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SILVER NANOCOLLOID FOR CONTROL CULEX QUINQUEFASCIATUS MOSQUITO LARVICIDE

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ABSTRACT

This study attempts to evaluate larvicidal activity of synthetic Silver nanoparticles (AgNPs) aiming to find cheap, and environment friendly control substance. The preparation of silver nanoparticles was implemented by using simple, cheap, and clean electrochemical method, where two high purity silver rods were supplied with DC electrical power (20-12 volt) ,and immersed in distilled water .UV-Visible spectrophotometer used to detect the distinct absorption spectrum. In literature this method found to produce 12 nm particle size of Colloidal silver solution. Different concentrations of AgNPs (28, 14, 7, 4, 2, and 1ppm) were tested against the late third instars larvae of Culex quinquefasciatus mosquito ,The synthesized nanoparticles were found effective against the larvae of Culex quinquefasciatus mosquito with LC_{50}(4.172 ppm), and LC_{90}(6.86 ppm).

KEYWORDS: *Culex quinquefasciatus, Mosquitoes, Silver , Nanoparticles*

1. INTRODUCTION

Mosquitoes which transmit a number of diseases such as malaria (Anopheles), filariasis (Culex, Mansonia), and dengue (Aedes aegypti) etc., causing millions of deaths every year, are the most important group of insects in term of public health [1]. According to World Health Organisation WHO, there were about 219 million cases of malaria in 2010 (with an uncertainty range of 154 million to 289 million) and an estimated 660,000 deaths (with an uncertainty range of 490,000 to 836,000). Malaria mortality rate has fallen by more than 25% globally since 2000 and by 33% in the WHO African region. Most deaths occur among children living in Africa [2]. Lymphatic filariasis is a widely distributed tropical disease with around 120 million people infected worldwide and 44 million people have lymph edema of the upper or lower limb, breast, scrotum or genitals or hydrocele[3].In the Sudan the disease was restricted to Krodofan, Darfur and the Blue Nile states, but recently it is widely distributed to include most of the states[4].

Larval stages of mosquitoes are targeted, because it is easy to deal with them in the water sources[5].conventional insecticides have created a number of ecological problems, such as the development of resistant insect strains, ecological imbalance, and harm to mammals[3].Repeated use of conventional insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It has also resulted in the development of resistance [6], undesirable effects on non target organisms, fostered environmental and human health concerns [7], which initiated a search for alternative control measures. Nanoparticles have attracted considerable attention [8].

Nanostructure materials have been the focus of intense research in recent decades due to their unique size-dependent physical and chemical properties [9] the synthesis of metal nanoparticles has been widely discussed in the literature due to their distinctive chemical and physical properties, which have many potential purposes [10]. Silver is one of the most studied metals include Stability, morphology, and particle size [11]. Silver has been known to be a disinfectant for several centuries and has been widely used in the treatment of clinical diseases, including newborn eye prophylaxis and topical burn wounds [12]. Silver serves as a potent antibacterial agent, acting against an exceptionally broad spectrum of bacteria while exhibiting low toxicity to mammalian cells [13]. Since silver therapy is of significant clinical benefit in the control of bacterial infections, various forms of new agents medical, biological and pharmaceutical preparations [14] Due to the above mentioned reasons, the present study was carried out to evaluate the acute toxicity of Silver nanoparticles for the control of Culex mosquito larvae.

Electrochemical methods have been implemented for the synthesis of metallic nanoparticles due to the advantages in experimental conditions control [15]. Control of the silver nanoparticles size could be achieved by choosing the reducer and concentrations and even the speed of addition, as well as by adjusting the concentration of the silver ions. Here the concentration of Silver nanoparticles (AgNPs) was controlled by stirring time[7].

1- MATERIALS AND METHOD

2.1 synthesis of silver nanoparticles:-

Two high purity Silver rods (99.99%)were used as electrodes by immersing in (200 ml) distilled water (pH=7,30C^0) in a glass beaker. A differential potential of 20-12 V was supplied between the electrodes. The system was kept under constant magnetic stirring to inhibit the formation of precipitates[16].The (AgNPs) Nanocolloid solution was examined optically using UV-Visible spectrophotometer.

