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Silver Nanoparticles Affect Biochemical Parameters in Tissues of Mosquitoes Larvae

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Abstract

This work is concerned with studying the effect of Green synthesized Silver Nanoparticles (GSNPs) on the mosquito larvae of *Culex pipiens*. The lethal concentration L_{C10}, L_{C50} and L_{C90} were obtained from the established regression log concentrate-response lines after 24 hours of exposure. Results implied significant increasing mortality rate upon increasing the lethal concentrations of GSNPs which recorded 0.576 ppm, 1.719 ppm and 5.129 ppm for L_{C10}, L_{C50}, and L_{C90} respectively. For investigating the root cause of mortality, data indicated that there was a significant increase of lipid peroxidation level in tissue homogenates of larvae treated with the increasing of above mentioned lethal concentrations of GSNPs, which recorded 17.25 nmol /g.tissue, 18.0 nmol /g.tissue and 25.0 nmol /g.tissue for L_{C10}, L_{C50}, and L_{C90} respectively, compared to control sample which recorded 16.0 nmol /g. tissue. At the meantime, data also revealed a significant increase in levels of total protein, albumin and globulin levels in tissue homogenates of larvae previously treated with the increasing of above mentioned lethal concentrations of GSNPs , which recorded, 0.231 g/dl , 0.109 g/dl and 0.122 g/dl for L_{C10}, 0.735 g/dl, 0.115 g/dl and 0.62 g/dl for L_{C50}, 0.93 g/dl ,0.125 g/dl and 0.805 g/dl for L_{C90} for total protein, albumin and globulin, respectively, compared to control samples which recorded 0.21 g/dl , 0.089 g/dl and 0.121 g/dl, for the same biochemical parameters respectively.

Keywords: Green Silver nanoparticles; Biochemical parameters in mosquitoes; mosquitoes' lipid peroxidation.

Introduction

Nanoscience is a current multidisciplinary subject that depends on the basic physical and chemical properties displayed by nano level objects [1]. They have been shown to display amazing electromagnetic, surface optical and chemically catalytic characteristics compared to the macro bulk material owing to their extremely low volume to surface area ratio [2]. Metal nanoparticles such as silver show different colors due to their Surface Plasmon Resonance (SPR) phenomenon. It is a group oscillatory behavior of the free electrons of the metal nanoparticles having the same frequency of the light wave interactions, resulting in resonance, causing the SPR band to form in the infrared and visible region [3]. Silver Nanoparticles, SNPs, has found increasing use as an attractive application for medical requirements because of their exclusive characteristics like their quantum properties, good surface to mass ratio which is greater than that of other particles and the capability to adsorb and transport other compounds such as proteins, drugs and probes. The field of NPs is being explored continuously in an exponential manner and it is amazing to find that SNPs are receiving the greatest amount of attention [4]. Interestingly, nanoparticles are playing an increasing role in the development of novel diagnostic methods and in the advanced design of drug delivery systems [5]. Silver nanoparticles (SNPs) in particular, show excellent anti-microbial properties and therefore are

