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UV Irradiation-induced Silver Nanoparticles as Mosquito Larvicides

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Abstract: Silver nanoparticles have a great potential for use in biological control including antimicrobial activity. The pest control of mosquito *Aedes aegypti* by means of larvicidal is still necessity in order to diminish the vector of some life-threaten diseases. In this study, polymethacrylate (PMA)-stabilized silver nanoparticles were synthesized by UV irradiation, characterized by Surface Plasmon Resonance (SPR), Transmission Electron Microscopy (TEM) and zeta potential measurement and evaluated for their larvicidal activity toward *A. aegypti* larvae. Through the processes of characterization and larvicidal assay, silver nanoparticles were spherical and in nanoscale size (= 10 nm). The larvicidal activity of silver nanoparticles was concentration-dependent and supposed to arise from the penetration of the nanoparticles into the larval membrane. The PMA-capped silver nanoparticles at a concentration of 5 ppm exhibited less than 10% survival of larvae within 3-h exposure time. The study suggests that the silver nanoparticles synthesized by UV-irradiation can be employed in biocontrol of pest including mosquito larvae.

Key words: *Aedes aegypti*, irradiation, larvicide, polymethacrylate, silver nanoparticles

INTRODUCTION

From the current situation of global warming and contaminated fresh water pools, a number of mosquitoes are markedly increasing in concurrence with a high incidence of dengue fever (Halstead, 2007). A decrease in dengue vector breeding by using larvicides against vector *Aedes aegypti* (Culicidae) is a strategy to control this pandemic life-threaten disease. The common mosquito larvicides nowadays include an organophosphate temephos, methoprene and soil bacterium, *Bacillus thuringiensis israelensis* and *B. sphaericus*. However, the high amount of chemical larvicides could lead to long term residual effect to the environment while the use of bacterial protein is in high cost and requires a complex purification process (Aiub *et al*., 2002; Pinheiro and Tadei, 2002). More importantly, the incidences of resistance to larvicides of mosquito larvae have been reported (Braga *et al*., 2004; Melo-Santos *et al*., 2010). Thus, attempts to develop novel materials as mosquito larvicides are still necessary.

Silver nanoparticles are emerging as one of the fastest growing materials due to their unique physical and chemical properties, small size and high specific surface area. They can be employed in material and engineering such as microelectronic (Zhang *et al*., 2005), catalyst (Wang *et al*., 2005), biosensor (Frederix *et al*., 2003) and in biomedicine especially for antibacterial agent (Lok *et al*., 2006; Panáček *et al*., 2006; Kim *et al*., 2007; Shrivastava *et al*., 2007; Choi *et al*., 2008; Guzmán *et al*., 2008; Rai *et al*., 2009) and antiviral agent (Elechiguerra *et al*., 2005). Silver nanoparticles can be synthesized by using chemicals such as sodium borohydride for reduction of divalent silver atom to zerovalent silver atom (Solomon *et al*., 2007). Recently, an alternative of green chemistry to silver synthesis becomes

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more attractive since the remediation of environmental toxic waste is unnecessary. The silver nanoparticles capped with polymethacrylic acid (PMA) and produced by UV irradiation was previously synthesized (Dubas and Pimpan, 2008; Spadaro *et al*., 2009) and used for sensing application (Dubas and Pimpan, 2008). The biocompatibility and harmlessness of the PMA polymer make it appropriate to be employed in biomedical application (Li and Lee, 2009). Accordingly, the use of PMA-capped silver nanoparticles synthesized by photoreduction for larvicidal activity towards *A. aegypti* is our objective for this study.

MATERIALS AND METHODS

Chemicals: Polymethacrylic acid (PMA) was purchased from Sigma-Aldrich (USA). Silver nitrate ($AgNO_3$) and temephos (*O,O,O′,O′*-tetramethyl-*O,O′*-thiodi-*p*-phenylene diphosphorothioate) were supplied from Carlo Erba, Italy and Chemfleet, Thailand, in orderly. The acetic-acetate buffer (pH = 4.75) was prepared by mixing equal volumes of 10 mM acetic acid (Sigma-Aldrich, USA) and 10 mM sodium acetate (Riedel-de Haen AG, Switzerland) solutions.

