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REMAKABLE ENHANCEMENT OF CYHALOTHRIN UPON LOADING INTO SILVER NANOPARTICLES AS LARVICIDAL

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Abstract

This work has been devoted to develop a novel synthetic scheme to produce pesticide nanocomposite of very high efficiency compared to its original ones. laboratory experiments conducted in NanoTech. company, Dreamland, October city, Egypt and Department of plant protection, Fac. of Agric. Alazhar Univ. Assiut. Egypt. The method is based on using silver nanoparticles (AgNPS) as a pesticide carrier by loading pyrethroid pesticide cyhalothrin (CYN) into the surface of prepared AgNPS. The nature of binding of the pesticide into the nanoparticles surface has been investigated via TEM and FT-IR techniques. The new formulation of the pesticide nanocomposite AgNPS@CYN has been tested for its larvicidal property against three strains larvae of *Culex pipiens* (*C. pipiens*) (i.e. susceptible, field and tolerant strains). Our findings indicate that silver- cyhalothrin nanocomposite is more efficient in controlling mosquito larvae than that of free cyhalothrin. The required concentration for mosquito control decrease more than 100 times. This approach might be successful ones for safer pesticide and clean for the environment.

Keywords: silver nanoparticles, cyhalothrin, *C. pipiens.*, Larvicidal, *LC50*.

1. INTRODUCTION

Mosquito, a biological vector, is responsible for transmission of some serious and dreadful diseases in the whole world. Vectors of the genera Anopheles, Culex and Aedes are principally responsible for spread of diseases like malaria, filariasis, dengue, yellow fever, etc... These diseases are endemic to mainly tropical countries causing millions of deaths. In tropical countries including Egypt the anopheles stephensi is the major vector of malaria. Every year, about 300–500 million people of the world are estimated to be affected by malaria, and this dreadful disease threatens about 2.4 billion of the world's population with a death rate of about 1.1–2.7 million (W H O 2005). Culex pipiens is the vector of Wuchereria species that causes lymphatic filariasis, having a global distribution and infected about 120 million people in the tropical countries (Bernhard L. et al 2003). There is an urgent need to check the proliferation of the population of vector mosquitoes in order to reduce the frequency of vector borne diseases by the application of suitable control methods(Kuppusamy C and Murugan K. 2009. Insecticide applications, although highly efficacious against the target vector species control, is facing a threat due to the development of resistance to chemical insecticides resulting in rebounding vectorial capacity (Liu W.T. 2006). Vector control requires new and improved mosquito control methods that are cheap, ecofriendly and non poisonous to non target organisms. Three strains of mosquito *Culex pipiens* bioassays were also conducted to assess the mortality of mosquitoes. The act of tethering a synthetic organic molecule such as cyhalothrin to a nano silver core allows for effective tracking of organics in complex biological matrices such as mosquitoes. This is a paradigm-shifting technology and offer new possibilities for vector and pathogen control.

Lambda-cyhalothrin is a synthetic pyrethroid insecticide, which used worldwide in agriculture, home pest control, protection of food stuff and disease vector control. The overuse of pesticides in bulk form has led to the contamination of ground waters, soil, sediments, plants, and animals, besides damaging any non-target organisms. The bulk form of pesticides suffers from lack of specificity, i.e. harm non-target organisms, which are beneficial to the environment. On the other hand, many pesticides are poorly soluble in water for which large amounts of organic solvents are required to solubilize them. Most of the organic solvents are hostile to the environment. The resistivity of insect pests to chemical insecticides is increase in costs of materials, and the problems of environmental/personal exposure have hampered the control of vectors and other insects (Salahuddin S. et al 2004).. Pesticides in nano-particular form present an attractive solution for this problem. Their effective concentration is expected to be much lower compared to that of bulk materials and they can be formulated without the use of organic solvents. However, although several approaches were reported on development of nano-pesticide formulations during the last years (Kuzma J. et al 2008 and Bhattacharyya A. 2010)., the research on nano-pesticide formation and applications is still scarce. One major problem with using traditional insecticides for vector control is that, over time, the insects develop resistance. Because insecticides either inhibit acetylcholinesterase (organophosphates and carbamates) or modulate the voltage gated sodium channels (pyrethroids and DDT), the development of resistance to one molecular target means many insecticides are rendered ineffective. One of the most important properties of nanoparticles is their high surface area to volume ratio. For many deferent nanoparticle types, this particular property results in high surface reactivity. 48 Metal-based nanoparticles, such as silver, are unique because they offer the possibility of altering their surfaces in order to introduce specific functionalities for environmental applications. (Nam Y, Lead J R. 2008 , Schmid G and Corain B. 2003, Brust M, Kiely C. 2002 and Haick H, Phys J. D 2007). The ultimate goal of nanosilver synthesis for real world applications is to achieve nanoparticles with the following characteristics:

(1) uniform and narrow size distribution, (2) well-defined shape, (3) known chemical composition with no impurities, and (4) no aggregation or agglomeration [8]. By utilizing a capping agent that acts as a colloidal stabilizer and enhances water suspend ability, these highly desirable characteristics can be achieved for silver nanoparticles (Poole C. P and Owens F. J. 2003 and Rotello V. M. Nanoparticles 2003). In addition, because silver is an electron dense metal. This work proposes a novel synthetic scheme to produce a nanoparticle pesticide core-shell nano-composite to be used as an active agent against insect vectors, such as mosquitoes. Lab synthesized ~20 nm stable silver nanoparticles were surface functionalized with cyhalothrin as the capping agent resulting in a stable colloidal suspension. All the prepared samples were characterized via Transmission Electron Microscopy (TEM) as a primary tool for measuring the particles size, structure and shape, and the plasmonic effect were detected via UV-VIS spectroscopy. The nature of linkage between pesticide and AgNPS were investigated using IR-Spectroscopy. The LC50 of AgNPS@CYN compared with CYN only to three strains of C. Pipiens susceptible, field and tolerant strains respectively were also measured.

2. MATERIALS AND METHODS

All chemicals were reagent grade and were of highest available purity (A.P.); deionized water and ethanol were used to prepare all the solutions. All glassware was thoroughly cleaned with aqua regia and rinsed with deionized water prior to use. Silver nitrate ($AgNO_3$-98%) and poly vinyl prrolodoine (PVP, with average molecular weight = 22,000) were purchased from Sigma–Aldrich. Lambda-cyhalothrin is a synthetic pyrethroid insecticide ($C_{23}H_{19}ClF_3NO_3$). CAS chemical name [a-cyano-3-phenoxybenzyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclo-propanecarbox-ylate], CAS registry number 91465-08-6. The active ingredient lambda-cyhalothrin, was purchased from Kafezayte Co. (Egypt).

2.1. Larvae Mosquito

larvae of field strain were collected from Arab ELmadabegh zone, Assiute, Egypt. The larvae of susceptible and tolerant strains were taken from an Entomological Research Laboratory in plant protection department, Alazhar University, Assiute. Egypt. The larvae were identified as *C . pipiens* . The larvae were acclimatized under suitable temperature and humidity for a period of 24 h (W H O 2005). The larvae were fed with glucose and yeast mixture.

2.2. Synthesis of Nanosilver Core Particles

Nanosilver particles were synthesized by reducing silver nitrate salt solution ($AgNO_3$, 1×10^{-3} M) with ethanol and PVP as capping and reducing agent using the microwave irradiation method (MWI). In a typical experiment, the ethanolic solution of $AgNO_3$ and PVP was heated by in the microwave (Kim J S. 2007). After few minutes the color of the mixture slowly turned from colorless to yellow indicating the reduction of Ag^+ ions (Pillai Z. S and Kamat P. V. 2004). The solutions were removed from the microwave and allowed to cool.

Conjugation of the AgNPS Core Particle with Cyhalothrin

2.3. Cyhalothrin (DM, 1.25% was then tethered to the surface of the resultant nanosilver particle suspension via a vigorous stirring. The color of the mixture slowly turned from yellow to light orange, indicating of the surface functionalization on the AgNPS.

2.4 Characterization of Nanosilver and AgNPS@CYN

The resultant mono-dispersed AgNPS suspension was subsequently surface functionalized with cyhalothrin molecules. These cyhalothrin molecules also proved to be colloidal stabilizers. The size, morphology, crystallinity, absorbance, and zeta potential as well as IR spectroscopy of the spherical AgNPS and the pesticide encapsulated silver nano particles (AgNPS@CYN) were measured.

2.4.1.Transmission Electron Microscopy (TEM imaging)

