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Application of biosynthesized silver nanoparticles as a novel vector control agent

Anima Nanda*, M. Lakshmipathy, Devan Elumalai[$], P. K. Kaleena[$] and B. K. Nayak[@]

Faculty of Bio & Chemical Engineering, Sathyabama University, Chennai, India
[$]Department of Zoology, Presidency College, Chennai, Tamil Nadu, India
[@]Department of Botany, KM Centre for PG Studies (Autonomous), Pondicherry, India

ABSTRACT

Microbe mediated synthesis of silver nanoparticles (AgNPs) constitutes a reliable, eco-friendly approach ameliorating green-chemistry principles. In this study, stable silver nanoparticles were synthesized by exposing aqueous silver ions to extracellular exudates of B. subtilis A1 under optimized laboratory conditions. The synthesized nanoparticles were characterized by spectroscopic and microscopic techniques. The plasmon resonance and diffraction patterns revealed the typical characteristics of silver with an average size of ~35.42nm and spherical in shape. Larvicidal assay performed using AgNPs showed significant results toward fourth instars of Anopheles stephensi and Aedes aegypti larvae. The results suggest that the B. subtilis A1 mediated synthesis of AgNPs possess excellent antimicrobial and larvicidal properties that may represent promising bio-control agent with improved biomedical applications.

Key words: *B. subtilis*; Silver nanoparticles; antimicrobial, *Anopheles stephensi, Aedes aegypti,* larvicidal activity**.**

INTRODUCTION

Nanotechnology and its applications have attracted a great interest among researchers, medicos and engineers to explore and re-design nanomaterials for a potential human and ecosystem exposure [1]. The unique chemical, optical, catalytic, electrical and magnetic properties of the nanomaterials have extended its application in medical and clinical field, a major breakthrough in bio-materials research [2]. Among panoptic range of nanomaterials, silver is widely exploited for its broad spectrum bactericidal and fungicidal activity [3]. It is noteworthy to mention that nanosilver based technologies to date have the highest degree of commercialization. This increase in nanosilver utilization for developing commercial products has emphasized industry and scientific community in parallel to study on syntheses [4] and properties for enhanced environmental applications [5].

In recent years there are increasing numbers of infectious diseases outbreaks caused by unusual strains of bacteria, fungi and viruses have emerged as a global threat. According to Centers for Disease Control and Prevention threat report 2013 nearly 2 million people get infected with drug resistant strains of bacteria with a death toll of 23,000 annually in the US. The growing prevalence and incidence of multidrug resistance related infections stems from irrational use of antibiotics within human and veterinary medicine [6,7]. As a result of increase in selective pressure the resistant bacteria survive and the susceptible one ceases to death. To prevent further spread of infectious bacterial strains, appropriate disinfection methods need to be followed despite a push for new antimicrobials [8].

Nanobiotechnology provides an extensive insight on transforming silver into silver nanoparticles with enhanced biocidal action than its bulk counterpart [9]. However, the mechanisms by which the silver nanoparticles paralyze the cell remain hypothetical, wherein this antimicrobial potential would lead to the development of a novel nanobioformulation surmounting drug resistance. The resistance pattern was not only observed in micro organisms but also evidenced in vector borne diseases caused by *Anopheles, Aedes* and *Culex* genera. A strong directional selection pressure that builds upon intensive use of insecticide accounts for its ineffectiveness [10]. Moreover, the application of synthetic insecticides such as organochlorine and organophosphate to control proliferation of vectors was found cumbersome due to technical, operational, ecological and economic factors. These consequences have resulted in an urge to hunt for developing cheap, eco-friendly, biodegradable and effective bio-control agent using nanotechnologies albeit most of the biological control methods [11]. Despite, the use of nanosilver in health-related fields, emphasis on the future prospect of silver nanotechnologies applications toward ecological considerations remains to be unexploited. This paper aims to study bacterial mediated synthesis of silver nanoparticles and its toxicological assessment toward fourth instar larva of malarial and dengue vectors.

