Research Article

Behavioral Toxicity of Biosynthesized Silver Nanoparticles on *Culex* Mosquito Larvae

Kapil Singh*, Pritam Singh Shekhawat, AshishRanjan Singh
Research and Development Department, Nanobeach, New Delhi, India.
***Corresponding author's E-mail:** b.kapilsingh@gmail.com

Accepted on: 07-05-2013; **Finalized on:** 31-07-2013.

ABSTRACT

Rapid progress and early acceptance of nanobiotechnology leads many harmful effects on all creatures. One of the crucial steps before developing any such technology is to test for any harmful effects of these nano sized objects on the general behavior and mortality of organisms. In the present investigation, Silver nanoparticles were bio-synthesized and examined for toxic effects on the general behavior, activities and mortality of Eukaryotic system (*Culex* mosquito larva). *A.nigar* fungus *was* used for bio-synthesis the nanoparticles and the Characterizations showed that average size of nanoparticles was 70 nm with heterogeneous dispersion and cubic crystal structure. For the toxicity testing, nanoparticles were added in different concentrations (5, 20, 35 and 50 mg/L) to larva's natural habitat (stagnant water) and were observed for the mortality and activity levels of different behaviors of larvae and pupae that were previously observed in control namely: light block alarm response; disturbance response; feeding activity and overall movements. For the mathematical representation of behavior, continuously decreasing scores were assigned for subsequent week behavior compared to control. There were three larvae taken in triplicate setup and observations were conducted after every 3 hours for 2 days. Silver nanoparticles showed toxic effects on mortality and behavior of *Culex* mosquito larvae in dose and time dependent manner while pupae were unaffected from nanoparticles. The pattern of changes in all behavioral activities seems in same way. On the 5 mg/L shows only highly alterations but no lethal effects whereas, in 20 mg/L remarkable alteration found but it did not cause death when compared to control. Remaining both concentrations (35 and 50 mg/L) showed a steep fall in the prepared graph of all activities that was more lethal from other groups which lead to death.

Keywords: Behavior, Biosynthetic, *Culex* sp., Larvae, Mosquito, Silver Nanoparticles.

INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern materials science. Nanoparticles have silently crept everywhere into our lives, and soon are about to get inside our bodies and brains too, for repairing damaged cells or targeting medicine or transmitting signals for imaging and interfacing with machines. Despite the rapid progress and early acceptance of nanobiotechnology, the potential effects of their widespread use in the consumer and industrial products are just beginning to emerge [1,2] and the environmental impact of nanomaterials is expected to increase substantially in the future. Whatever be the way one of the crucial steps before developing any such technology is to test for any harmful effects. These nano sized objects might have on the general activity, behavior and mortality of organisms. To address the same issue we used mosquito (*Culex*species) larvae as an experimental organism, and for the first step we tested biosynthetically prepared silver nanoparticles on them.

Silver nanoparticles are the crystalline aggregates of silver atoms in the size range of 1 to 100 nanometers. There are many methods available for synthesis of Silver nanoparticles but Bio-route synthesis have many advantages such as such as eco-friendly, easy procedural, easy controlled economic viability, and no need to use high pressure, energy, temperature and toxic chemicals in synthesis protocol. Silver nanoparticles have found tremendous applications in the field of high sensitivity bio molecular detection and diagnostics, antimicrobials and therapeutics, Catalysis and micro-electronics.[3-7]

In spite of the wide usage of Ag-np there are very few reports on the toxicity of silver nanoparticles are available.[8,9] Reported studies showed the toxicity and bioaccumulation of nanoparticles in prokaryotic system and eukaryotic cell lines which leads to high mortality rate. The potential for adverse health effects due to prolonged exposure at various concentration levels in living organisms and their behavior has not yet been established. So, experimental analysis needs to be conducted on higher organisms using different concentrations of nano-dimension materials so as to get a database of toxic and nontoxic nano-materials and safe concentration for potential use in biological applications.

