also support the efficacy of phenolic and terpene-rich alcoholic extracts, such as linalool and linalyl acetate, which showed an alcohol concentration ranging from 36 to 140 ppm against *Culex pipiens* and *Aedes* spp. larvae (experimental sources 2022–2024). These differences explain the apparent superiority of nanoparticles due to their physicochemical properties, such as their nanoscale size, large surface area, and reactive activity, which enhances the production of reactive oxygen species (ROS), membrane penetration, and damage in the midgut via advanced oxidation mechanisms. These properties are based on studies of environmentally extracted zinc oxide nanoparticles (ZnO-NPs) during the period 2022–2023 [41]. Therefore, this study provides strong evidence that environmentally extracted ZnO-NPs offer potent, multi-stage toxicity against *Culex*

pipiens mosquitoes, while alcohol extracts offer a supportive biological option within practical integrated pest management strategies

Table 3 shows that the effectiveness of *Lavandula angustifolia* extracts against *Culex pipiens* mosquito stages directly depends on both the type of extract and the concentration used, as mortality rates increased significantly with increasing concentrations in all stages ($p < 0.05$), and the alcoholic extract recorded a stronger effect compared to the aqueous extract, as the highest values were reached at the concentration of 25 mg/mL, at $80.0 \pm 10.4\%$ in the first larva, followed by the second larva ($72.5 \pm 8.4\%$) and eggs ($52.7 \pm 10.3\%$), while it gradually decreased in the late stages until the pupa ($19.4 \pm 3.2\%$). This is attributed to the increased concentration of active compounds extracted with alcohol, such as linalool and linalyl acetate, which possess high toxic activity and cause physiological and cellular disturbances. Furthermore, early larval stages are more sensitive due to the thinner cuticles and weaker internal defenses compared to later stages, which explains the gradual decline in mortality rates. These results confirm that increasing concentrations enhances the biological effectiveness of extracts, especially alcoholic ones, which supports their use as a promising biological option within integrated mosquito management programs [44,45].

The results of Table 4 indicate that zinc oxide nanoparticles prepared using lavender extract showed a clear and non-cumulative insecticidal effect on immature stages of *Culex pipiens* mosquitoes (eggs, larvae, and pupae). A direct relationship was observed between the

Table 4. Effect the plant type and concentration of ZnO- nanoparticles on mortality rate of *Culex pipiens* mosquitoes.

Extract	Con.	Mortality rate					
		Egg stage	First larval stage	Second larval stage	Third larval stage	Fourth larval stage	Pupa stage
Mean±S.D							
Zn nanoparticles - <i>Lavandula angustifolia</i>	0	5.0±1.0 a	2.5±0.1 a	2.5±0.1 a	2.5±0.1 a	2.5±0.1 a	5.0±1.0 a
	4	12.5±3.0 b	66±9.1 c	36.1±2.4 b	13.9±1.4 b	5.6±1.1 b	5.0±1.0 a
	6	32.5±7.9 c	70±6.9 c	45.0±9.9 c	40.0±6.6 d	22.5±5.4 c	8.1±2.0 b
	8	52.7±10.3 e	80±10.4 d	62.5±6.2 d	57.5±12.4 e	47.5±8.2 e	15.6±4.2 c
	10	66.6±8.5 e	82.5±6.6 d	82.5±6.6 f	72.5±8.4 f	57.5±12.4 f	24.9±5.1 d

Means within a column followed by different letters indicate statistically significant differences at $P < 0.05$.

concentration of nanoparticles and mortality rates at all stages. The mortality rate in the egg stage reached 66.6%, in the first larvae 82.5%, in the fourth larva 57.5%, and in the pupae 24.9% at the highest concentration (10 mg/ml). This indicates that ZnO-NPs have an effective ability to penetrate biofilms and affect physiological processes such as the generation of reactive oxygen species (ROS) and disruption of cell functions. These results are consistent with the study reported by [46], which demonstrated the toxic effect of ZnO particles prepared from *Azadirachta indica* on *Aedes aegypti* larvae, as well as the study by [47] documented the efficacy of ZnO-NPs extracted from *Capparis spinosa* against *Culex pipiens*. While study showed [48] also that ZnO particles prepared from *Ocimum sanctum* caused high larval mortality due to ROS production. [49] confirmed the insecticidal properties of plant-derived ZnO particles prepared from *Nostoc* sp., finally study [50] supported their efficacy against *Culex quinquefasciatus*. Together, these results confirm that the green synthesis of ZnO particles using plant extracts is a promising and environmentally friendly option for disease vector control within integrated mosquito management programs.

CONCLUSION

We conclude from our study that zinc oxide nanoparticles prepared using synthetic nano zinc lavender extracts showed remarkable insecticidal activity against immature stages of *Culex pipiens* mosquitoes, with mortality rates clearly increasing with increasing particle concentration, reflecting a direct relationship between concentration and biological effect. These results demonstrate that these nanoparticles can be a promising natural alternative to traditional chemical pesticides in disease vector control programs, given their effectiveness, selectivity, and binding to plant materials. These results are consistent with the Sustainable Development Goals by contributing to enhancing public health, reducing reliance on harmful chemical pesticides, and protecting ecosystems from pollution, making them a sustainable option compatible with modern environmental trends in pest management and disease control.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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