Synthesis of silver nanoparticles: The UV irradiation-induced silver nanoparticles were synthesized by previously described method (Dubas and Pimpan, 2008). The 10 mM PMA in 10 mM acetic-acetate buffer solution at a volume of 25 mL was added to another 25 mL of 10 mM $AgNO_3$ in 10 mM acetic-acetate buffer solution. The mixture was stirred at room temperature for 5 min before exposed to low intensity (8 W) UV lamp for approximately 90 min. The solution of synthesized silver nanoparticles appeared in deep purple color and contained 540 ppm of Ag atom.

Characterization of silver nanoparticles: The nanoparticles were characterized for surface plasmon resonance (SPR) by UV-Visible spectroscopy (Specord S100, Analytikjena, Germany). The morphology and particle size of nanoparticles were visualized by Transmission Electron Microscopy (TEM) (H-7650, Hitachi, Japan). The surface charges of the particles were measured at 25°C using a zetasizer apparatus (NanoZS, Malvern, UK).

Larvicidal activities of silver nanoparticles

Culture of mosquito larvae: The larval culture was done on *A. aegypti* eggs obtained from Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. To start the colony the larvae were hatched and reared in plastic cups containing dechlorinated tap water at room temperature. Mosquito larvae were fed with powdered nutrient broth once a day. After 4 days the hatched larvae turned into larvae in early fourth larval stage and were subjected to be employed in further experiment.

Larvicidal test: Mosquito larvae were placed in 8 batches of 20 larvae each in plastic cups containing 100 mL of the test solutions which were dilute PMA-capped silver nanoparticles at various concentrations, 0.01, 0.1, 1 and 5 ppm Ag atom. The solutions of 1 ppm $AgNO_3$ and 1 ppm PMA were also studied for comparison. The tap water was used as a negative control (0% mortality of larvae) while the positive control (100% mortality of larvae) was the commercial larvicide, 1 ppm temephos solution. A number of dead larvae in each batch were counted every hour for 24 h exposure period. After soaked with ethanol, xylene and permount agent for each 15 min, the treated larva was mounted on a slide and examined under a microscope (Meiji EMZ-TR, Japan) for image capture on a DS-Fi1 digital camera (Nikon, Japan). Moreover, the effect of silver nanoparticles on the hatchability of *A. agypti* eggs was determined by hatching a hundred of eggs in plastic trays containing either water or 5 ppm silver nanoparticles and after 2 days, the newborn larvae were observed.

RESULTS

Synthesis and characterization of silver nanoparticles: The PMA-capped silver nanoparticles synthesized by photoreduction were in purple color with a corresponding absorption peak at 525 nm (Fig. 1a). The TEM image of silver nanoparticles illustrated the uniform distribution of spherical particles with approximately 5-10 nm in diameter (Fig. 1b). The zeta potential of -27.7±3.07 mV represented the negative charges of PMA stabilizer surround the particles.

Larvicidal bioassay: The effectiveness of silver nanoparticles as mosquito larvicides was determined from a number of live larvae with exposure time periods (Fig. 2). From the result, PMA-capped silver nanoparticles at concentrations of 0.01 and 0.1 ppm caused no death of larvae. The nanoparticles at 1 ppm slightly decreased the survival of larvae to 88±11% after 24 h exposure while more than 90% of mortality was observed in larvae after in contact with 5 ppm silver nanoparticles for 3 h. The solution of 1 ppm silver nitrate gradually killed the larvae over the experimental period and approximately less than 10% survival was found after 17 h. In contrast, the PMA

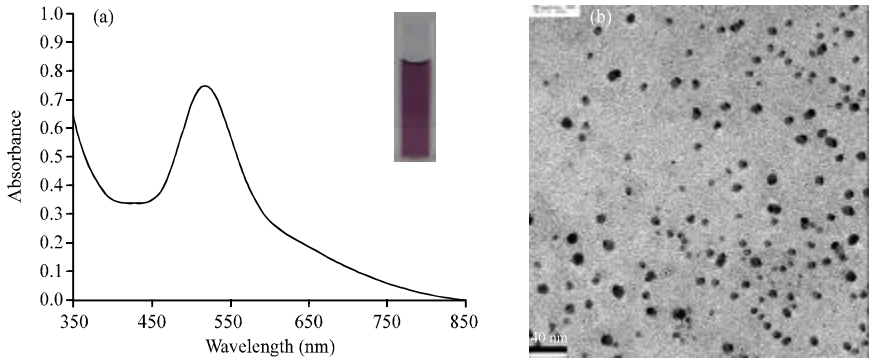

Fig. 1: (a) The appearance and UV spectrum and (b) the TEM image of PMA-capped silver nanoparticles