There were many studied on prokaryotes and very few on eukaryotic cell lines already conducted for determination of biological activity and toxicity of Silver nanoparticles.[10,14] But in the present study, we explore the effects of biogenic silver nanoparticles on mortality and general activities and behavior of *Culex* larvae. As need of study on eukaryotes *Culex* sp. Larvae was taken as modal organism in this study to test the dose dependent toxic effect of Silver nanoparticles. *Culex* is a higher organism and can be found easily in India. Its larvae can also be classified from other species easily by simple microscopic investigation of morphological specifications. No more studies on effects of silver nanoparticles on insect's life and behavior have been carried out although a few studies on effect of some parameters on mosquito larvae have been done.[15-17] Biogenic silver nanoparticles were prepared by the use of *Aspergillusnigar* fungal extract because *A. nigar* is an easy and fast growing microorganism.

MATERIALS AND METHODS

Sample collection

A.nigar fungal strain (MTCC 10180) was obtained from microbial type culture collection and gene bank in IMTECH Chandigarh,and cultured in Research and development laboratory, Nanobeach, New Delhi. Mosquito samples were collected from stagnant water in campus ofNanobeach, New Delhi.

Preparation of Fungal Extract

Fungalstrain was spread on patryplates filled with PDA medium and incubated for 5 days on 37°C. After the significant growth of fungi, the fungi material was scratched out from plates using specula. 10 gm wet biomass was mixed in the 100 ml MQ water taken in conical flask. This mixing was done on rotating shaker at 150 rpm for 24 hours at 25 °C. After this mixing we filtered it by using ordinary filter paper. Again the solution was filtered using whatman filter paper. The obtained solution is desired fungi extract.

Synthesis of Silver Nanoparticles

2 mM silver nitrate ($AgNO_3$) solution was prepared. The process of weighing AgNO3 and then dissolution was done in dark. In a typical biosynthesis production scheme of silver nanoparticles, 100 ml of fungal extract was mixed with 300 ml silver nitrate solution and shake on rotating shaker for 3 days at 28°C. 2 ml of mixture volume was taken periodically for UV-Vis Spectroscopic inspection of nanoparticle synthesis. In this process silver nanoparticles were produced through reduction of the silver ions to metallic silver. After the shaking for 3 days, mixture was centrifuged at 12000 rpm for 15 min at 25°C. Obtained palette was triple washed with MQ water by centrifugation. Palette of Pure nanoparticles was dried by vacuumed drier and then crushed to get in powder form.

Characterization of nanoparticles

The reduction of silver ions was routinely monitored by visual inspection of the solution as well as by measuring the UV-Visible spectra of the solution by periodic sampling of aliquots (2 *mL*) of the aqueous component. The UV-Vis spectroscopy measurements were recorded on a Shimadzu dualbeam spectrophotometer (model UV-2450). An X-Ray Diffraction (XRD) spectrum is taken by X'Pert Pro x-ray diffractometer (PAN alytical BV, The Netherlands)operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation in a θ- 2 θ configuration.The nanoparticle films were formed on carbon coated copper grids (40 *µm*× 40 *µm*mesh size), Atomic Force Microscopy (AFM) images of the film were scanned on nanosurf.

Experimental design to examine the behavioral toxicity

The observation was recorded on the basis of 4 behaviors

i. Light block alarm response: an artificial light source was switched on 10 minutes before every observation so that the larvae gets acclimatized and switched off for every observation. Repeated stimulation produces "stimulus satiation". So as to avoid this, enough large time period of three hour between each observation was taken.
ii. Disturbance alarm response: container is disturbed a little for observation of activities.
iii. Feeding movement
iv. Overall movement (including thrashing and filtering movements).

For observation for each behavior, 5 containers containing 50 ml stagnant water (pH 6.8) mixed with different concentrations of Ag Nanoparticles: control without Silver nanoparticles; 5, 20, 35 and 50 mg/L Ag nanoparticles respectively. Prepared nanoparticles were precisely weighed and appropriately mixed with stagnant water in all containers. 3 *Culex* larvae and 1 pupa were placed in each container which was covered with filter paper so as to ensure that air could pass. The above setup was triplicated for consistency of the results (total 9 larvae and 3 pupas in all three containers of one set). The organisms under experimental setup were observed regularly with an interval of 3 hours between every observation for 48 hours.

The amount of behavioral activity observed based on the overall response and movement ability. Behavioral activity was divided on a qualitative basis into 5 levels and assigned score as follows:

Highly active (score = 4)

As observed generally in the larvae with showing all the 4 different types of behavior as in natural or control conditions. The score assigned for larva was 4.

Relatively less active (score = 3)

The different types of movements were observable but the velocity and frequency of activity was reduced. The score assigned for larva was 3.

