Larvicidal Activity of Nano- Encapsulated Lambda – Cyhalothrin Against Susceptible Mosquito Larvae (*Culex Pipiens*) in Comparison with Conventional form

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ABSTRACT

Encapsulated lambda – cyhalothrin nanoparticles loaded in polyethylene glycol were prepared by melt dispersion method in concentration 4% and 8% of their tested conventional lambda. Larvicidal activity of encapsulated lambda nanoparticles was investigated in comparison with its conventional form against susceptible larvae of *Culex pipeins* after 24, 48 and 72 hrs. exposure periods. The conventional lambda showed LC_{50} values equal 7×10^{-8}, $1.8 \times 10^{-8}$and 1×10^{-9}M, during the exposure time, respectively. The reduced tested concentrations of nanoparticles (4% and 8%) were highly toxic against mosquito larvae with LC_{50} values equal 4.6×10^{-8}, $2.9 \times10^{-9}$and 7.8×10^{-10}M (for 4%) and 1.8×10^{-8},5.3×10^{-9} and 1.2×10^{-9}M (for 8%) after 24, 48 and 72 hrs. of exposure periods, respectively. The toxic effects of the low concentrations from lambda nanoparticles against *Culex pipens* may be due to the diffusion released of cyhalothrin with amounts equal 1.84×10^{-9}, 1.16×10^{-10} and 3.12×10^{-11} (in case of 4%) and 1.44×10^{-9}, 4.24×10^{-10} and 9.6×10^{-11} M (for 8%) after 24,48 and 72 hrs., respectively. These results proposed that the encapsulated lambda-cyhalothrin nanoparticles can serve as an effective larvicide against *Culex pipeins* larvae at lawer concentrations avoiding the different problems of conventional form.

Keyword: lambda-cyhalothrin, poly ethylene glycol, susceptible *Culex pipiens* larvae.

INTRODUCTION

The frequent use of pesticides in conventional form has been causing the contamination of the environment and damaging many non-target organisms. The conventional form of pesticides lack of specificity which can harm the beneficial organisms to the environment. On the other hand, many pesticides are least soluble in water which required large amounts of organic solvents for solubility. Lambda – cyhalothrin is a synthetic pyrethroid insecticide, which used worldwide in agriculture, home pest control and disease vector control. The overuse of lambda in conventional form may produces different problems in the environment. Pesticides in nanoparticles form give an attractive solving for this problem (Liu *et al*., 2008 andAbouelkassem *et al*.,2016). The effective concentration of nanoparticles is expected to be much lower than of conventional materials and can be more soluble in water without the use of organic solvents. Most methods reported on preparing pesticides nanoparticles involve their encapsulation within polymers (Liu *et al*.,2008; Boehn*et al*.,2003; Perez-de-Luque and Rubials,2009). However, polymeric nanoparticles of poorly soluble pyrethroid, bifenthrin were prepared (Liu *et al*., 2008) by flash nanoprecipitation achieving particle size in the range 60 – 200 nm. (Anjali *et al*., 2010) prepared nano permethrin with particle size of nano-dispersion in water equal 151±27nm.for larvicidal studies against *Culex quinquefasciatus*.(Bhan *et al*., 2014) evaluated the larvicidal activity of nano- encapsulated temephos and imidacloprid with average size equal 129.5nm. against *Culex quinquefasciatus*. However, nanotechnology deals with structures sized of 1 to 100 nm., but in the presence of other phenomena such as transparency or turbidity, ultra-filtration, stable dispersion, molecular structure, etc.; that extend the upper limit are considered and the use of the prefix nano is accepted for dimensions smaller than 500 nm. (Aleman*et al*.,2007; Michel, *et al*.,2012). Silver nanoparticles loaded cyhalothrin (Ag Nps @ CYN) was very effective as larvicide against the fourth instar larvae of (*Culex pipiens*) with 100 % activity at 1 mg/ L as a much lower concentration than that required for the free cyhaothrin without using any additives such as organic solvent (Abouelkassem *et al*.,2016).