MATERIALS AND METHODS

Silver nitrate ($AgNO_3$, 99.9% pure, AR grade) was purchased from Qualigens, India. Nutrient Agar (NA) and Luria Bertani (LB) medium were supplied by HiMedia, India. Disposable 200mL cups were purchased from local supplier.

Synthesis and Characterization

Silver nanoparticles were synthesized using an environmental isolate, *Bacillus subtilis* A1 extract under optimized laboratory conditions and characterized by UV-visible spectroscopy, X-ray diffraction, field emission scanning electron microscopy as reported in our previous study [12].

Larvicidal bioassays

The preliminary screening of *Anopheles* and *Aedes* larvae was performed using the samples collected from roadside ditches, paddy fields, stagnant water around Tiruvallur, Tamilnadu. They were identified morphologically by using Atlas of Mosquito manual and further confirmed in Zonal Entomological Research Centre, Vellore, Tamilnadu. The identified *Anopheles* sp. and *Aedes* sp. mosquito larvae were maintained and reared in the laboratory.

The larvicidal potency of crude extract was evaluated using the standard protocol recommended by WHO (1996) [13] with slight modification and as per the method described by Kamaraj *et al*., [14]. 4.9mL of sterile water containing 20 fourth instar larvae of *Aedes aegypti* and *Anopheles stephensi* were added to beakers containing 195mL of sterile water and 100µL of sample in desired concentration (0.1–0.02 gL^{-1}). The tests of each sample concentration were repeated at least 5 times. The tap water was used as a negative control. The number of dead larva was counted after 24h and the percentage of mortality was assessed to determine the acute toxicity on fourth instar larvae of *Anopheles* and *Aedes* sp.

Dose-dependent assay

Based on the preliminary screening, synthesized AgNPs were subjected to dose-dependent toxicity test by placing 20 mosquito larva (*Anopheles* and *Aedes* sp.) in 195mL of sterile double distilled water and 100µL of AgNPs at desired concentration 0.01–0.002gL^{-1}). The tests of each sample concentration were repeated at least 5 times along with control. The experiment was performed on larva under starvation condition.

Statistical analysis

The data on the larvicidal potency were subjected to probit analysis (SPSS ver. 16.0) for calculating LC_{50}, LC_{90} and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and r^2 values calculated [15]. Results with $p<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Screening of mosquito larva

In a systematic screening of mosquito larva, *Anopheles* larva rest parallel to and immediately below the surface of water. The presence of very short siphon with a rudimentary breathing tube was found characteristic of *Anopheles stephensi*. On the other hand, *Aedes* larvae typically hang on water surface obliquely. The presence of short and thick siphon and a pair of hair tufts showed the characteristic feature of *Aedes aegypti*.

Larvicidal Bioassay

The silver nanoparticles synthesized were tested toward drug resistant clinical isolates of bacteria has yielded significant results. In continuation with the previous study larvicidal bioassay was performed to check vectors causing diseases like malaria and dengue. *B. subtilis* A1 mediated synthesis of AgNPs was found effective against the fourth instar larvae of *Anopheles stephensi* and *Aedes aegypti*, vectors of Malaria and Dengue fever on par with the crude extract. The crude extract of A1 showed moderate larvicidal effects toward fourth instar larvae of *Anopheles stephensi* (LC_{50} 51.72 mg/L, LC_{90} 153.85 mg/L and r^2 = 0.996) and *Aedes aegypti* (LC_{50} 57.07 mg/L, LC_{90} 171.12 mg/L and r^2 = 0.978) respectively. Whereas significant larvicidal activity was observed from the AgNPs synthesized using *B. subtilis* A1 extract towards fourth instar *Anopheles stephensi* larvae (LC_{50} 4.13 mg/L, LC_{90} 12.89 mg/L and r^2 = 0.955) and *Aedes aegypti* (LC_{50} 4.27 mg/L, LC_{90} 14.11 mg/L and r^2 = 0.969) (Table 1).