The transmission electron microscopy (HR-TEM) images were carried out in Nanotech Company for photo-electronic, Dreamland, 6-October, Egypt. The HR-TEM is JOEL JEM-2100 operating at 200 kV equipped with Gatan digital camera Erlangshen ES500. UV-visible Absorption Spectroscopy: UV-visible absorption spectra were recorded on a PERKIN- ELEMER LAMBDA 40 spectrophotometer using 1 cm matched quartz cell over a wavelength range of 200 to 1000 nm. Dynamic Light Scattering: The size distribution and the hydrodynamic diameter of the AgNPS particle and AgNPS@CYN suspension were measured using a Zetasizer, Malvern, UK, and Model: Zetasizer nano series (Nano ZS), Size range (nm):0.6:6000 nm. Agriculture research centers. Cairo, Egypt. Particle Charge: Particle charge or zeta potential of the the AgNPS particle and AgNPS@CYN suspension were measured using a Zetasizer, Malvern, UK, Model: Zetasizer nano series (Nano ZS), Size range (nm):0.6:6000 nm. Agriculture research centers. Cairo, Egypt. Fourier Transform Infrared Spectroscopy, Solutions of AgNPS particle and AgNPS@CYN on infrared spectrometers (Gasco FT-IR Japan). Spectra were recorded in transmission mode with a resolution of 4 cm^1. The scanning performed from 4000-400 cm^{-1} .

2.5. Larvicidal activity of AgNPS@CN against *C. Pipiens*

Fourth instar larvae for three strains of *C. pipiens* were treated with different concentrations of AgNPS loaded cyhalothrin (AgNPS@CYN) and bulk cyhalothrin, following the standard larval susceptibility test method (W H O 2005). Twenty larvae of *C. pipiens* were placed in 250 ml sterile beaker containing 200 ml of water. Bulk cyhalothrin and synthesized (AgNPS@CYN) were added separately to the beaker containing larvae. A control set was also kept. All the samples were maintained in room temperature. The larvicidal effects of the pesticides were monitored by recording the mortality after 24 h of the exposure period. Dead larvae were identified when they failed to move. Each test was performed in six replicates. The corrected mortality percentages were statistically computed according to Finney (Finney, D.l 1971).

2.6. Statistical analysis

The percentages of larval mortality and standard error were calculated for each concentration of AgNPS@CN and bulk cyhalothrin. The LC50 was determined at the 95% confidence level ($P < 0.05$) using Probit analysis. A student's t-test was performed to find out the significance between the concentration of AgNPS@CYN and the mortality at 24 h. Results with $P< 0.05$ were considered to be statistically significant.

3. Results and DISCUSSION

3.1. Synthesis and Characterization of AgNPS and AgNPS@CYN

A primary goal of nanosilver synthesis for practical applications is to produce mono-dispersed nanoparticles with a well-defined shape. Therefore, careful selection of the reducing agent and stabilizer are critical steps which can be more easily controlled when the nanoparticles are synthesized. Hence, we were able to successfully synthesize water-soluble, highly mono-dispersed, spherical AgNPS with a known chemical composition. For these experiments, PVP served the dual role of both a reducing agent and a stabilizer. The well-defined AgNPS core particles were then conjugated with CYN, resulting in AgNPS@CYN, which was also produced in ethanol as opposed to harsh nonpolar solvents. Characterization of both the AgNPS and the AgNPS@CYN are shown in detail.

3.1.1. Transmission Electron Microscopy (TEM) imaging

The size and shape of the silver colloid particles have been measured by TEM imaging. A representative TEM image of these particles is given in Figure 1(A). The particles are mostly spherical. From the sizes of a great number of particles, measured on the TEM images, an average size (diameter) of the synthesized nanoparticles is 16-18 nm. Upon loading the CYN into AgNPs, gradual colors change from yellow to orange. The color change, as an effect of agglomeration (assembly of the particles), is a well-understood phenomenon (Mohamed M.B. et al 2012), as shown in figure 1 (B).

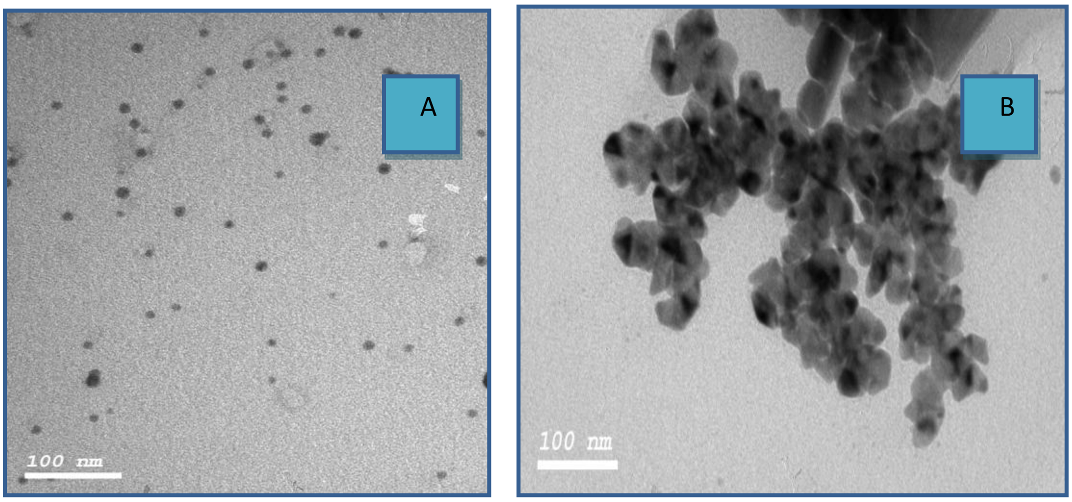


Figure 1: The transmission electron micrographic image of (A) AgNPS only and (B) AgNPS@CYN