Extremely less active (score = 2)

The movement becomes retarded the larvae could still browse but the typical thrashing activity is completely lost. The larvae tend to remain suspended form the surface. And could come back to surface when forcefully sunk to bottom but the time period of retrieval exceeded the normal limit and even reach up to 85 seconds. The score assigned for larva was 2.

Nearly dead/No activity (score = 1)

No type of movement observable, larvae remained in resting state when forcefully sunk to the bottom the larvae were unable to come up and eventually died due to lack of oxygen. Larvae in this stage when observed under microscope showed active respiratory pulsation in the longitudinal trunk meaning the larvae were still alive but there was severe retardation of movement. The score assigned for larva was 1.

Dead (score = 0)

Bodies floating at the surface inverted or tilted sideways, or settled to bottom. The score assigned for larva was 0.

For each type of four behaviors, each larva was assigned with corresponding activity level score (4, 3, 2, 1 or 0).Scores obtained for all 9 larvae in three containers of a set were added to form a mathematical representation in data table of behavioral observation. Data tables of behavioral observation were prepared with all mathematical score values of behavior obtained by larval group in stress condition provided by deferent concentration of Silver nanoparticles. The comparative dose dependent effect of silver nanoparticles could be seen by derived graphical plots by the use of mathematical values of behavior in prepared data tables. Total number of dead larva in timeline of observation was placed in a Quantitative data table for total mortality and a graph plot for total mortality was also prepared from the mathematical data of total mortality table.

RESULTS AND DISCUSSION

Confirmation of *Culex* Species

Selection and identification of *Culex* Species from collected Mosquito samples was done in research and development laboratory, Nanobeach, New Delhi. The microscopic picture of *Culex* Larvae is showed in Figure 1.

Figure 1: *Culex* larvae under microscope

Characterizations of Silver nanoparticles

Visual inspection of synthesis of Silver nanoparticles

The appearance of yellowish brown color clearly indicates the formation of silver nanoparticles in the biosynthesis.[18,19] The characteristics brown color of colloidal silver solution is due to the excitation of surface Plasmon vibrations in the nanoparticle and provides a convenient spectroscopic signature of their formation.[20,21]

UV-Vis Spectra analysis of Ag nanoparticles

It is well known that colloidal dispersions of metallic nanoparticles exhibit absorption bands in the UV-vis region, due to collective excitations of the free electrons (surface Plasmon band).[22] The formation of Ag nanoparticles can be analyzed easily by periodic observation with UV-Vis spectroscopy.[23] Figure 2 shows the UV-Vis spectra recorded from the three periodic sampling. The Peak is seen at around 422 nm, suggesting the synthesis of silver nanoparticles.[24] Broadening of peak indicated fairness in the mono dispersion of particles.[24]It was observed that the silver nanoparticles of silver were stable for 6 month and onward with no aggregation.

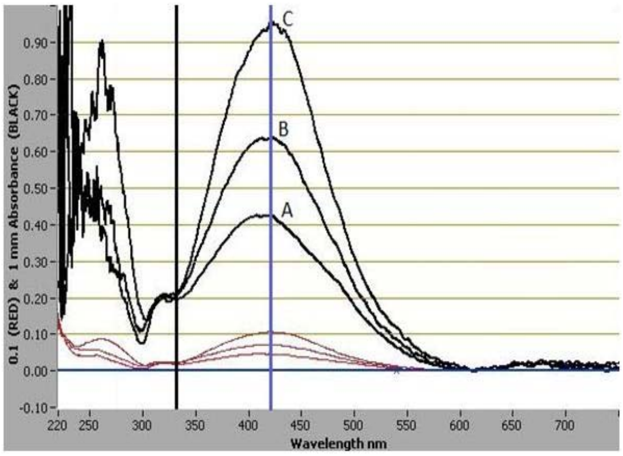


Figure 2: UV-Vis spectra recorded at various times (A: 24 hours, B: 48hours, C: 72 hours resp) after the start of the reaction of 300 ml of 2 mM AgNO3 solution with 100 ml of *A. nigar* extract (aqueous).

