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Research Article

Toxicity of polymeric nanocapsules prepared from *Moringa oleifera* leaf extract against the larvae of *Culex pipiens* (Diptera: Culicidae)

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ABSTRACT: The study aimed to prepare a nanocapsules formulation from the acetonic extract of *Moringa oleifera* leaves, using polymeric capsules, and test its toxicity against the third instar larvae of *Culex pipiens* mosquitoes. The leaf extract was prepared using acetone as a solvent, and the nano polymeric capsules were prepared using the synthetic polymer polyethylene glycol 4000. The results showed the successful preparation of nano polymeric capsules from the leaf extract, with an average particle size of 259.2 nm, and a nanocapsule diameter of 263.83 nm, as determined by DLS and SEM analysis, respectively. The toxicity results indicated that the nano polymeric capsules of the leaf extract exhibited higher mortality rates, reaching 97.6% at a concentration of 1333 ppm, with a median lethal concentration (LC_{50}) of 421.56 ppm. In comparison, the traditional leaf extract showed higher mortality rates of 100% at a concentration of 6125 ppm at 72 h of treatment, with a median lethal concentration (LC_{50}) of 1719.67 ppm. These results demonstrate that the nano polymeric capsules of the leaf extract are more efficient than the traditional extract, even at lower concentrations, and could serve as an environmentally-friendly and effective means of mosquito control.

KEYWORDS: *Culex pipiens,* nanocapsules, *Moringa oleifera*, plant extracts, toxicity

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INTRODUCTION

The mosquito *Culex pipiens* belongs to the family Culicidae of the order Diptera. It is considered one of the most important medically significant insects due to its ability to transmit many human and animal disease agents (Ferraguti *et al.*, 2021). This species is distributed in diverse geographical areas in many countries worldwide (Brugman *et al.*, 2018). Due to its association with the transmission of numerous pathogenic agents in humans and animals, various control methods have been used, the most important of which is synthetic chemical pesticides (Bellini *et al.*, 2014). Despite the significant benefits of using pesticides to control the spread of mosquito species, their negative effects, such as environmental pollution, the killing and destruction of natural enemies and non-target organisms, the emergence of insect resistance, and their negative impact on public health (Aktar, 2009), have led researchers to seek alternative methods to synthetic chemical pesticides, with a focus on using biological control, especially bacterial agents such as *Bacillus thuringiensis* and other microbial agents like fungi, including *Beauveria bassiana* (Hegazy *et al.*, 2022). Plant extracts and their secondary compounds have also been used in mosquito control, such as neem compounds from the *Azadirachta indica* (Kudom *et al.*, 2011). The moringa plant, *Moringa oleifera* has attracted attention recently due to its importance and multiple uses, as it contains many secondary compounds. Its products have been used in various health and nutritional aspects, in addition to being used in pest control (Elghandour *et al.*, 2023; Prabhu *et al.*, 2011; Jasem *et al.*, 2024; Jassim *et al.*, 2024). Despite the significant benefits and high features of using plant extracts and their products in pest control, they have some limitations that reduce their efficiency at the practical level, such as their rapid degradation, inability to withstand different environmental conditions, and thus the loss of many of their pesticidal properties (Souto *et al.*, 2021). The development of the usability of plant extracts and their secondary products has been the goal of many researchers in finding ways to reduce the rapid fading, degradation, and loss of plant secondary compounds for their properties. One of these methods is the use of nanotechnology to develop nano products that, in insect control, rely on the use of secondary compounds from plant extracts (Rai *et al.*, 2012; Nie *et al.*, 2023; de Andrade *et al.*, 2022) and oils extracted from seeds such as Castor Oil (Al Hilfy *et al.*, 2022ab), fungi such as

Metarhizium anisopliae (Hammed *et al.*, 2024; Hammed *et al.*, 2022), and bacteria such as *Pseudomonas fluorescens* and *Bacillus thuringiensis* (Ali *et al.*, 2024; Kamil *et al.*, 2023; Kamel *et al.*, 2022) which are characterized by their high efficiency and ability to preserve active substances and control their release, thus allowing for effective field-level use. From this perspective and the importance of finding alternative methods to pesticides in mosquito control, and developing the use of secondary compounds found in the *Moringa* plant, the study aimed to develop a nano product in the form of biodegradable nanocapsules and to study some of its properties, as well as to study its toxicity against mosquito larvae as one of the strategies based on environmentally friendly nanotechnology in pest control.