3.1.2. UV-Visible Absorption Spectroscopy

Figure 2 shows the measurements from the UV visible absorption spectroscopy during the synthesis of AgNPS, the solution mixture turned from colorless to yellow (Figure 2 inset). This color change is indicative of the reduction of Ag^+ ions as revealed by the surface plasmon (SPR) band at 405 nm that characteristic for AgNPS. On conjugating CYN to the AgNPS, the color of the solution mixture changed once again from yellow to orange also a remarkable blue shift was observed for λ_{max} of SPR ~ 410 nm with broadening in the band, indicative of the surface functionalization and successful conjugation (Figure 2 inset) (Mohamed M.B. et al 2012).

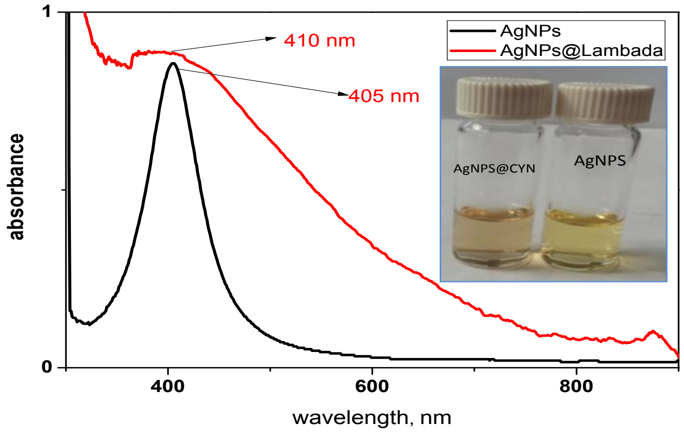


figure 2: The UV-Vis spectra of AgNPS only and AgNPS@CYN, the inset figures showed the Photo images for AgNPS on left side and AgNPS@CYN on the right side

3.1.3. Dynamic Light Scattering

The results obtained from the dynamic light scattering spectra showing a shift in the hydro-dynamic diameter from AgNPS to AgNPS@CYN particles. Both samples exhibit a bimodal size peak at 51 and 135 nm, respectively. These results suggest that AgNPS@CYN particles have a larger hydro-dynamic radius and a higher aggregation potential when compared to AgNPS only indicating the attachment of cyhalothrin molecules to the AgNPS (see supplemental information).

The overall charge that the particle acquires in a particular medium can be determined by measuring the zeta potential of the suspension. The resulting repulsive force can be used to predict the colloidal stability and agglomeration state of nanoparticles. Particles with a large positive or negative zeta potential repel each other leading to a non-aggregated solution with high stability. Conversely, low zeta potential values result in the tendency of nanoparticles to flocculate, or coagulate loosely thereby causing agglomeration(Wang L. et al 2008). The zeta potential of AgNPS and AgNPS@CYN were found to be around 3mV and 90 mV, respectively verifying that both the AgNPS and AgNPS@CYN particles were charged and stable.

3.1.4. Fourier Transform Infrared Spectroscopy (FT-IR)

The IR spectrum of the AgNPS@CYN is compared with this of the (Figure 2). The difference between the spectrum of the free AgNPS and that of the AgNPS@CYN can fall into the following observations:

C=C stretching vibrations for hetero-aromatic compounds observed in the region 1600-1400 cm^{-1} (Silverstein R.M. and Webster F.X. 1998). The CYN and (AgNPS@CYN)show this band at 1635$_{sh}$ (1636$_{sh}$) cm^{-1} respectively (Fox S. A and Martin A. E. 1940), have found that aliphatic C-H appeared as two strong bands at 2962+10 cm^{-1} and 2872+10 cm^{-1} corresponding to asymmetrical and symmetrical stretching modes. υCH absorption bands arising from asymmetrical vibrations are stronger than symmetrical ones (Avram M. and G . Mateescu D. 1972). The CYN showed these bands (sharp bands) at 2983 and 2904 while these bands appeared as very weak bands at (2985 and 2904$_{sh}$) cm^{-1} this change may be due to the effect of neighboring carboxylic group which involved in linkage with AgNPS.