XRD Analysis of Ag nanoparticles

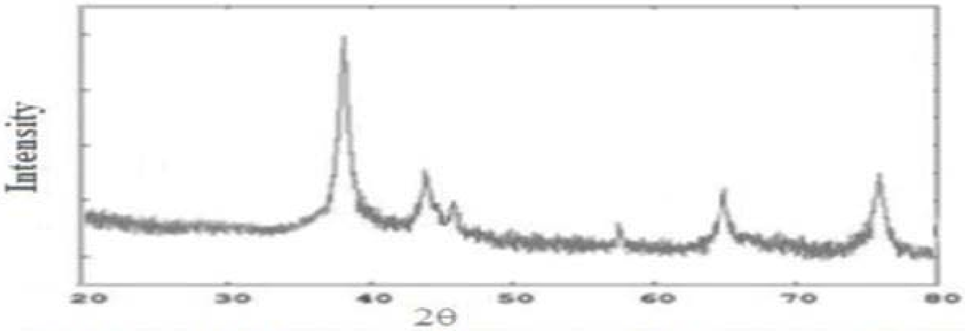
Biosynthesized Silver nanoparticles by employing *A. nigar* Fungal Extract were further confirmed by the characteristic peaks observed in XRD spectra (Fig 3). The XRD pattern showed intense peaks in a whole spectrum of 2θ value ranging from 20 to 80. The intense peaks observed in the spectrum agree to the Braggs's reflection of Silver Nano crystals reported in literatures.[25-27] The typical XRD pattern (Fig 3) suggests that the prepared silver nanoparticles are biphasic (cubic and hexagonal structure) in nature.[28-29]

AFM imaging and analysis

Further insight in the morphology and size details of the silver nanoparticles was provided by AFM imaging of sample. A representative AFM image recorded from the silver nanoparticles is shown in Figure 4 A-B. The silver nanoparticles were nearly spherical in shape. All the nanoparticles are well separated and no agglomeration was noticed. The average size of silver nanoparticles has been obtained as 70 nm From the AFM micrograph.[30]

Effect of Silver nanoparticles on activity and behavior of mosquito larvae

The Data observed clearly indicates that the larval activity was severely reduced in dose and time dependent manner. Mortality was also increases with time of exposure and concentration of Nanosilver while no reduction in activity of larvae under control conditions was seen in any behavioral observation and mortality. Similarly no reduction in the activity of pupae was observed. Toxicity on all Groups was as shown. Table 1 shows data table of different behavioral observations prepared by all mathematical values of behavior obtained by larval group in stress condition provided by deferent concentration of Silver nanoparticles. Graphical plots in Figure 5 A-D show comparative graphical representation of alternation in behavior of larvae.



S.No.	2θ Value	Plane	Element	Phase
1	38.1	111	Ag	Cubic
2	44.4	200	Ag	Cubic
3	45.9	100	Ag	Hexagonal
4	57.7	103	Ag	Hexagonal
5	64.7	220	Ag	Cubic
6	77.8	311	Ag	Cubic

Figure 3: X-ray diffraction pattern of the silver nanoparticles. Silver nanoparticles were synthesized by reaction of 300 ml of 2 mM AgNO3 solution with 100 ml of aqueous extract of *A. nigar.*

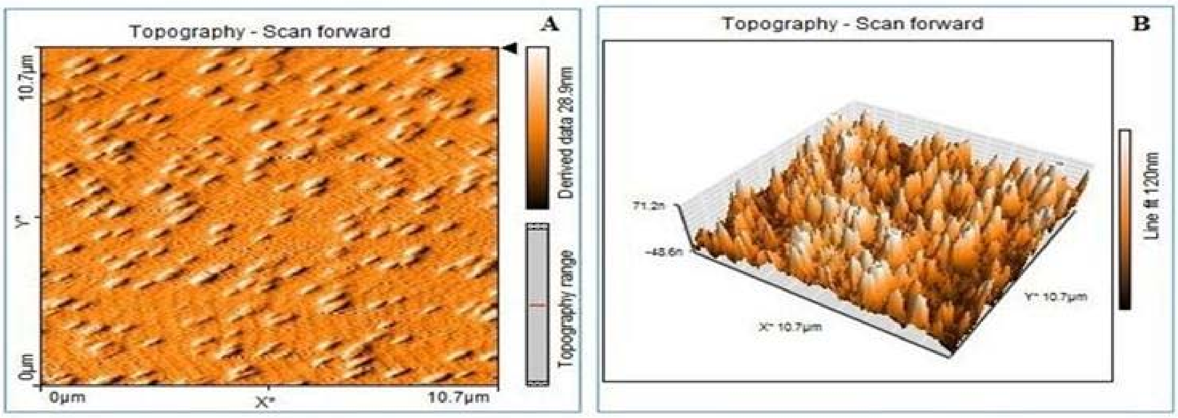


Figure 4: A. AFM inset image of as prepared silver nanoparticles; **B.** AFM topographical image of silver nanoparticles.