MATERIALS AND METHODS

Rearing of *C. pipiens* mosquito

The laboratory colony of mosquitoes was established by collecting larvae and pupae from various aquatic environments in Baghdad. They were then reared in plastic containers (30 x 20 x 10 cm^3) containing chlorine-free water. The containers were placed in glass-covered basins, and the growth and development of the larvae were monitored while providing them with food periodically in the form of ground bread until they transformed into pupae and emerged as adults. After emergence, the adults were transferred to a glass organic box containing a live pigeon for mating purposes, and the females were fed to lay eggs. Glass beakers measuring 250 ml containing chlorine-free water were used to collect the eggs laid by the females. The egg rafts were then gently transferred to plastic containers containing chlorine-free water, as mentioned above and monitored until hatching and the appearance of larvae, at which point they were provided with food. Samples of the mosquitoes were sent to the Natural History Museum for diagnosis, and it was determined that they belonged to *C. pipiens*

Preparation of acetonic extract of *M. oleifera* leaves

Moringa leaves were collected from trees in the botanical garden of the College of Science at the University of Baghdad. The leaves were then dried in the shade under laboratory conditions for one week and ground using an electric grinder. One hundred grams of Moringa leaf powder was transferred to a Soxhlet apparatus for extraction, using 500 ml of acetone as the solvent at a temperature of 45°C for approximately 10 cycles. The extract was then transferred to a rotary evaporator for concentration and removal of the solvent at a temperature of 45°C. A thick liquid was obtained, which was left in the laboratory to remove any remaining solvent residues from the extract. Finally, the crude extract was obtained and placed in a plastic bag for use at a temperature of 25°C.

Preparation of polymeric nanocapsules of *M. oleifera* leaf extract

To prepare polymeric nanocapsules of *Moringa* extract, the melting dispersion method was used with some modifications (Peng *et al.*, 2008). Polyethylene glycol with a molecular weight of 4000 was used as the synthetic polymer, which is a white crystalline solid. 48 g of polyethylene glycol crystals were placed in a 100 ml glass beaker and heated with stirring using a magnetic stirrer at a temperature of 65°C for one hour to melt the crystals and obtain a thick liquid. Two grams of the crude extract of Moringa leaves were then added to the thick liquid of the polymer with continuous stirring for two hours to ensure homogeneity of the crude extract with the polyethylene glycol polymer and to ensure the encapsulation of the crude extract with the mentioned polymer. The resulting thick liquid containing the *Moringa* extract was left to dry and solidify, and the solid mass containing the polymer and *Moringa* extract was ground to obtain a very fine powder representing the polymeric capsules of *Moringa* extract coated with the biodegradable polyethylene glycol polymer.

Identification of some properties and characteristics of prepared polymeric nanocapsules

Some properties and characteristics of nanopolymeric capsules, including size, shape, and distribution of the polymeric capsules, were tested using a scanning electron microscope (SEM) and a Dynamic Light Scattering (DLS) device. Additionally, the functional groups of both the acetonic extract of *M. oleifera* leaf and the synthetic polymer polyethylene glycol with a molecular weight of 4000 were determined, along with the nanopolymeric capsules of the *Moringa* leaf extract using Fourier-Transform Infrared (FTIR) spectroscope.

Toxicity of polymeric nanocapsules of *M. oleifera* extract and non-nanocapsule extract on *C. pipiens* mosquito larvae

This study aimed to evaluate the toxicity of nano polymeric capsules containing moringa extract compared to traditional non-nano polymeric extract on *C. pipiens* mosquito larvae. The larvae were selected at the third stage for testing purposes. Twenty larvae were transferred to small plastic cups containing chlorine-free water, with a total of 3 replicates. Four concentrations of nanopolymeric capsules were used: 107, 267, 667, 1067and 1333 ppm, while the following concentrations were used for non-nanocapsule *moringa* extract: 306, 765, 1530, 3060, and 6125 ppm. The control group was treated with water only. The mortality rates were calculated daily for 72 h and corrected using the Abbott formula (Abbott, 1925). The lethal concentration LC_{50} was determined using Probit analysis (Finney, 1952) software.