C-H deformation (δCH_3) appeared as strong multiple bands of high intensity around 1380 cm^{-1} and 1465 cm^{-1} for symmetric and asymmetric deformation, respectively (Avram M. and G . Mateescu D. 1972 and Kalsi P. S. 2002). The CYN and (AgNPS@CYN) under investigation showed asymmetric deformation vibration δCH_{3asymm} bands at 1407 and 1375 cm^{-1}. This band may be also merged with C-F band that may be appeared at 1000 - 1400 cm^{-1}.

The unbounded "free" hydroxyl group shows strong absorption band in the 3650-3580 cm^{-1} region. The CYN and (AgNPS@CYN) showed bands at 3458 and 3453 cm^{-1} attributed to OH of solvent or moisture.

υC=O of saturated aliphatic esters fall in the range 1750-1730 cm^{-1}. The CYN and (AgNPS@CYN) showed the C=O band at 1739 and 1738.5 as very sharp band. The υC=O of AgNPS@CYN becomes very stronger than free CYN which can be taken as an evidence for the participation of carbonyl ester group in linkage with AgNPS. The C-O stretching vibrations of esters consists of two asymmetric coupled vibration =C-(C=O)-O and O-C-C, the formers being more important. Esters of aromatic acids absorb strongly in the 1310-1250 cm^{-1} region [19]. The C-O for CYN and AgNPS@CYN displayed at 1074 cm^{-1}, while it associated with huge weakness for AgNPS@CYN on comparing with the free CYN, indicating the contribution of the C-O group in the chelation with AgNPS**.** The IR spectra for CN bond ascribed to be appeared around 2210-2260 cm^{-1}**.** This band appeared at 2361 cm^{-1} for free CYN and tends to disappear for AgNPS@CYN. This may be indication for involving nitrile group in linkage with AgNPS**.**

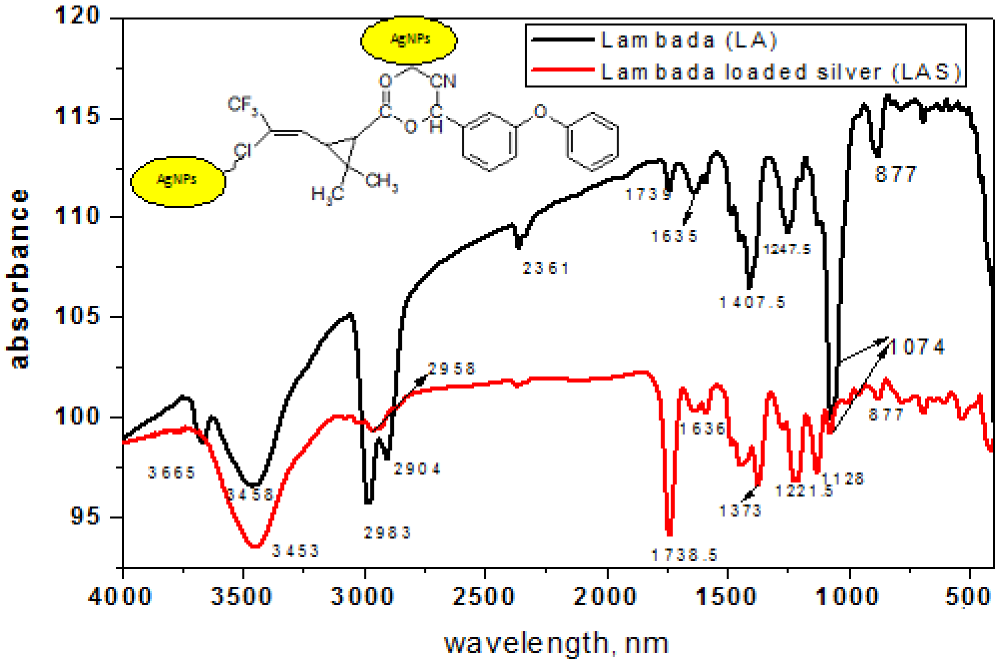


Figure 3: The FT-IR spectra for lambada only and AgNPS@CYN, the inset figure represent the sites of binding of lambada to silver