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rapidly being incorporated into a wide array of consumer products such as textiles, cosmetics or packaging materials [6]. Moreover, SNPs are playing a major role in the field of nanomedicine [7]. They have been used for infection prevention in medical field. They have potential antimicrobial activity toward many pathogenic microbes [8]. The antimicrobial effect of SNPs revealed a significant effect against the bacteria *Klebsiella pneumonia*, *Escherichia coli*, and *Pseudomonas aeruginosa* [9].Moreover, Silver nanoparticles imply molluscicidal effect on *Biomphalaria alexandrina*, the intermediate hosts of the trematode *Schistosoma mansoni* [10]. Meanwhile, vector borne diseases are among the major causes of illness and death in tropical and subtropical countries and worldwide more than one million people die due to vector borne diseases every year. Mosquitoes are one of the most important vectors transmitting a variety of diseases for human and domesticated animals. In Egypt, the mosquito *Culex pipiens* is the main vector of some arboviruses [11], and an important vector for periodic lymphatic filariasis [12], St. Louis encephalitis [13], West Nile virus in the USA [14], western equine encephalitis, Japanese encephalitis, and Rift Valley fever [15,16,17]. *Culex pipiens*, therefore, is the main target in control programs against these diseases. In Egypt, the intensive use of insecticides often leads to emergence of resistance. Insecticide resistance is one of the major obstacles in the control of medical and agricultural arthropod pests [18]. The appearance of such problems has been accompanied by growing interest to use of novel pesticides with a new mode of action specially when dealing with undesired biological entities [19]. Green synthesized Nanoparticles, GSNPs, has been developed via different biological systems, including bacteria, fungi, yeasts, algae or plants [20, 21, 22]. The extracts from living organisms can act as reducing and stabilizing agents. There are many advantages in the synthesis of SNPs via biological extract, such as eco-friendliness, low cost, mild reaction conditions and compatibility for biomedical applications.

The present work investigated the larvicidal efficacy of Green Silver nanoparticles (GSNPs) against mosquitoes larvae (*Culex pipiens*), under laboratory condition in Egypt.

2-Experimental

2.1 Preparation of Green Silver nanoparticles (GSNPs)

Ginger extract was prepared by cutting 5.0 g of rhizome into small pieces, which were refluxed with 100 mL of 70% ethanol at 70°C for 2 hours. After cooling, the obtained extract was filtered through Whatman filter paper and centrifuged. The supernatant was collected and stored at 4°C. For the biosynthesis of SNPs, 1 mL of ginger extract was added to 20 mL of AgNO3 solution (1 mmol/L) in a round-bottom flask. The mixture was heated at 85°C and color change of the solution was recorded within 20 minutes. Thereafter, SGNPs were characterized by UV–visible (vis) spectroscopy on a UV-2550 spectrophotometer in the range of 200–800 nm. The size and shape of GSNPs were analyzed by transmission electron microscopy (TEM, JEM 1011) at an acceleration voltage of 100 kV [23].

2.2 The larvicidal efficacy of Green Silver Nanoparticles (GSNPs)

Larvae used in this study belonging to the species *Culex pipiens*. They were obtained from Research Institute of Medical Entomology, Giza, Egypt. They were maintained in the water of their biotopes under laboratory conditions (75% relative humidity and 25°C). All 3rd instars larvae selected for larvicidal activity came from the same generation and from the same breading sites. The methodology of the toxicity tests was based on the WHO standardized sensitivity tests [24, 25]. Using the previously prepared green silver nanoparticles, a series of concentrations (0.7, 0.8, 1.0, 2.0, 3.0, 5.0 and 10.0 ppm/ml) were prepared using dechlorinated water as a diluent. Twenty five 3rd instar larvae were put into a small beaker (500 ml) containing the test solution of each concentration. Four replicates were performed for each concentration. In control experiments, larvae were placed into dechlorinated water only. Larval mortalities were determined 24 hours post treatment. A larva was considered dead if it did not move when prodded with a fine wooden dowel [26]. Lethal concentrations were determined by mortality rates after 24 hours of exposure. Probit analysis [27] was used for lethal concentrations and obtaining slope values using Polo-PC Plus v.3.1 statistical software. Data were corrected for control mortality using Abbott's formula [28].

2.3 Biochemical investigations

After *Culex pipiens* larvae were treated with different lethal concentrations of green silver nanoparticles for 24 hours of exposure period, subsequently alive treated larvae were homogenized using UP 200H ultrasonic processor, one gram tissue in 5ml phosphate buffer solution (PBS). The obtained suspensions were centrifuged at 4000 rpm for 45 minutes. The pellets were discarded while the aliquots of supernatants were involved in different cellular and biochemical assays. Cellular

investigations regarded endogenous lipid peroxidation as an indicator of oxidative stress which was estimated spectrophotometrically following the method described by Okhawa *et al.,* [29]. The biochemical assay tests were carried out as follows: Total protein was determined according to the method of Domas [30]. Albumin was determined according to Gustafsson [31]. Calculation of globulin was determined by subtracting the amount of albumin from the total protein. The concentration of each parameter was detected at its relevant wave length using Photometer 5010, Serial number 3666, ROBERT RIELE GmbH&Co KQ, made in Germany. Finally, A/G ratio was calculated by dividing albumin levels by globulin levels each at its relevant lethal concentration.