Statistical analysis

A factorial experiment was carried out according to a Completely Randomized Design (CRD), and the differences between the means of the treatments were determined according to the value of the Least Significant Difference (LSD) at the probability level of 0.05. The Data were analyzed according to the Genstat.12. Statistical software

RESULTS

Determining the shape and diameter of polymeric nanocapsules of *M. oleifera* leaf extract

The shape and diameter of the polymeric nano-capsules for the *M. oleifera* plant extract were determined using scanning electron microscopy. The SEM image Figure 1 shows the irregular spherical shape of the polymeric capsules with a diameter ranging from 72.64 to 634.9 nm, with an average of 263.83 nm. It is evident from the results that the diameter of the polymeric nano-capsules falls within the defined nanoscale range for nanomaterials in pesticides, which is specified to be between 1 and less than 1000 nm. The size of nano-particles prepared using periodic table elements usually varies between 1-100 nm, different from the size range of other nanomaterials such as polymeric nano-capsules.

Determining the size and distribution of polymeric nanocapsules for the extract of *M. oleifera* using Dynamic Light Scattering (DLS)

Determining the size and distribution of polymeric nanocapsules for the extract of *M. oleifera* using Dynamic Light Scattering (DLS) reveals the following results in Figure 2. The size of the nanocapsules ranges from 49.2 to 519.9 nm, with an average of 259.2 ± 173.2 nm. The results obtained from scanning electron microscopy are consistent with the findings from DLS, confirming the nanoscale nature of the nanocapsules in the *M. oleifera* leaf extract. In a similar study, Jasim (2023) reported an average size of 331 nm for nanocapsules derived from the Pân plant extract. The variation in size may be attributed to aggregation processes, which tend to increase the overall size. Generally, smaller-sized capsules or particles exhibit higher efficiency and stability.

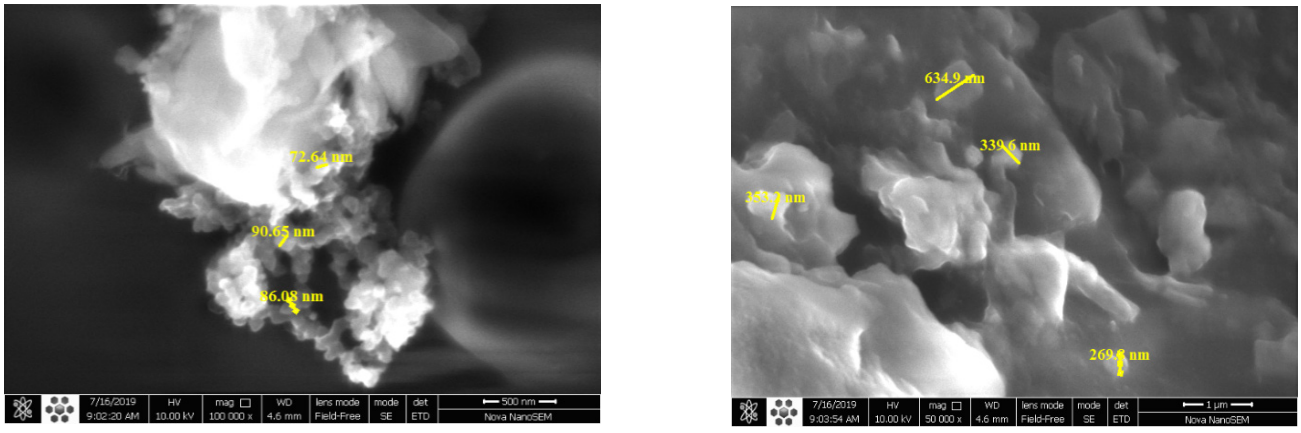


Figure 1. Shape and diameter of polymeric nanocapsules using a scanning electron microscope (SEM) of *M. oleifera* leaves extract.