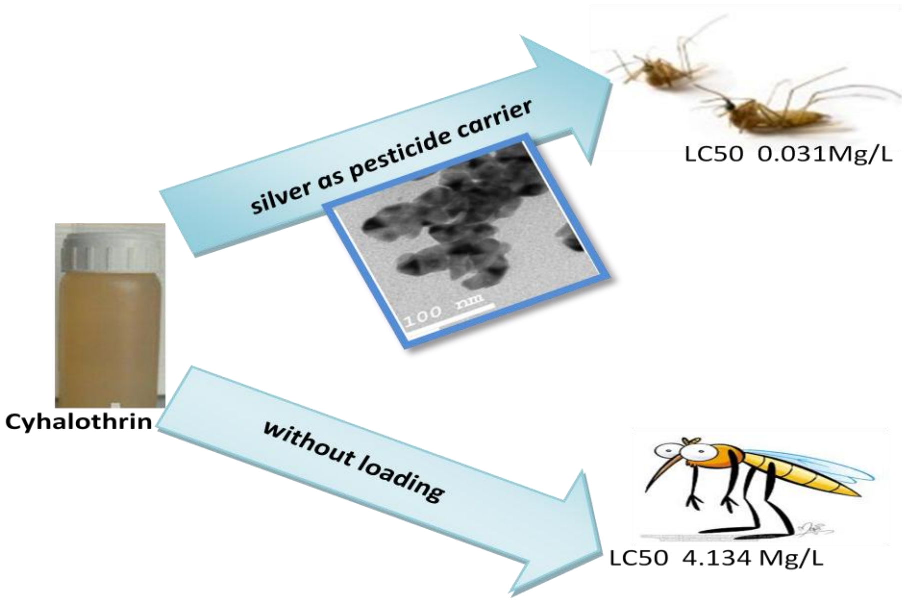


Figure 4: art figure represent the LC$_{50}$ of CYN only and AgNPS@CYN, and its TEM image of their assembly.

3.2. Larvicidal activity of nanocyhalothrin against *Culex pipiens*

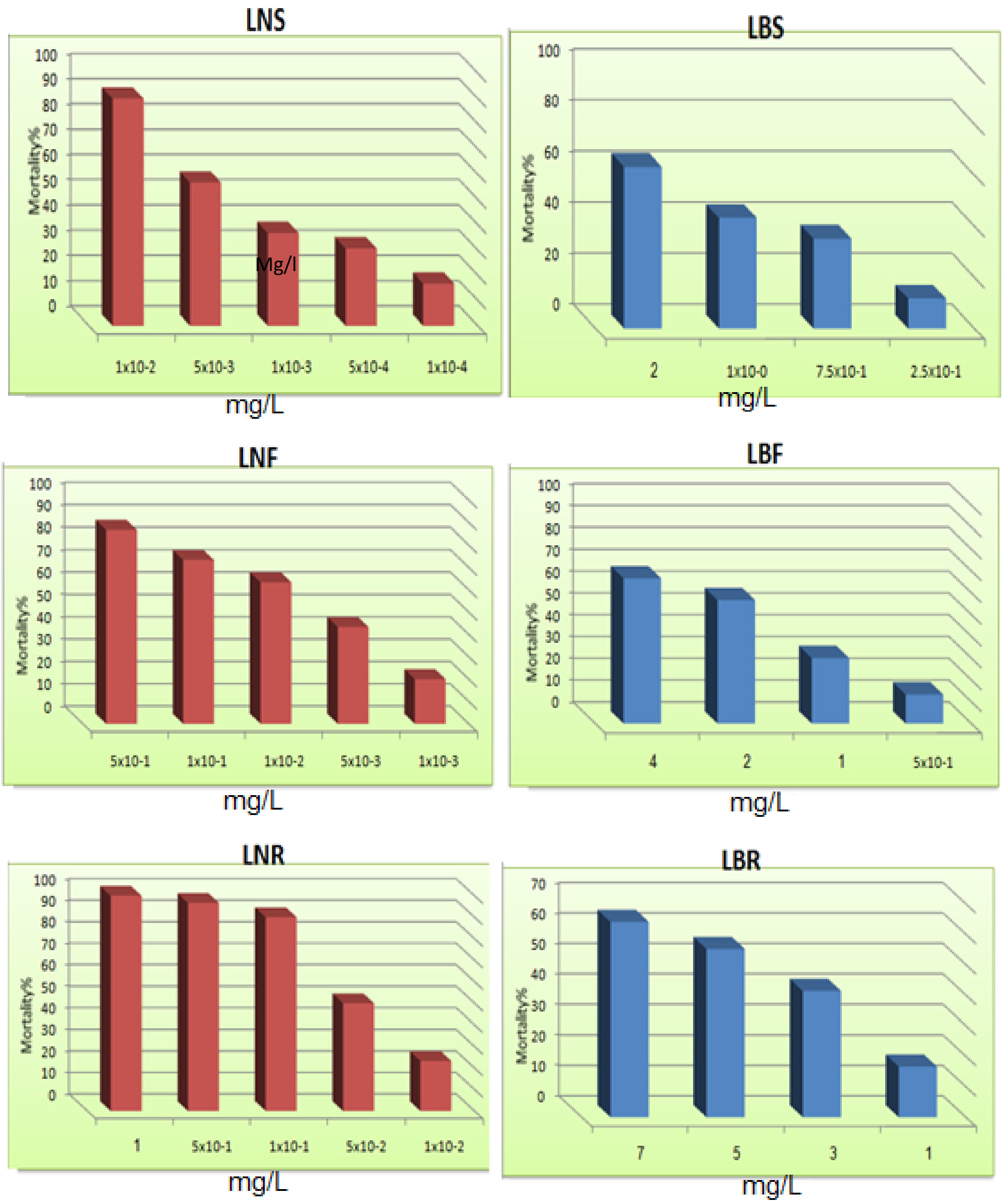
The larvicidal activities of silver nanoparticles loaded cyhalothrin (AgNPS@CYN) and bulk form of cyhalothrin were studied against three stains, fourth instar of *Culex pipiens*. The results of the present study suggested that, the maximum (100%) percentage of larvicidal activity was identified with silver nanoparticles loaded cyhalothrin (AgNPS@CYN) at 1 mg/L for resistant strain of *Culex pipiens*, fourth instar larvae (Table 1).