2.2. Rearing of Culex quinquefasciatus larvae:-

Egg rafts of Culex mosquitoes were collected from breeding sites arondd Atbara, Sudan. Then they transported to laboratory in plastic cups (7cm in diameter and 7cm deep) containing de-chlorinated (three days old) tap water, kept under a light bulb (60 watt) to enhance the hatching. The hatched larvae were kept in clean plastic dishes (25cm in diameter and 7cm deep containing distilled water,and fed on a mixture of autoclaved milled bread and yeast (3:1) once a day in small amounts. larvae were transferred daily to cleaned container by means of clean plastic droppers. To be sure that all the mosquitoes used in this piece of work belong to *Culex quinquefasciatus* Say, samples of the fourth instars larvae were identified following the key devised by Sirivanakarn and White [17]. All larvae needed were reared in the laboratory conditions where temperature was kept at 26 ± 2^0C and relative humidity fluctuating between 65 and 80%. The laboratory was artificially lit with diffused light; however, the natural cycle of day and night was kept most of the time.

Larval bioassay:-

Following the conventional methods recommended by[18] WHO (2005), five concentrations (28, 14, 7, 4, 2, and 1ppm) of Silver Nanocolloid solution were used to give a complete rundown mortality from 0 to 100 % in mosquito larvae. Every concentration was tested with a total of 80 late third or early fourth instars laboratory reared larvae executed as four replicates each with 20 larvae in 300 ml glass jar containing 200 ml of the test solution. The control was set up with 200 ml of distilled water

2.5. Statistical analysis:-

The concentration and mortality percentage were found to form a linear relationship. The regression equation (y = a + bx) was, therefore calculated by regression analysis. In the equation:

y = mortality percentage

x = concentration.

a = constant = the intersect of the regression line with the Y axis.

b = constant = the slope (tan theta θ) the regression line makes with the horizontal.

3. RESULTS

3.1 CHARACTERIZATION OF SYNTHESIZED (AGNPS):-

The Characterization Of (AgNPs) nano colloidal (NCS)was carried out by using UV-Visible spectrophotometer as shown in Figure 1 .the absorption spectra bands for the Silver colloid found to lie around (390-400nm). The figure.1 shows maximum absorbance in some band of spectra. We see dominate plasmonic resonance absorption peak at (400 nm). The plasmonic resonance absorption peak at (400 nm) indicates the existent of Silver nanoparticles are stable in the distilled water at room temperature without changing their properties [19].

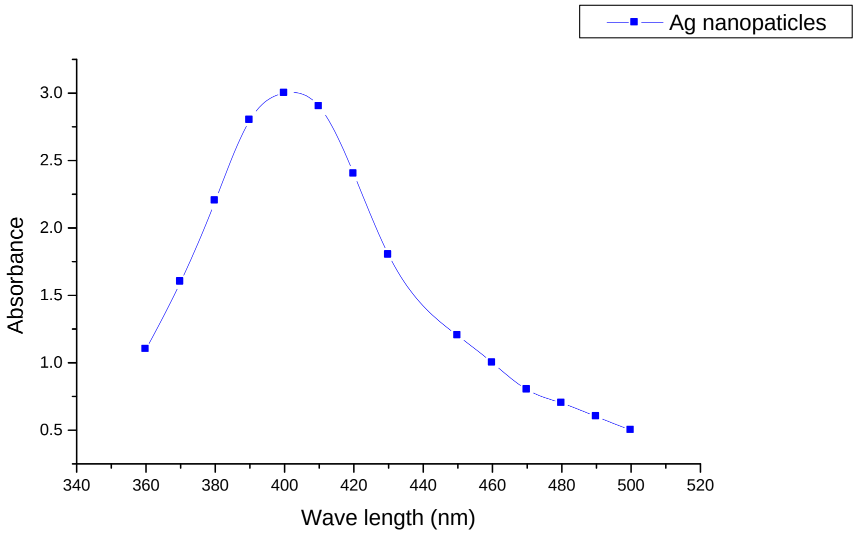

Fig. 1. The UV-VIS absorption spectra of Ag Nanoparticles

3.2 larvicidal activity:-

The mathematical relationship between concentrations of (AgNPs) Nanocolloid solution used and the resulting mortalities of mosquito larvae was evaluated by subjecting the data to regression analysis, and the dose/response regression equations were determined. In addition, the LC_{50} and LC_{90} were calculated. The results of the analysis shown in Table.1 and Figures 1.