Data was subjected to analysis of variance (ANOVA) and of the significant differences using the method of Sokal and Rohlf (1969) [32].

3- Results

The larvicidal efficacy of Green Silver Nanoparticles (GSNPs) against mosquito larvae (*Culex pipiens*) was evaluated by determining the levels of L_C of different concentrations and the slope of the log-concentration Probit response lines after 24 hours and the results were tabulated in tables (1&2). Results implied that GSNPs were effective against mosquito larvae. The levels of $L_{C10,}$ L_{C50} and L_{C90} were 0.576 ppm, 1.719 ppm and 5.129 ppm, respectively. The ratio of L_{C90} / L_{C50} simply represents the steepness of the log-concentration Probit lines in reversal way to the slope value. Data indicated that the slope of efficacy regression line of silver nanoparticles was (2.699± 0.174), and L_{C90}/L_{C50} ratio were 2.98.

For investigating the root cause of mortality, data in table (3) & figure (1) indicated that there was a significant increase ($p<0.05$) of lipid peroxidation levels in tissue homogenates of larvae treated with the increasing above tested lethal concentrations of GSNPs, which recorded 17.25nmol /g.tissue, 18.0nmol /g.tissue and 25.0nmol /g.tissue for $L_{C\mathbf{10}}$, $L_{C\mathbf{50}}$, and $L_{C\mathbf{90}}$ respectively, compared to control sample which recorded 16.0nmol/g. tissue.

At the mean time, data in table (4) and figures (2, 3& 4) implied significant increasing levels ($p<0.05$) of total protein, albumin and globulin in tissue homogenates of larvae treated with the above tested lethal concentrations of GSNPs, which recorded, 0.231 g/dl , 0.109 g/dl and 0.122 g/dl for L_{C10}, 0.735 g/dl, 0.115 g/dl and 0.62 g/dl for L_{C50}, 0.93 g/dl ,0.125 g/dl and 0.805 g/dl for L_{C90} for total protein, albumin and globulin, respectively compared to control samples, which recorded 0.21 g/dl for total protein , 0.089 g/dl for albumin and 0.121 g/dl for globulin.

4- Discussion

In the recent years, ongoing research has focused on development of nano-scale objects as efficient larvicidal agents. Among the various nanoparticles, silver nanoparticles have gained much attention due to their unique larvicidal properties. However, concerns about the synthesis of these materials such as use of precursor chemicals and toxic solvents, and generation of toxic byproducts have led to a new alternative approach, green synthesis [33]. This eco-friendly technique incorporates use of biological agents, plants or microbial agents as reducing and capping agents [23].