Mortality rate of *Culex pipiens* increased with the increase of concentrations tested to both bulk and silver nanoparticles loaded cyhalothrin (AgNPS@CYN). The percentages mortality in susceptible strain of *Culex pipiens* ranged from 16.67 to 90 % with concentrations 0.0001 and 0.01 mg/l, compared with 20.0 and 86.67 % with concentrations 0.25 and 2.0 mg/l. for silver nanoparticles loaded cyhalothrin (AgNPS@CYN) and bulk cyhalothrin, respectively (Fig 6). In concerning to failed strain, the percentages mortality recorded 20.0 and 86.67 with concentrations 0.001 and 0.5 mg/l, compared with 13.33 and 66.67 % with concentrations 0.5 and 4.0 mg/l for silver nanoparticles loaded cyhalothrin (AgNPS@CYN) and bulk cyhalothrin, respectively (Fig 7). Further, the same trend of previous results achieved with resistant strain of *Culex pipiens* where recorded 23.33 to 100.00 % with 0.01 and 1.0 mg/L concentrations, compared with 16.61 and 64.04 % with 1.0 and 7.0 mg/L concentrations (Fig 8).

The results of the concentration dependant assay suggested that, the value LC50 was identified as 0.0016, 0.0088 and 0.031 mg/L for susceptible, field and resistant strains of *Culex pipiens*, respectively, for silver nanoparticles loaded cyhalothrin (AgNPS@CYN). The corresponding LC50 values were 1.260, 1.960 and 4.134 mg/L. for susceptible, field and resistant strains of *Culex pipiens*, respectively, for bulk cyhalothrin (Table 1). The results of upper confidential level (UCL) and lower confidential level (LCL) values are mentioned in Table 1.

Table 1: Toxicity effect of bulk cyhalothrin comparative with silver nanoparticles loaded cyhalothrin (AgNPS@CYN) against three strains of *Culex pipiens* at various concentrations

Bulk cyhalothrin						(AgNPS@CYN)					
Strains	Conc. Mg/L	M %	LC50 Mg/L	C.L		Strains	Conc. Mg/L	M %	LC50 Mg/L	C.L	
				L	U					L	U
Susceptible	0.25	11.70	1.260	0.9079	2.162	Susceptible	0.0001	16.67	0.0016	0.0009	0.0029
	0.75	35.18					0.0005	30.67			
	1.00	43.27					0.001	36.67			
	2.00	63.25					0.005	56.67			
							0.01	90.00			
Field	0.5	13.33	1.960	1.431	3.027	Field	0.001	20.00	0.0088	0.0036	0.0184
	1.0	30.00					0.005	43.33			
	2.0	56.67					0.01	63.33			
	4.0	66.67					0.1	73.33			
							0.5	86.67			
Tolerant	1.0	16.61	4.134	2.894	6.628	Tolerant	0.01	23.33	0.031	0.0196	0.0461
	3.0	41.33					0.05	50.00			
	5.0	55.16					0.1	90.00			
	7.0	64.04					0.5	96.67			
							1.0	100.00			



LBS : lambada bulk susceptible strain LNS: lambada nano susceptible strain

LBF : lambada bulk field strain LNF: lambada nano field strain
LBR : lambada bulk resistant strain LNR : lambada nano resistant strain

Figure 5: mortality percentages of bulk cyhalothrin at tested concentrations against susceptible, strain, field and resistant of *C. pepines* respectively comparative with silver nano composite cyhalothrin (AgNPS@CYN).