Control

All larvae seemed healthy and active for all time of observation (2 days). Change in activity of any larvae under control conditions in any behavioral observation was non-significant. Similarly, Mortality level also was null for larval system in control condition. No reduction in the activity of pupae was observed.

5 mg/L concentrative stress

On the concentration of 5 mg/L of silver nanoparticles, larvae show same pattern of change in all four behavioral activities with the reference of time. Larvae shows no serious alternation in behavior for half of observation time but High alterations in all activities took place in other half at this low stress condition.No serious lethal effects were seen within observation time. Pupae were observed with no reduction in any activity with null mortality. Overall low toxicity could be seen at this low concentration stress.

20mg/L concentrative stress

On this condition more significant alterations of decrement in all four behavioral activities were found with the reference of time but it did not cause significant level of mortality when compare to control. Pupa was normal as control.

35mg/L concentrative stress

As the other concentrations, larvae show same pattern of change in all four behavioral activities with the reference of time on this relatively higher concentrative stress. Fast decrement in behavioral activities was seemed with comparatively high level of mortality. No reduction in the activity of pupae was observed when compared to control.

50mg/L concentrative stress

A steep fall in the prepared graph of all activities could be seen at this high concentrative stress. This concentration showed more lethal effects from other groups which lead to death. Most of larva died very early of observation time. This high concentration showed highest degree of toxicity. As the other concentrative stress, Pupae were not affected.

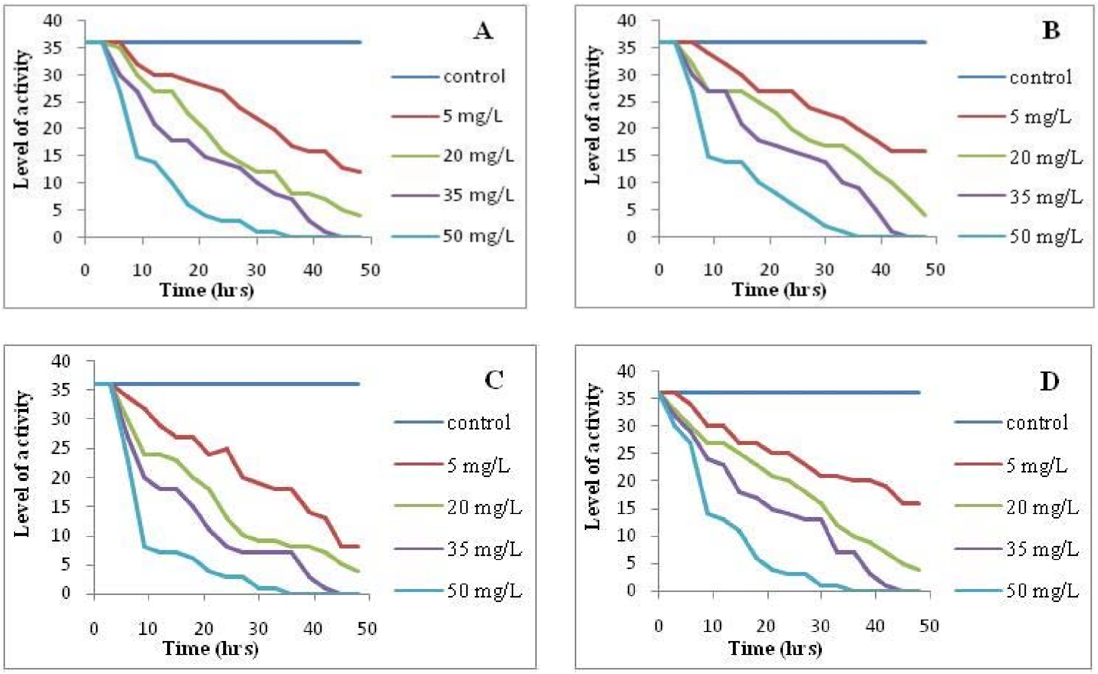


Figure 5: Dose and time dependent effect of biosynthetic silver nanoparticles on *Culex* sp. larval acivity and behavior **A.** light block alarm Response; **B.** disturbance alarm Response; **C.** feeding activity; **D.** overall movement.