In this study encapsulated nanoparticles of lambda – cyhalothrin (insecticide) loaded with poly (ethylene glycol) were prepared by melt dispersion method. Structural morphology and size were analyzed with transmission electron microscopy (TEM – 1400 plus). Larvicidal effects against susceptible mosquito larvae (*Culex pipiens*)of lambda in conventional form compared to the encapsulated nano-particles at 4 % and 8% of the tested conventional concentrations, were investigated.

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MATERIALS AND METHODS

I- Preparation of Nanoparticles:

a-Materials:
-Lambda cyhalothrin 95%
-Poly (ethylene glycol) PEG 6000
b-Equipments:
-200 mesh sieves
-Transmission Electron Microscopy (TEM-1400 plus)
c-Procedure:

The preparation of encapsulated nanoparticles (melt dispersion method) in which poly (ethylene glycol) (PEG) loaded with lambda was carried out according to Bhan *et al*. (2014) and Peng *et al*. (2008) with some modifications. PEG 6000 was heated in two different parts 46.5 and 42.0gm separately at 65ºC. After melting 4% and 8% (w/v) of lambda were mixed separately with the melted PEG, respectively, and stirred gently to ensure even distribution of the mixture. The mixture was cooled at room temperature, then grounded or blended completely in a mortaror blender and sieved using a 200 -mesh sieve. The powders were placed in airtight glass container and stored at room temperature in desiccator containing calcium chloride to prevent moisture absorption. The nanoparticles size, distribution and morphology were examined on Transmission Electron Microscope (TEM-1400) at Faculty of science. Alexandria University.

II. Larvicidal activity measurements: Tested Insects:

Susceptible mosquito larvae of *Culex pipiens* strain was obtained from Research Institute of Medical Entomology (RIME), Dokki, Giza and had been maintained in laboratory under suitable conditions. Then, 3rd instar larvae were taken for the experiments.

1-Tested compounds:

Conventional lambda – cyhalothrin and their encapsulated nanoparticles at 4% and 8% of the tested conventional concentrations.

2-Methods of experiments:

Larvicidal activity of lambda (cyholothrin) against susceptible strain of mosquito larvae (*Culex pipiens*)wasconducted according the standard WHO procedure (WHO,2005) with few modifications as follows:

Twenty 3rd instar larvae were separately transferred gently to different concentrations of the conventional lambda with a control parallel- four replicates were used to ambient temperature (27 – 28˚C). A small aliquot of yeast powder was supplied for nutrition.

The encapsulated nanoparticles coated with PEG loaded with 4% and 8% ratios of the tested lambda were also evaluated for their larval toxicity against *C.pipens*,with the same procedure with the conventional lambda. The tested concentrations were prepared by dissolving the nanoparticles in distilled water at ratios (4% and 8%) of the tested conventional concentrations. Control was concurrently conducted larving the same nanoparticle composition (PEG) and larvae number without the tested lambda.

The mortality data in both treatments and control were recorded after 24,48 and 72hr. of exposure periods. The larvae were considered dead if they were immobile and unable to reach the water surface (Macedo *et al*. 1997).

The experiments with more than 20% mortality in control were discarded. With mortality range from 5% to 20% in control, the mortality data were corrected by applying the following Abbot' sformula (Abbot' s 1925), so as to remove the factors other than the tested compound:

Abbot' formula = T – C/100 – C × 100
Where, T= The percent mortality in test concentration
C= The percent mortality in control

The lethal concentrations caused 50% mortality (LC50) were estimated by probit analysis (Finney, 1971).

The amount of the compound released has been calculated by the following formula (Bhan *et al*. 2014).

The amount released= LC50 × the % of compound used in encapsulated formulation / 100

Statistical analysis:

The mortality data statistically analyzed with F. test and analysis of variance of treatments differences according to Steel and Torrie (1980) and SAS software package (version 9.13, 2007).