CONCLUSIONS

Our find indicate the feasibility of using nano silver cyhalothrin composite (AgNPS@CYN) in controlling mosquito larvae, at much lower doses than that required for the free cyhalothrin without using any additives such as organic solvent. These results may be extrapolated to suggest that AgNPS@CYN could serve selectively as a potential larvicide.

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REFFERENCES

Avram, M,. & Mateescu, D. Gh. (1972). Infrared Spectroscopy, Applications in Organic Chemistry, John Wiley, New York.

Bernhard, L., Bernhard, P. & Magnussen, P. (2003). Management of patients with lymphoedema caused by filariasis in northeastern Tanzania: alternative approaches. Physiotherapy, 89, 743–749.

Bhattacharyya, A., Bhaumik, A., Usha Rani, P., Mandal, S. & Epidi, T. T. (2010). Nanoparticles—a recent approach to insect control. African J. Biotech, 9, 3489–3493.

Brust, M. & Kiely, C. J. (2002). Some Recent Advances in Nanostructure Preparation from Gold and Silver Particles. A Short Topical Review . Colloids and Surfaces A, 202, 175.

Finney D.l. (1971). Probit analysis. (3rd ed). Cambridge University Press, London. 333 P.

Fox, S. A. & Martin, A. E. (1940). Investigation of infera red spectra (2.5- 7.5 M) Absorption of water Proc. Proc. Roy. Soc. A175, 208

Haick, H. (2007). Chemical sensors based molecularly modified metallic nanoparticles. Journal of Physics D Applied Physics, 40, 23, 7173-7186

Kalsi P. S. (2002). Spectroscopy of Organic Compounds (5th Ed).

Kim, J. S. (2007). Reduction of Silver Nitrate in Ethanol by Poly(N-vinyl pyrrolidone J. Ind. Eng. Chem, 13, 4 , 566-570

Kuppusamy, C. & Murugan K. (2009). Mosquitocidal effect of Andographis paniculate-nees against the malaria vector Anopheles stephensi Liston (Diptera: culicidae. Int. J. Integr. Biol,.5, 2, 75–81.

Kuzma, J., Romanchek J. & Kokotovich, A. (2006). Upstream oversight assessment for agrifood nanotechnology: a case studies approach. Risk Anal,. 28, 1081–1098.

Liu, W. T. (2006). Nanoparticles and their biological and environmental applications. J. Biosci. Bioeng, 102, 1–7.

Ju-Nam, Y. & Lead, J. R. (2008). Manufactured nanoparticles: an overview of their chemistry, interactions and potential environmental implications. Sci. Total Environ, 1., 400, 1-3: 396–414.

Mohamed, M. B., Adbel-Ghani, N. T., El-Borady, O. M. & El-Sayed, M. A. (2012). j. of Nanomedicine & Nanotechnology, 3, 7, 146-153.

Pillai, Z. S. & Kamat, P. (2004). What Factors Control the Size and Shape of Silver Nanoparticles in the Citrate Ion Reduction Method? , *J. Phys. Chem. B* **,** *108*, 3, 945-951

Poole C. P. & Owens F. J. (2003). Introduction to Nanotechnology; Wiley-Interscience: Hoboken, NJ. 2003.

Rotello, V. M. (2003). Nanoparticles: Building Blocks for Nanotechnology,(1st ed), Springer, New York.

Salahuddin, S., Siti Hajar. A., & Hidayatul fathi, O. (2004). Residual efficacy of insect growth regulators pyriproxyfen, triflumuron and s-methoprene against Aedes aegypti (L.) in plastic containers in the field. Trop. Biomed, 21, 97–100.

Schmid, G, Corain B. Eur. J. Inorg. Chem. 2003; 17: 3081–98. USEPA, 2008.

Schmid, G. & Corain, B. (2003). Nanoparticulated gold: Syntheses, structures, electronics, and reactivities. Eur J Inorg Chem. 17, 3081–98. Pesticide issues in the works: nanotechnology, the science of small.

Silverstein, R. M. & Webster, F. X. (1998). Spectroscopic Identification of Organic Compounds. 6th Ed., New York.

Wang. L., Zhao. W. & Tan, W. (2008). Bioconjugated Silica Nanoparticles: Development and Applications. Nano Res1 , 2 , 99-115.

W H O. (2005). Guidelines for Laboratory and field testing of Mosquito Larvicides. (CDS/WHOPES/GCDPP/05.13).