Herein, Green Silver Nanoparticles (GSNPs) proved a promising larvicidal effect against mosquito larvae of *Culex pipiens*, under laboratory conditions in Egypt concerning some cellular and biochemical parameters. Meanwhile, the efficacy of GSNPs as insecticidal agent was reported by several investigators. Kadarkarai *et al.,* [34] studied the larvicidal and pupicidal activity of carbon nanoparticles (CNPs) and silver nanoparticles (SNPs) against *Culex quinquefasciatus*. They reported that in laboratory assays, L_{C50} values ranged from 8.72ppm (first-instar larvae) to 18.676ppm (pupae) for silver nanoparticles and from 6.373 ppm (first-instar larvae) to 14.849 ppm (pupae) for carbon nanoparticles. In addition, Marimuthu *et al.,* [35] evaluated the acute toxicity of indica leaf extract and biosynthesized SNPs against larvae of malaria vector *Anopheles subpictus*, the dengue vector *Aedes albopictus* and Japanese encephalitis vector *Culex tritaeniorhynchus*. The indica leaf aqueous extract, biosynthesized SNPs showed higher toxicity against *Anopheles Subpictus*, *Aedes albopictus* and *Culex tritaeniorhynchus* with L_{C50} values of 31.56, 35.21 and 38.08 ug/ml, respectively. Moreover, Hatem *et al.,* [36] reported that silver nanoparticles of *Cassia fistula* fruit pulp were effective against the larvae instar and pupae of *Aedes albopictus* and *Culex pipienspallens* , after 24, 48and 72hours of treatment. The value of $L_{C50}$for *Aedes albopictus* ranging from 8.3 mg/L for inster and 17.3 mg/L for pupae, while the value of L_{C50}*for Culex pipienspallens* ranging from 1.1 mg/L for instar and 19.0mg/L for pupae. Recently, Natayae*t al.,* [37] investigated the larvicidal activity of *Curcuma zedoaria* essential oil (ZEO) and biosynthesized silver nanoparticles using this essential oil (ZEO- SNPs) against *Culex quinquefasciatus* larvae. The larvicidal activity against both insecticides susceptible and resistant strains of *Culex quinquefasciatus* larvae of (ZEO) were investigated and compared with (ZEO- SNPs).

The (ZEO- SNPs) offered complete larvae mortality within 24 hours of exposure. The values of L_{C50} and L_{C99} against the susceptible strain, were 0.57 and 8.54 ppm, while for resistant strain recorded 0.64 and 8.88 ppm for L_{C50} and L_{C99}, respectively. But, after 24 hours of exposure, L_{C50} and L_{C99} of (ZEO) against susceptible strain were 36.32 and 85.11 ppm, L_{C50} and L_{C99} of (ZEO) against resistant strain were 37.29 and 76.79 ppm, respectively.

All obtained results came in accordance with number of studies that revealed that SNPs interfere with cellular functions; cause toxic effects and may interfere with specific biological systems [38]. Hence, this may explain the cause of increasing the rate of mortality upon increasing the GSNPs concentration in this study. In order to elucidate GSNPs mode of action, three well-defined mechanisms have been proposed so far: (i) cell membrane damage, (ii) intracellular penetration and damage, and (iii) oxidative stress [33].As known, one of the main functions of the cell membrane is to protect the organism against the environmental threats and to maintain homeostasis while still permitting the transport of nutrients inside the cell. The interaction between GSNPs and larvae starts with adhesion of GSNPs to the larval cell membrane, which is based on electrostatic attraction between the negatively charged cell membrane and positively or less negatively charged GSNPs [33]. GSNPs have been found to attach to the surface of the larval membrane, cause structural changes of the membrane, and finally lead to death of the cells [39]. Meanwhile, lipid peroxidation can be defined as the oxidative deterioration of lipids containing any number of carbon-carbon double bonds, and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides are unstable and decompose to form a complex series of compounds including reactive carbonyl compounds. Damage to lipids alters and modifies cellular membranes and, therefore, cellular function [40]. Lipid Peroxidation is highly destructive to the functioning of cell and its survival. It decreases membrane fluidity and changes the phase properties of the membranes. Also, it causes lysosomes to become fragile or leaky. Moreover, it causes decrease in activities of enzymes associated with membranes [41].This may elucidate another one cause of the observed increasing mortality rate of the treated larvae in the present study. It is most likely that GSNPs induced lipid peroxidation in larval cells; thereby the nanoparticles could damage the intracellular DNA and proteins [42]**.** Thus, upon investigating total protein levels among different GSNPs concentrations, results revealed significant increase in levels of total protein upon increasing GSNPs concentration. This can be explained according to the proteomic study of Lok *et al.*, [43] which pointed out that the interaction of SNPs with sulphur containing membrane-bound proteins and enzymes led to inactivation of these molecules. Additionally, organisms started to express a series of envelope proteins, heat shock proteins and periplasmic components responsible for protecting the cell against the entry of foreign substances as a stress response [33].Meanwhile, dehydration and increasing mortality rate of the treated larvae with mentioned lethal concentration of GSNPs may be also attributed to the significant increase of albumin levels among these concentrations. Albumin has a number of important roles in the body, including maintaining oncotic pressure and binding a variety of molecules. It also contributes to the pool of amino acids used for protein synthesis, buffers extra vascular fluids, aids in preventing pathologic thrombus formation, and helps maintain normal microvascular permeability [44, 45]. Similarly, globulins are a varied group of proteins that are produced by the immune system to help fighting infection and transport nutrients [46]. Upon examining globulin levels in larvae treated with GSNPs, results revealed significant increase in globulin levels upon increasing GSNPs concentration. These increasing levels of globulin may be attributed to the increasing concentrations of GSNPs that entered the larval cells thus induce immune responses against the invading agent and consequently the adverse effects it induced in their tissues. Finally, calculating A/G ratio compares the amount of albumin with globulin. Changes in this ratio can provide a clue as to the cause of the change in protein levels [47].