RERSULTS AND DISCUSSION

I.Physical properties of nanoparticles measurements:

Transmission Electron Microscopy (TEM) studies revealed that sizes of encapsulated nanoparticles of lambda, cyhalothrin (8%) were 100.01, 111.15, 117.87 and 157.05nm with average size equal 121.52. The structural morphology of the encapsulated nanoparticles predominate in spherical shapes with smooth edges (Figure 1)

I.Larvicidal activity against susceptible mosquito larvae (*C. pipeins*):

The larvicidal activity of conventional lambda and its encapsulated nanoparticles (4% and 8%) were investigated against the susceptible mosquito larvae (*Culex pipeins*) during 24, 48 and 72 hrs. of exposure. (Table 1,2 ,3and 4).

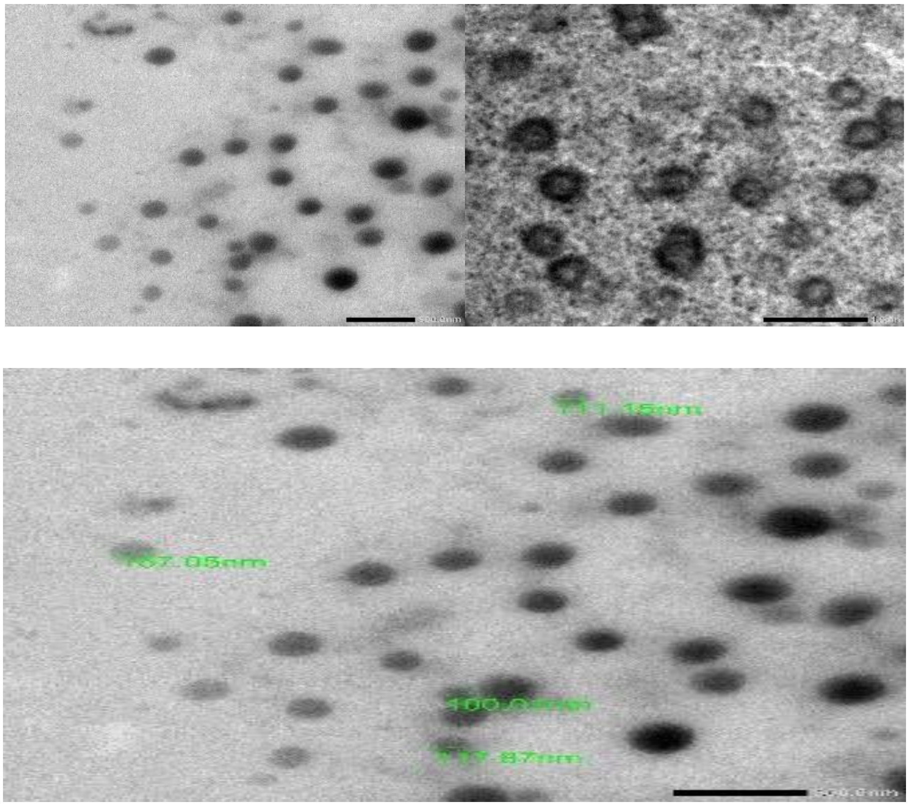

Figure 1. Sizes of encapsulated nanoparticles of lambada, cyhalothrin (8%)

Table (1) showed the statistical data of lambda – cyhalothrin which indicated that high significant differences between the mortality mean through the different concentrations. There were also high significant differences between the mean of mortality caused by conventional lambda and lambda nanoparticles in both ratio 4% and 8%. The exposure periods exhibited high significant differences between 24,48 and 72 hrs.

Larvicidal activity of conventional lambda (cyhalothrin) with different molar concentrations exhibited that the mortality percent gradually increased with increasing the tested concentrations with significant differences during all the periods of exposures, which caused LC_{50}values equal 7×10^{-8}, $1.8 \times 10^{-8}$and 1×10^{-9}M (7×10^{-5}, 1.8×10^{-5} and 1×10^{-6} ppm) , during 24 , 48 and 72 hrs. exposure, respectively. These results indicated that lambda (cyhalothrin) was more effective against the larvae after 72,48 and 24 hr. in descending order, which may be due to constantly active release of cyhalothrin with increasing the time of exposure (Table 2).