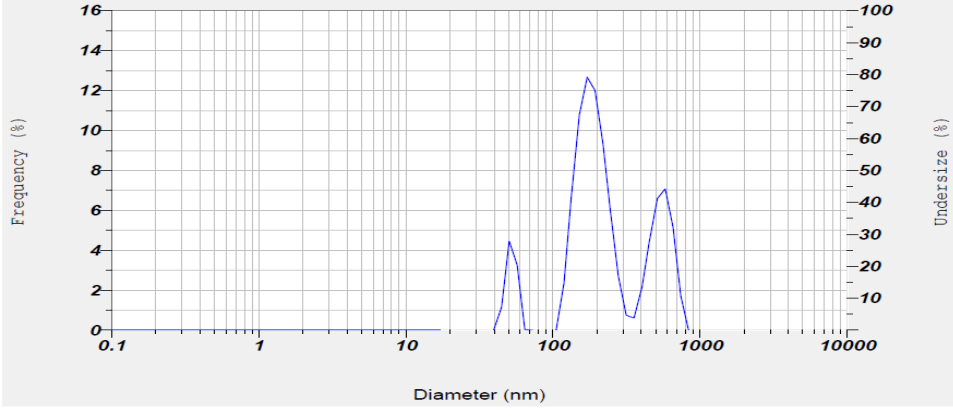


Figure 2. Size and distribution of polymeric nanocapsules by dynamic light scattering (DLS).

Determination of functional groups in polymeric nanocapsules and *M. oleifera* leaf extract by FTIR

Figure 3 shows the functional groups in the acetonic extract of *M. oleifera* leaves analyzed by Fourier transform infrared spectroscopy using Fourier transform, noting the presence of a weak peak at 600 cm-1 representing a C-Cl alkyl halide functional group, and the presence of a medium peak in the region 825 cm-1 representing a C=C alkene functional group. A strong sharp peak is observed at 900 cm-1 representing a 1,3-disubstituted (C-H) group. Medium peaks were also observed at 1125 and 1200 and 1275 and 1350 and 1500 cm^{-1} representing C-O alcohol, C-N amine, aromatic amines C-N and alcohols and phenols O-H and alkanes C-H, respectively. Weak peaks were also observed at 1950 and 2100 and 2175 and 2250 and 2700 and 2775 and 3450 cm^{-1} representing aromatic hydrocarbons C-H, alkynes C≡C, ketone C=C=O, nitrile C≡N, alcohol O-H and amine N-H functional groups, respectively. A strong peak was also observed at 2850 cm^{-1} representing carboxylic acids O-H or amine N-H groups. Figure 4. shows the functional groups of polyethylene glycol 4000 polymers, noting a medium peak at 1050 cm^{-1} representing a primary alcohol C-O group, in addition to weak peaks at 1200 and 1275 and 1350 and 1500 cm^{-1} representing C-O alcohol, aromatic esters C-O, alcohol O-H and alkane C-H groups, respectively. A medium peak was also observed at 1725 cm^{-1} representing an aromatic C-H group. Weak peaks were also observed at 2925 and 3000 cm^{-1} representing alcohol O-H groups. The medium peak at 3375 cm^{-1} indicates an alcohol O-H group. Figure 5 shows the functional groups of the nanoscale polymeric capsules of *M. oleifera* leaf extract, noting medium peaks at 825 and 900 and 975 and 1125 and 2850 cm^{-1} representing C=C alkenes, 1,3-disubstituted (C-H), alkynes and amine C-N and alkane C-H groups. Weak peaks were observed at 1200 and 1275 and 1350 and 1500 and 1950 and 2700 cm^{-1} representing C-O alcohol, primary amines C-N, phenols O-H, alkanes C-H, aromatic compounds C-H and alcohol O-H functional groups, respectively.

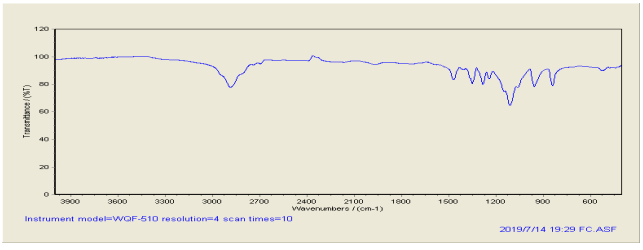


Figure 3. Functional groups in the acetone extract of *M. oleifera* leaves analyzed by FTIR.