5- Conclusion

Green Silver Nanoparticles, GSNPs, affect the survival of the larvae of vector borne disease, *Culex pipiens*, via induction of chains of lipid peroxidation reactions and observed changes in vital biochemical parameters tested in their tissue homogenates post incubation with GSNPs. This may open a new, smart and safe avenue for green synthesized nanoparticles to be used as larvicidal agents.

6- Conflict of interests

There are no conflicts of interests to declare.

7- Formatting of funding

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8- Acknowledgments

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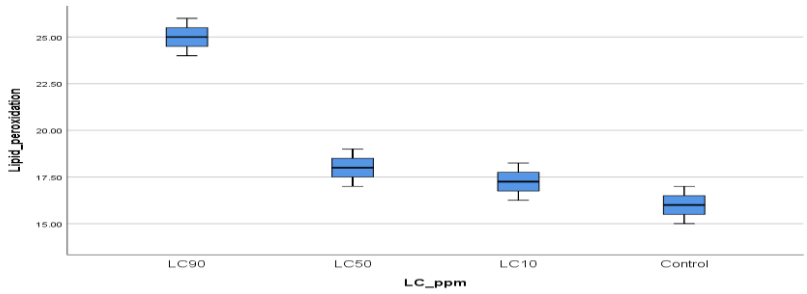


Fig. 1: Lipid Peroxidation (LP) levels among different lethal concentrations of GSNPs against larvae (*Culex pipiens*).

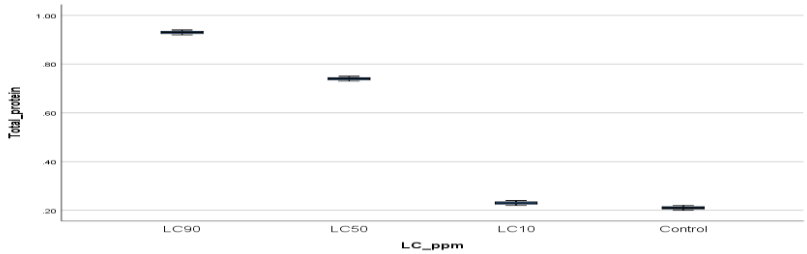
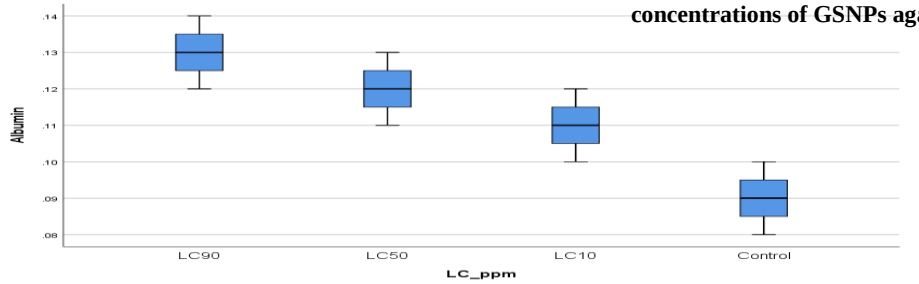


Fig.2:
Total protein levels among different concentrations of GSNPs against *Culex pipiens* larvae.