Larvicidal activity of encapsulated nanoparticles lambda cyhalothrin at 4% and 8% of the tested conventional concentrations were significantly effective against the susceptible mosquito larvae (*Culex pipiens*).They showed LC_{50} values equal 4.6×10^{-8}, $2.9 \times 10^{-9}$and 7.8×10^{-10}M (4.6×10^{-5}, 2.9×10^{-6} and 7.8×10^{-7}ppm) by 4% ratio and 1.8×10^{-8},5.3×10^{-9} and 1.2×10^{-9}M (1.8×10^{-5},5.3×10^{-6} and 1.2×10^{-6}ppm)by 8% ratio during 24,48 and 72 hr. of exposure periods,respectively. (Tables 3 and 4).

It was noted that, although the tested concentrations of encapsulated nanoparticles (4% and 8%) of the conventional concentrations) were very low, they were highly toxic on the tested mosquito larvae (*C.pipiens*) in comparison with the conventional lambda. The toxicity of both encapsulated nanoparticles lambda and its conventional form ascendingly increased with increasing the exposure periods. Lambda – cyhalothrin encapsulated nanoparticles loaded with poly (ethylene glycol)are releasing slowly and persistent very active against mosquito larvae up to 72 hr. These results are in agreement with the view of Patil *et al*. (2011); Bhan *et al*. (2014) and Abouelkassem*et al*.; 2016).

However, the encapsulated lambda nanoparticles at the reduced tested concentrations (4% and 8% of the conventional concentrations) showed an increase in their toxic effects against *C. pipiens* larvae during the exposure periods by their diffusion release from PEG matrix with amounts equal $1.84 \times 10^{-9,}$ 1.16×10^{-10} and 3.12×10^{-11} M (1.84×10^{-6} 1.16×10^{-7} and 3.12×10^{-8} ppm)by 4% and 1.44×10^{-9}, 4.24×10^{-10} and

Table 1. Statistical analysis of lambda (cyhalothrin) between concentrations, exposure periods and different formulations, for mortality means

Means between periods *	Means between compounds **	Means of concentrations, periods and compounds of main lambda, nano 4% and nano 8%					
		Conc. 1	**Conc. 2**	**Conc. 3**	**Conc. 4**	**Conc. 5**	**Conc. 6**
After 24 hr. 12.78 B	Conventional lambda 12.61 B	5.67	8.67	10.67	14.33	17.67	19.67
After 48 hr. 12.11 C	Lambda nano 4% 12.22 C	8.33	12.33	15.00	17.00	20.00	00.00
After 72 hr. 13.38 A	Lambda nano 8% 13.44 A	10.33	15.00	16.67	18.33	20.00	00.00
Means of concentrations		8.11 E	12.00 D	14.11 C	16.56 B	19.22 A	6.56 F

LSD (Least significant difference) of periods = 0.351
LSD (Least significant difference) of compounds = 0.2937
LSD (Least significant difference) of concentrations = 0.4233
*at the fixed compound and concentration
**at the fixed concentration

Table 2. Larvicidal activity of conventional lambda (cyhalothrin) against the 3rd instar of susceptible mosquito larvae (*Culex pipiens*) during different exposure periods shown as mortality percentages, LC_{50} values.

Exposure periods	Concentration in molar (M)						LC_{50}	
	2×10^{-9}	2×10^{-8}	2×10^{-7}	2×10^{-6}	2×10^{-5}	3.3×10^{-5}	M	ppm
24 hrs.	25	45	55	65	80	99.75	7×10^{-8}	7×10^{-5}
48 hrs.	30	55	75	80	99.75	-	1.8×10^{-8}	1.8×10^{-5}
72hrs.	50	85	95	95	99.75	-	1×10^{-9}	1×10^{-6}

Table 3. Larvicidal activity of encapsulated lambda nanoparticles (4%) against the 3rd instar of susceptible mosquito larvae (*Culex pipiens*) during different exposure periods shown as mortality percentages, LC_{50} values and the amount of cyhalothrin released