Different concentrations of aqueous AgNPs (28, 14, 7, 4,2 ,1 ppm) were tested against the larvae of Culex mosquito . The larvae of Culex mosquito were found highly susceptible to the synthesized AgNPs. The late third instar larvae have shown the 100% mortality after 24 h of exposure at concentrations(25, 12,and 7 ppm) .

Larvicidal activity is judged with a measurement called LC_{50}. "The LC_{50} value is the concentration that kills 50 percent of mosquito larvae in 24 hours," Lower LC_{50} means higher activity, because it takes a lower concentration to kill larvae in the same amount of time.Silver Nanocolloid solution concentration had LC_{50} values with (4.172 ppm) .And lethal concentration that kills 90% of the exposed larvae was (6.86 ppm) Table 2. Statistical analysis of the results showed that there is appositive correlation between concentration of Silver Nanocolloid solution, and mortality of mosquito larvae with presence of almost a perfect positive correlation between concentrations and response in all cases (Coefficient factors R^2=0.918 close to +1) Table1.

Table 1.Effect of Silver nanocolloid solution concentration on *Culex quiquefasciatus* larval mortality after 24 hours of exposure.

Concentration AgNPs (ppm)	Empirical Larva mortality 24 h							Calculated mortality%
	R 1	R 2	R 3	R 4	Total	Average	%	
Control	0	0	0	0	0	0	0	---
28	20	20	20	20	80	20	100	---
14	20	20	20	20	80	20	100	---
7	20	20	20	20	80	20	100	92.08
4	6	8	4	6	24	6	30	47.44
2	4	4	4	6	20	4.5	22.5	17.67
1	2	0	2	2	2	1.5	7.5	2.80
Regression analysis equation	y=a+bx y=14.88x-12.08 R^2= 0.918							

Table .2 Silver nanocolloid solution concentration that kills 50% and 90% of the exposed *Culex quinquefasciatus* larvae.

LC_{50} lethal concentration that kills 50% of the exposed larvae.	4.172 ppm
LC_{90} lethal concentration that kills 90% of the exposed larvae .	6.86 ppm