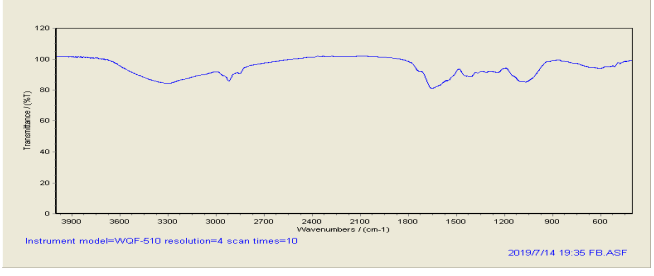


Figure 4. Functional groups of the polymer polyethylene glycol 4000 upon Analysis by FTIR.

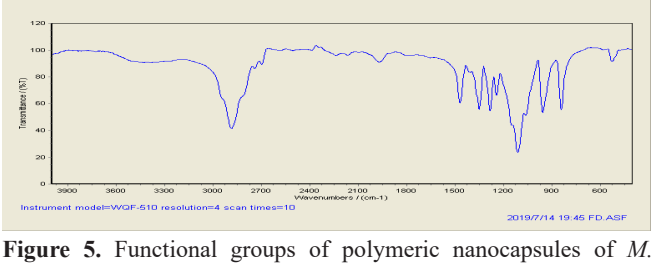


Figure 5. Functional groups of polymeric nanocapsules of *M. oleifera* leaf extract analyzed by FTIR.

Effect of different concentrations of polymeric nanocapsules of *M. oleifera* leaf extract on mortality of third instar larvae of *C. pipiens* mosquito

The results recorded in Table 1 indicate that the nanoscale polymeric capsules of *M. oleifera* leaf extract cause toxic effects on third instar larvae of *Culex* mosquito proportional to the increase in concentration and exposure time, where the highest mortality rate was 80.1% and the lowest mortality rate was 0.4% at concentrations of 1333 and 107 ppm after 24 h of treatment, while the mortality rate increased to 97.6% at a concentration of 1333 ppm after 72 h of treatment, while the mortality rate did not increase at the lowest concentration of 107 ppm after 72 h where it recorded 2.3%. The results of statistical analysis showed significant differences in mortality rates at different concentrations with superiority of the third, fourth and fifth concentrations, although the superiority of the last concentration was not significant. The statistical analysis results also showed that the mortality rates increase with increasing exposure time with a significant difference, and the interaction between the studied factors significantly affects the mortality rates. The results recorded in Table 2 show that the acetonic extract of *M. oleifera* leaves causes toxic effects on mosquito larvae proportional to the increase in concentration and exposure time, noting that the highest mortality rate was recorded at a concentration of 6125 ppm where it reached 100%, while the lowest mortality rates were recorded at concentrations of 306 and 765 ppm where they were 2.5% and 2% after 72 h, respectively. The results of statistical analysis indicated significant differences.

Toxicity of polymeric nanocapsules of *M. oleifera* extract and non-nanocapsule extract against *C. pipiens* mosquito larvae

The results in Table 3 indicate that the median lethal concentration (LC_{50}) for polymeric nanocapsules was 421.56 ppm, while the sub-lethal concentration (LC_{30}) was 286.11

ppm, and the concentration killing 90% (LC_{90}) was 1086.96 ppm, the results also showed that the LC_{50}, LC_{30} and LC_{90} for the non-Nano capsule extract were 1719.67, 1071.15 and 5468.65 ppm respectively. It is also noted from the results that there are no significant differences between toxicity points and toxicity lines as they passed through most toxicity points, indicating the quality of fitting when drawing the toxicity line in Figures 6 and 7. In summary of the results, polymeric nano capsules of *Moringa* extract outperformed the conventional extract in their effect on mosquito larvae, which is evident from the lower LC_{50} value and other toxicity values by about five times compared to toxicity values when treated with the conventional extract. This is an indicator of obtaining a nanoscale, environmentally friendly product from natural sources that is biodegradable without leaving any toxic effects in the environment with high efficiency in mosquito control.