Fig. 3: Albumin levels among different lethal concentrations of GSNPs against *Culex pipiens* larvae.



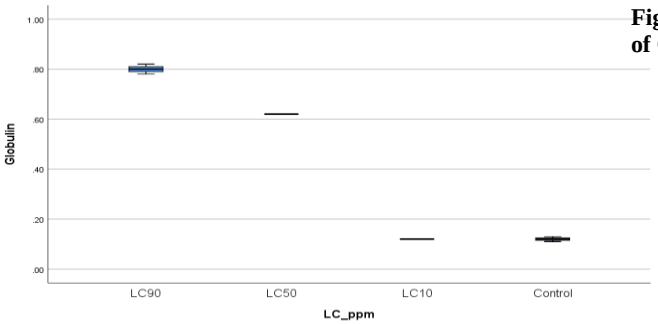


Fig. 4: Globulin levels among different concentrations of GSNPs against *Culex pipiens* larvae.

Table 1: Response of larvae (*Culex pipiens*) to different concentrations of green silver nanoparticles.

Concentration(ppm)	No. of larvae tested	Died	Alive	Mortality%
0.7	100	16	84	16
0.8	100	20	80	20
1.o	100	24	76	24
2.0	100	50	50	50
3.0	100	80	20	80
5.0	100	91	9	91
10.0	100	97	3	97
0.0	100	0.0	100	0.0

Table 2: Efficacy of different lethal concentrations of Green Silver Nanoparticles against larvae (*Culex pipiens*).

Lc values(ppm)				
Lc_{10}(lower-upper)	Lc_{50}(lower-upper)	Lc_{90}(lower-upper)	$Lc_{90/}Lc_{50}$	Slope ± SE
0.576 (0.445- 0.699)	1.719(1.513-1.955)	5.129(4.203-6.677)	2.98	2.699 ± 0.174

Table 3: Lipid Peroxidation (LP) levels among different lethal concentrations of GSNPs against larvae (*Culex pipiens*).

Green Silver nanoparticles concentrations (ppm)	Lipid peroxidation level (nmol/g. tissue)
LC10 (0.576)	17.25
LC50 (1.719)	18.00
LC90 (5.129)	25.00
0.00	16.00

Table 4: Total protein, albumin, globulin levels and calculated A/G ratios among different lethal concentrations of GSNPs against *Culex pipiens* larvae

Green Silver Nanoparticles concentration (ppm)	Total protein level (g/dl)	Albumin (g/dl)	Globulin(g/dl)	A/G ratio
LC10 (0.576)	0.231	0.109	0.122	0.89
LC50 (1.719)	0.735	0.115	0.62	0.185
LC90 (5.129)	0.93	0.125	0.805	0.155
0.00	0.21	0.089	0.121	0.74

Table 5: ANOVA test conducted at different lethal concentrations of GSNPs among different parameters of *Culex pipiens* larvae.

Parameter(s)	Source of variance (S.V.)	Sum of Squares	df	Mean Square	F	Sig.
Lipid	Between Groups	147.141	3	49.047	49.047	0.00
peroxidtion	Within Groups	8.000	8	1.000		
	Total	155.141	11			
Total protein	Between Groups	1.189	3	.396	3964.75	0.00
	Within Groups	.001	8	.000		
	Total	1.190	11			
Albumin	Between Groups	.003	3	.001	8.750	0.007
	Within Groups	.001	8	.000		
	Total	.003	11			
Globulin	Between Groups	1.093	3	.364	2914.40	0.00
	Within Groups	.001	8	.000		
	Total	1.094	11			

Where: df= degree of freedom, F= variance and the mean difference is significant at 0.05 level (Results are statistically significant if sig. or p- value ≤ 0.05).