Table 1: Prepared data table and mathematical representation of different behaviors under different concentrative stress.

Activity	Concentration of Ag Nanoparticles (mg/L)	Time (Hr.)																
		0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48
light block alarm Response	0 (control)	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36
	5	36	36	36	32	30	30	29	28	27	24	22	20	17	16	16	13	12
	20	36	36	35	30	27	27	23	20	16	14	12	12	8	8	7	5	4
	35	36	36	30	27	21	18	18	15	14	13	10	8	7	3	1	0	0
	50	36	36	27	15	14	10	6	4	3	3	1	1	0	0	0	0	0
disturbance alarm Response	0 (control)	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36
	5	36	36	36	34	32	30	27	27	27	24	23	22	20	18	16	16	16
	20	36	36	32	27	27	27	25	23	20	18	17	17	15	12	10	7	4
	35	36	36	30	27	27	21	18	17	16	15	14	10	9	5	1	0	0
	50	36	36	27	15	14	14	10	8	6	4	2	1	0	0	0	0	0
feeding activity	0 (control)	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36
	5	36	36	34	32	29	27	27	24	25	20	19	18	18	14	13	8	8
	20	36	36	30	24	24	23	20	18	13	10	9	9	8	8	7	5	4
	35	36	36	27	20	18	18	15	11	8	7	7	7	7	3	1	0	0
	50	36	36	23	8	7	7	6	4	3	3	1	1	0	0	0	0	0
overall movement	0 (control)	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36
	5	36	36	34	30	30	27	27	25	25	23	21	21	20	20	19	16	16
	20	36	33	30	27	27	25	23	21	20	18	16	12	10	9	7	5	4
	35	36	32	29	24	23	18	17	15	14	13	13	7	7	3	1	0	0
	50	36	30	27	14	13	11	6	4	3	3	1	1	0	0	0	0	0

Table 2: Quantitative data tables of total mortality

Time (hr)	Control	5 mg/L	20 mg/L	35 mg/L	50 mg/L
0	0	0	0	0	0
3	0	0	0	0	0
6	0	0	0	0	2
9	0	0	0	1	3
12	0	0	1	2	4
15	0	0	1	2	4
18	0	0	2	3	5
21	0	1	2	4	5
24	0	1	2	4	6
27	0	2	3	5	6
30	0	2	3	5	7
33	0	3	4	6	8
36	0	3	4	6	9
39	0	3	5	7	9
42	0	4	6	8	9
45	0	4	6	9	9

Effect of Silver nanoparticles on mortality of mosquito larvae

The mortality of larvae of groups (control, 5, 20, 35 and 50 mg/L concentrative stress groups) were continue increased on dose and time dependent manner. At the high concentrations (35 and 50 mg/L) of nanoparticles, lethal effects on larvae were observed. All larvae were dead in observation time on these high concentrative stresses. Larvae showed very less mortality on low concentrative stress (5 mg/L) where as at the concentration of 20 mg/L, mortality was higher than 5 mg/L but maximum of larvae remained live after observation time (Figure 6, Table 2). The low concentrations (5 and 20 mg/L) seem safe in the regard of mortality but serous negative effects on general activity and behavior could be seen.

The larvae did not die due to starvation caused by reduction in the microorganism population as a response to increase in the Nano Ag concentration in water, as it was previously observed that the larvae could remain alive under starvation for even 4 days. Also the larvae did not die due to changes in pH as the pH of the setup was observed to be between the optimal pH of larval growth i.e between 6.5 to 8.5 pH for all the time of observation.

As the pupa showed no reduction in the activity in any concentrative stress condition, we could conclude that the larvae did not die due to choking of spiracles by nanoparticles, rather the activity reduction in the larvae could be due to ingestion of nanoparticles, as the pupa is a non-feeding yet respiring stage. It was clearly observable that the mainly movement was severely obstructed hence we could hypothise that the muscular system was affected directly or indirectly because of nanoparticle ingestion hence the larvae were unable to keep themselves near the surface and died due to lack of oxygen.