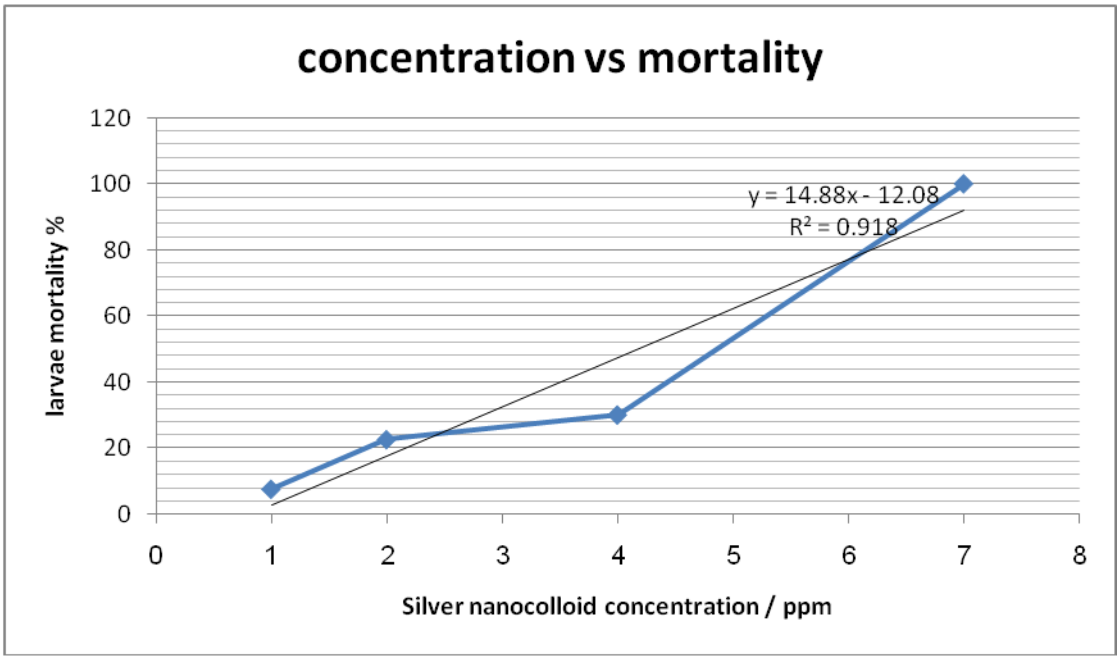

Fig .2 graph represent linear relationship between Silver nanocolloid concentration / ppm and *Culex quinquefasciatus* mortality% after 24 hours of exposure.

4-DISCUSSION

The Characterization Of (AgNPs) Nanocolloidal solution was carried out by using UV-Visible spectrophotometer as shows in Figure-1 it revealed a typical UV-visible absorption spectra for the silver colloid suspension. The figure shows maximum absorbance in some band of spectra. The dominate plasmonic resonance absorption reaches peak at (400 nm)[13].The distinctive colours of silver colloidal are due to a phenomenon known as plasmon absorbance. Incident light creates oscillations in conduction electrons on the surface of the nanoparticles and electromagnetic radiation is absorbed .When the 12 nm Ag nanoparticles, the maximum wavelength is near 400 nm[12]. In general, as the particles become larger the absorption maximum shift to longer wavelengths and the peaks broaden. The peak width at half the absorption maximum is PWHM. The plasmonic resonance produces a peak near 400 nm[12,19]. The wavelength of the plasmonic absorption maximum in a given solvent can be used to indicate particle size. The plasmonic resonance absorption peak at (400 nm) indicates the existent of Silver nanoparticles are stable in the distilled water at room temperature without changing their properties [19,20].

Larviciding is the application of chemicals to kill mosquito larvae or pupae in the water. It is generally more effective and target-specific than applying chemicals to kill adult mosquitoes (adulticiding). Continuous application of these insecticides results in control failures, incidences of resistance, and disease resurgence owing mainly to the development of resistance in the vectors [2]. nanoparticles can be an alternative source for the conventional chemical larvicides, because they constitute a potential source of bioactive chemicals and generally free from harmful effects. Further research is extended to find out the mass production, purification, physiochemical nature of biogenic nanoparticles and their invivo efficacy in laboratory and field trials. The AgNPs synthesized by using Electrochemical methods has been tested against the larvae of Culex mosquito . Statistical analysis of the results showed that there is appositive correlation between concentration, and mortality of mosquito larvae -responsible for toxic effects with a perfect coefficient factors (close to +1). By this approach, it is suggestive that this rapid synthesis of nanoparticles would be proper for developing process for mosquito control.

AgNPs showed 100% larvicidal activity against the *Culex quinquefasciatus* larvae within 24 hours of exposure at concentrations higher than 7 ppm. At concentration 4 ppm of AgNPs, the percentage mortality was dropped dramatically to 30 % for 24 h. The Lc_{50} of (AgNPs) Nanocolloidal solution against *Cx. quinquefasiatus* larvae as determined in the present work (Lc_{50}=4.172 ppm) was found to fall within the range calculated by other workers for mosquito larvae which according to [8] fall between 3.65, and 5.16ppm.

5.CONCLUSION

Electrochemical method was considered simply, cheaply, clean, and faster than other methods. This method found to produced particle size of Colloidal silver solution with 12 nm dimention . The larvicidal activity of the synthesized nanoparticles were found effective against the larvae of *Culex* quiquefasciatus mosquito. The synthesized silver nanoparticles have promising aplication in controlling the menace of mosquitoes in our environment, and potential to be used as an ideal environment friendly approach for the control of mosquito. However, the laboratory results need to be confirmed by field pilot trials before any large-scale applications are recommended.

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