Exposure periods	Concentration in molar (M)						LC_{50}		Amount of compound released	
	8×10^{-11}	8×10^{-10}	8×10^{-9}	8×10^{-8}	8×10^{-7}	4×10^{-6}	M	ppm	M	ppm
24 hrs.	25	35	45	70	90	95	4.6×10^{-8}	4.6×10^{-5}	1.84×10^{-9}	1.84×10^{-6}
48 hrs.	45	55	70	85	99.75	-	2.9×10^{-9}	2.9×10^{-6}	1.16×10^{-10}	1.16×10^{-7}
72hrs.	55	65	75	90	99.75	-	7.8×10^{-10}	7.8×10^{-7}	3.12×10^{-11}	3.12×10^{-8}

Table 4. Larvicidal activity of encapsulated lambda nanoparticles (8%) against the 3rd instar of susceptible mosquito larvae (*Culex pipiens*) during different exposure periods shown as mortality percentages, LC_{50} values and the amount of cyhalothrin released

Exposure periods	Concentration in molar (M)						LC_{50}		Amount of compound released	
	1.6×10^{-10}	1.6×10^{-9}	1.6×10^{-8}	1.6×10^{-7}	1.6×10^{-6}	8×10^{-6}	M	ppm	M	ppm
24 hrs.	35	50	60	80	95	99.75	1.8×10^{-8}	1.8×10^{-5}	1.44×10^{-9}	1.44×10^{-6}
48 hrs.	50	50	70	85	99.75	-	5.3×10^{-9}	5.3×10^{-6}	4.24×10^{-10}	4.24×10^{-7}
72hrs.	50	75	80	95	99.75	-	1.2×10^{-9}	1.2×10^{-6}	9.6×10^{-11}	9.6×10^{-8}

9.6×10^{-8} ppm) by 8%, during 24,48 and 72 hr. of exposure periods, respectively.

It is concluded that the encapsulated lambda nanoparticles at reduced concentrations (4% and 8% of the conventional form) were more toxic against mosquito larvae (*C.pipiens*) than the conventional lambda at higher concentrations. It was found also that, nanoparticles loaded with 8% lambda-cyhalothrin was more effective than 4% after 24hr, whereas 4% was more toxic active after 48 and 72 hrs.These results suggested that the encapsulated lambda-cyhalothrin nanoparticles can serve as an effective larvicide at the reduced concentrations avoiding the different problems of conventional form.

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الملخص العربي

النشاط الابادى ضد يرقات البعوض لمبيد لمباداسيهالوثرين فى صورة النانو على يرقات البعوض الحساسة بالمقارنة مع الناتج الاصلى

محمد عبد الفتاح دشيش – دعاء محمد المصرى – محمود مرشدى فرج – حمادة محمود يوسف

تم تحضير جزيئات النانو لمبيد الحشرات لمبادا سيهالوثرين محملا فى البولى اثيلين جليكول بواسطة طريقة الانتشار المذاب بتركيزات ٤% – ٨% من تركيز اللمبادا الاصلى حيث تم دراسة التأثير الابادى الحشرى على يرقات البعوض الحساسة لمركبات النانو المحضرة والمقارنة مع الصورة الاصلية لها على فترات تعرض ٢٤– ٤٨ – ٧٢ ساعة.

وقد أظهرت صورة اللمبادا الاصلية تاثير ساما حيث وجدت

LC_{50} (7×10^{-8}, 1.8×10^{-8} and 1×10^{-9}M)

خلال فترات التعرض الثلاثة على التوالى. اما فى حالة التركيزات المنخفضة ٤% – ٨% LC_{50} لصورة النانو فكانت أكثر فعالية ضد يرقات البعوض المختبرة بقيم أقل منها للصورة الاصلية خلال فترات التعرض الثلاثة، والتأثير السام لمركبات النانو المنخفضة التركيز قد يرجع الى الانتشار المتتالى للسيهالوثرين بكميات مؤثرة خلال فترات التعرض الثلاثة.

ومن النتائج ممكن اقتراح أن جزئيات النانو من اللمبادا يمكن استعمالها فى صورة مبيدات فعالة ضد يرقات البعوض عند التركيزات المنخفضة متفاديا المشاكل المختلفة لصورة المركب التقليدية.