Table 1: Larvicidal activity of crude extract of *B. subtilis* A1 and synthesized AgNPs against fourth instar larvae of *Aedes aegypti* and *Anopheles stephensi*

Extract	Species	Concentration (mg/L)	% Mortality* ± SD	LC_{50} (LCL– UCL) (mg/L)	LC_{90} (LCL– UCL) (mg/L)	r^2
Crude aqueous extract	*Aedes aegypti*	100	74±1.09	57.07 (49.5– 65.7)	171.12 (159.6-194.3)	0.978
		80	65±2.04			
		60	44±1.12			
		40	38±1.20			
		20	21±1.41			
	Anopheles stephensi	100	80±1.12	51.72 (44.3– 60.3)	153.85 (139.2-172.6)	0.996
		80	67±1.04			
		60	50±3.01			
		40	39±2.45			
		20	26±1.56			
Synthesized AgNPs	*Aedes aegypti*	10	100±0.00	4.27 (3.6– 5.3)	14.11 (11.2-17.0)	0.969
		8	72±4.23			
		6	59±2.03			
		4	47±0.82			
		2	32±2.55			
	Anopheles stephensi	10	100±0.00	4.13 (3.22– 4.9)	12.89 (10.89-14.3)	0.955
		8	81±3.21			
		6	72±4.23			
		4	39±0.72			
		2	28±2.30			

*Control – Nil mortality; LC_{50} & LC_{90}: lethal concentration that kills 50% and 90% of the exposed larvae, LCL: lower confidence limit, UCL: upper confidence limit, *Mean value of five replicates*

Several research studies on larvicidal potential have been investigated using phytochemicals [16] and silver nanoparticles toward fourth instars of *Anopheles*, *Aedes*, *Culex* and *Rhipicephalus* larva [17]. Mouchet *et al*., [18] reported that a high mortality (85%) was noted at the highest double walled carbon nanotubes concentration against the larvae of *Xenopus laevis.* However, the higher mortality rates at lower doses observed in the current study are comparable with earlier reports [19]. *B. subtilis* A1 mediated synthesis of AgNPs in the present study showed LC_{50} < 5mg/L which would be a crucial element in formulating a practical larvicidal dose.

The larvicidal potential brought about by nano-silver could follow the same mechanism of action as observed in bacteria. However, it has been proposed that nanoparticle mediated toxicity induces the formation of reaction oxygen species (ROS), lipid peroxidation and reduced Glutathione (GSH) [20].

As far the environmental issues are concerned, the break down products of the chemical pesticides also has detrimental effects over the edaphic microflora. Malaoxon, one of the highly toxic transformed products of organophosphate malathion is capable of inducing mutations and chromatid breaks in unicellular and multicellular life forms. Temephos, another organophosphate insecticide inhibits the action of group of enzymes called cholinesterases. These enzymes play most important role in controlling signal transmission between the nervous system and the musculoskeletal systems. In general the organophosphate insecticides are readily absorbed through the lungs, skin and digestive tract [21]. It would be beneficial to develop safe ways to control the vector population by reducing the widespread distribution of synthetic chemicals having negative impact on the environment.

The results concluded that the toxicity of AgNPs influences viability, metabolic activity, and oxidative stress. Further, the combinatorial activity of extract and AgNPs might focus on improved biomedical applications with environmental considerations to have a vigil over emerging pathogenic strains of bacteria and as a vector control strategy.

CONCLUSION

We demonstrated the synthesis of silver nanoparticles using a rhizosphere inhabiting, stress tolerant *B. subtilis* A1 and its antimicrobial and larvicidal potential investigated. The biogenic silver nanoparticles showed significant larvicidal activity toward *Anopheles stephensi* and *Aedes aegypti*. This novel bio-control approach toward the containment of emerging resistant strains of micro organisms and mosquito larva could keep the chemical biocides at bay with non-target population unaltered.

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