Table 1. Mortality rate (in percentage) of third instar larvae of *C. pipiens* when treated with different concentrations of polymeric nanocapsules of *M. oliefera* leaf extract

Concentration (ppm)	% Mortality/h			Mean
	24 h	48 h	72 h	
107	0.4	2.2	2.3	1.6 c
267	21.5	34.6	40.1	32.1 b
667	84.2	83.9	90.6	86.2 a
1067	80.3	81.3	92.9	84.8 a
1333	80.1	93.2	97.6	90.3 a
Mean	53.7b	59.0 b	64.3a	
LSD 0.05	**Conc.**	**time**	**Interaction**	
	9.92	**7.68**	**17.18**	

Table 2. Mortality rate (in percentage) of third-instar larvae of *C. pipiens* when treated with different concentrations of acetonic extract of *M. oleifera* leaves

Concentration (ppm)	% Mortality/h			Mean
	24 h	48 h	72 h	
306	2.5	2.5	2.5	2.5c
765	2	2	2	2c
1530	63.5	65.1	67.5	65.4 b
3060	60.2	77.0	78.6	71.9 b
6125	98.3	100	100	99.4 a
Mean	45.3 a	49.3 a	50.1a	
LSD 0.05	**Conc.**	**time**	**Interaction**	
	14.05	**10.88**	**24.33**	

Table 3. Toxicity of polymeric nanocapsules of *M. oleifera* extract and non-nanocapsules extract to *C. pipiens* mosquito larvae

Treatment	Lethal concentration		95 fiducial CI %		Chi-test (χ2) Sig.	Fitting
	LC	ppm	Lower	Upper		
M. oleifera nanocapsules	LC_{30}	286.11	206.013	397.34		
	LC_{50}	421.56	303.54	585.45	0.217	GOOD FIT
	LC_{90}	1086.96	782.69	1509.55		
M. oleifera plant ext.	LC_{30}	1071.15	734.36	1562.41		
	LC_{50}	1719.67	1178.97	2508.35	0.058	GOOD FIT
	LC_{90}	5468.65	3749.18	7976.70		

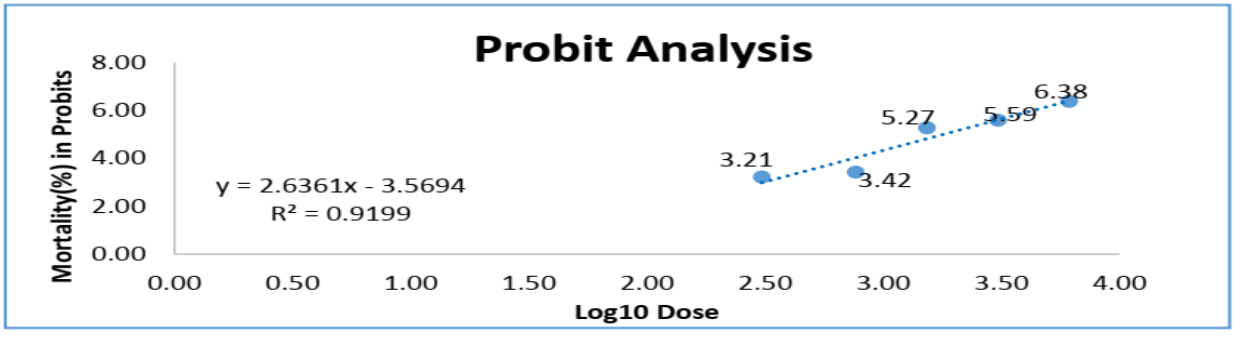


Figure 6. The relationship between the concentration log and the probits mortality values for *C. pipiens* mosquito larvae at Treatment with different concentrations of *M. oleifera* leaf extract.