The major reduction was on the bodily movements, mainly the thrashing/wriggling activity of larvae was lost early, other cognitive functions still remained intact until the movement was completely obstructed and larvae were unable to show any response.

Overall, at low concentrations around 5 mg/L only the activity of the larvae was reduced, but death could not occur even when kept in the same conditions regularly for 8 days. At higher concentrations (at and above 35 mg/L) the larvae died.

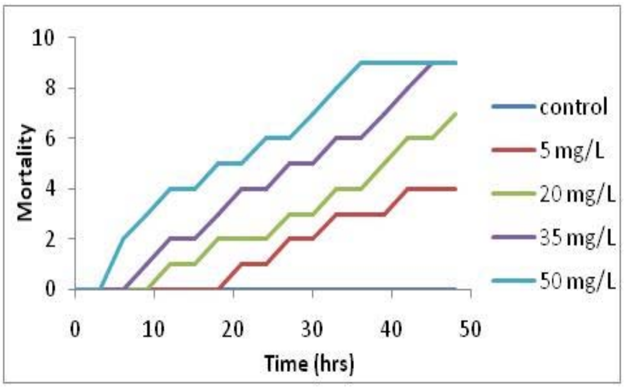


Figure 6: Total mortality of larvae on different concentrations of Silver nanoparticles.

CONCLUSION

Spherical Silver nanoparticles of 70 nm were biologically synthesized by aqueous extract of *A. nigar* fungi. Silver nanoparticles showed toxic effects on *Culex* mosquito larvae and its behavior in dose and time dependent manner.

Acknowledgements: The authors are very grateful to Head, Research and Development department, Nanobeach, for their support and help in this study.