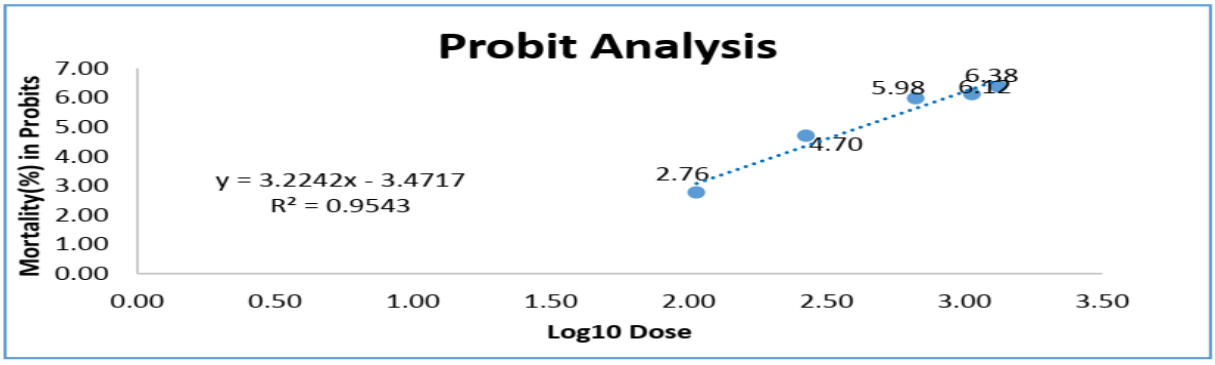


Figure 7. The relationship between the concentration log and the probits mortality values for *C. pipiens* mosquito larvae at Treatment with different concentrations of polymeric nanocapsule of *M. oleifera* leaf extract.

DISCUSSION

The shape and diameter of the polymeric nano-capsules for the *M. oleifera* plant extract were determined using scanning electron microscopy showing the irregular spherical shape of the polymeric capsules with an average of 263.83 nm. The nature of the polymer and its concentration used in the preparation of nano-capsules affects their size, as well as the composition and concentration of active substances and other additives (Zielińska *et al.*, 2020; Granata *et al.*, 2022). In a similar study, Jasim (2023) found that the average diameter of nano-capsules for the alcoholic extract of *M. oleifera* plant extract was 179.9 nm. The results obtained from scanning electron microscopy are consistent with the findings from DLS, confirming the nanoscale nature of the nanocapsules in the *M. oleifera* leaf extract. In a similar study, Jasim (2023) reported an average size of 331 nm for nanocapsules derived from the *M. oleifera* plant extract plant extract. The variation in size may be attributed to aggregation processes, which tend to increase the overall size. Generally, smaller-sized capsules or particles exhibit higher efficiency and stability. The type of functional groups present in the plant extract *M. oleifera* and polymer (PEG 4000) greatly affect the properties of the polymeric nanocapsules of the compound. For example, the interaction of hydroxyl and amine groups with functional groups in the plant extract affects the stability of the nanocapsules and the efficiency of compound release from them, using biodegradable natural polymers also enhances biocompatibility between them, in addition to controlling the release of active substances, The more stable and robust the polymeric capsule of the compound, the more compounds are preserved inside the capsule and protected from degradation and unsuitable conditions. (Sharma *et al.*, 2021; da S Pereira *et al.*, 2021). The overall results indicated that higher mortality rates in mosquito larvae require lower concentrations when using nanoscale polymeric capsules of Moringa plant extract compared to using the conventional extract. The reason may be the nanoscale size of the polymeric capsules and their rapid ability to penetrate and reach the sensitive site in the insect body compared to the conventional extract, which may degrade and decompose more quickly, affecting its toxicity level (Del Prado-Audelo *et al.*, 2022; Perlatti *et al.*, 2013). In summary of the results, polymeric nano capsules of *Moringa* extract outperformed the conventional extract in their effect on mosquito larvae, which is evident from the lower LC_{50} value and other toxicity values by about five times compared to toxicity values when treated with the conventional extract. This is an indicator of obtaining a nanoscale, environmentally friendly product from natural sources that is biodegradable without leaving any toxic effects in the environment with high efficiency in mosquito control.

CONCLUSION

The results of the current study clearly showed the possibility of preparing biodegradable polymeric nanocapsules from the industrial polymer polyethylene glycol that encapsulates the acetonic extract of plant leaves. The nanocapsules were characterized by sizes and shapes within the nanoscale specifications. The nanocapsules of the leaf extract also showed high toxicity against mosquito larvae at lower concentrations compared to non-nanocapsule extract. Therefore, they can be used as one of the alternative means to chemical pesticides as they are environmentally friendly and biodegradable, in addition to their high efficiency in causing mortality of mosquito larvae.

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