REFERENCES

1. Hoet PHM, HohlfeldIB, Salata OV, Nanoparticles-known and unknown health risks, Journal of Nanobiotechnology, 2, 2004, 12.
2. Nanoscience and nanotechnologies- Opportunities and uncertainties, UK Royal Society and Royal Academy of Engineering: Final Report, 2004.
3. Schultz S, Smith DR, Mock JJ, Schultz DA, Single-target molecule detection with non-bleaching multicolor optical immunolabels, Proceedings of the National Academy of Sciences USA 1, 97(3), 2000, 996-1001.
4. Rai M, Yadav A, Gade A, Silver nanoparticles as a new generation of antimicrobials, Biotechnology Advances, 27(1), 2009, 76-83.
5. Elechiguerra JL, Burt JL, Morones JR, Bragado AC, Gao X, Lara HH, Yacaman MJ, Interaction of silver nanoparticles with HIV-1, Journal of Nanobiotechnology, 29, 2005, 3-6.
6. Lansdown AB, Silver in health care: antimicrobial effects and safety in use, Current Problems in Dermatology, 33, 2006, 17-34.
7. Gittins DI, Bethell D, Nichols RJ, Schiffrin DJ, A nanometre-scale electronic switch consisting of a metal cluster and redox-addressable groups, Nature, 408(6808), 2000, 67-69.
8. Hunt PR, Marquis BJ, Tyner KM, Conklin S, Olejnik N, Nelson BC, Sprando RL, Nanosilver suppresses growth and induces oxidative damage to DNA in Caenorhabditiselegans, Journal of Applied Toxicology, 2013 May 2. doi: 10.1002/jat.2872. (Epub ahead of print).
9. AshaRani PV, Low G, Mun K, Hande MP, Valiyaveettil S, Cytotoxicity and genotoxicity of silver nanoparticles in human cells, American Chemical Society, 3(2) 2009, 279–290.
10. Shaluei F, Hedayati A, Jahanbakhshi A, Kolangi H, Fotovat M, Effect of subacute exposure to silver nanoparticle on some hematological and plasma biochemical indices in silver carp (Hypophthalmichthys-molitrix), Human & experimental toxicology, 2013 Apr 30. (Epub ahead of print.)
11. Elumalai EK, Prasad TNVKV, Nagajyothi PC, David E, A bird's eye view on Biogenic Silver nanoparticles and their applications, Der ChemicaSinica, 2(2), 2011, 88-97.
12. Lok C, Ho C, Chen R, He Q, Yu W, Sun H, Tam PK, Chiu J, Che C, Silver nanoparticles - partial oxidation and antibacterial activities, Journal of Biological Inorganic Chemistry, 12(4), 2007, 527-34.
13. Sondi I and Sondi B S, Silver nanoparticles as antimicrobial agent- a case study on *E. coli* as a model for Gram-negative bacteria, Journal of Colloid Interface Science, 275, 2004, 177.
14. Yeo MK, Kang M, Bull, Effects of nanometer sized silver materials on biological toxicity during zebra-fish embryogenesis, Korean Chemical Society, 29(6), 2008, 1179-1184.
15. Miron G, Walters L J, Tremblay R, Physiological condition and barnacle larval behavior- a preliminary look at the relationship between TAG/DNA ratio and larval substratum exploration in *Balanusamphitrite*, Marine Ecology Progress Series, 198, 2000, 303-310.
16. Roth AR, Yates WW, Lindquist AW, Preliminary studies of larvicides on snow-water mosquitoes, Mosquito news, 7(4), 1947, 154-156.
17. Clark TM, Flis BJ, Remold SK, Differences in the effects of salinity on larval growth and developmental programs of a freshwater and a euryhaline mosquito species (Insecta-Diptera, Culicidae), The Journal of Experimental Biology, 207, 2004, 2297-2304.
18. Durán N, Marcato PD, Alves OL, Gabriel IH, Souza DE, Esposito, Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusariumoxysporum* strains, E-Journal of Nano biotechnology, 3, 2005, 1-7.
19. Saifuddin N, Wong CW, NurYasumira AA, Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation, E-Journal of Chemistry, 6(1), 2009, 61-70.
20. Parashar V, Parashar R, Sharma B, Pandey AC, *Parthenium* Leaf Extract Mediated Synthesis of Silver Nanoparticles: A Novel Approach Towards Weed Utilization, Digest Journal of Nanomaterials and Biostructures, 4 (1), 2009, 45 – 50.
21. Elavazhagan T, Arunachalam KD, Memecylonedule leaf extract mediated green synthesis of silver and gold nanoparticles, International Journal of Nanomedicine, 6, 2011, 1265–1278.
22. Sharma VK, Yngard RA, Lin Y, Silver nanoparticles: Green synthesis and their antimicrobial activities, Advances in Colloid and Interface Science, 145, 2009, 83–96.
23. Wiley BJ, Im SH, Li ZY, McLellan J, Siekkinen A, Xia Y,Maneuvering the surface plasmon resonance of silver nanostructures through shape-controlled synthesis, The Journal of Physical Chemistry B, 110(32), 2006, 15666-75.
24. Jain D, Daima HK, Kachhwaha S, Kothari SL, Synthesis of plant-mediated Silver nanoparticles using Papaya fruit extract and evaluation of their antimicrobial properties, Digest Journal of Nanomaterials and Biostructures, 4(3), 2009, 723 – 727.
25. Shankar SS, Rai A, Ahmad A, Sastry M, Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (Azadirachtaindica) leaf broth, Journal of Colloid and Interface Science, 275, 2004, 496–502.
26. Lu H W, Liu S H, Wang X F, Qian J, Yin J, Jhu JK, Silver Nanocrystals by hyperbranched polyurethane-assisted Photochemical reduction of Ag^{+}, Materials Chemistry and Physics, 81, 2003, 104.
27. Jain D, Kachhwaha S, Jain R, Srivastava G, Kothari SL, Novel microbial route to synthesize silver nanoparticles using spore crystal mixture of Bacillus thuringiensis, Indian Journal of Experimental Biology, 48, 2010, 1152-1156.
28. Singh A, Jain D, Upadhyay MK, Khandelwal N, Verma H, Green Synthesis of Silver Nanoparticles Using Argemone Mexicana Leaf Extract And Evaluation Of Their Antimicrobial Activities, Digest Journal of Nanomaterials and Biostructures, 5(2), 2010, 483-489.
29. Govindaraju K, Tamilselvan S, Kiruthiga V, Singaravelu G, Biogenic silver nanoparticles by Solanumtorvum and their promising antimicrobial activity, Journal of Biopesticides, 3(1), 2010, 394 - 399.
30. Raman N, Sudharsan S, Veerakumar V, Pravin N, Vithiya K, Pithecellobiumdulce mediated extra-cellular green synthesis of larvicidal silver nanoparticles, SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy, 96, 2012, 1031-7.

Source of Support: Nil, **Conflict of Interest:** None.