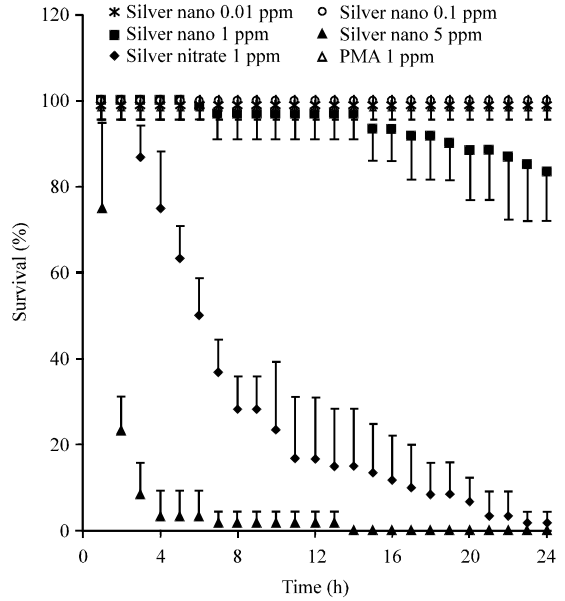

Fig. 2: The survival percentage of mosquito larvae after exposure to different concentrations of silver nanoparticles (silver nano), 1 ppm silver nitrate and 1 ppm PMA solutions (Mean±SD, n = 20)

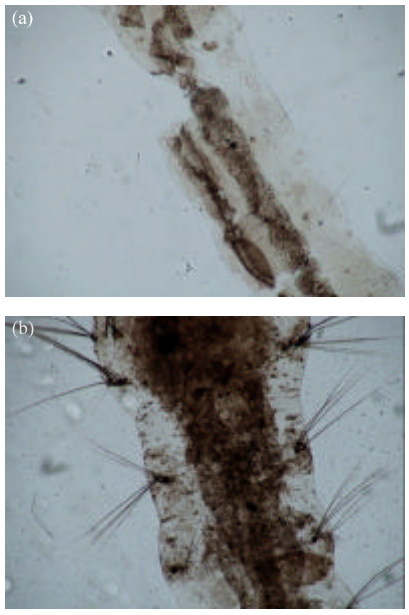

Fig. 3: The 40X magnificent image of abdomen part of *Aedes aegypti* larva (a) after untreated and (b) treated with 5 ppm silver nanoparticles

solution at the same concentration as silver nitrate was harmless to larvae. The dose of household use of 1 ppm temephos solution (positive control) could completely eradicate all the larvae within 3 h. The 40X magnification photos of the abdomen part of the dead larva were viewed under microscope as shown in Fig. 3a and b. The morphology of nanoparticle-treated larva was almost the same with untreated (water) larva except for some dark spots appeared throughout the larval body. Moreover, the spots were not observed in the larval body when exposed to silver nitrate or temephos solutions (data not shown). For hatchability assessment, it was found that there were a number of mosquito eggs hatched in solution of silver nanoparticles, however the newborn larvae were killed immediately after exposed to the nanoparticles.

DISCUSSION

Since silver nanoparticles are considered to be potential for biological applications including antibacterial agent with no bacterial resistant effect, we intend to evaluate whether they could apply for mosquito larvicidal agent. The nanoparticle synthesis is important as the instability or aggregation of nanoparticles would lead to a decrease in their biological activities (Kvítek *et al*., 2008). In contrast to a conventional method for synthesis

of silver nanoparticles in which many reagents are required, the solely use of UV irradiation can reduce the silver ion for nucleation of nanoparticle formation and promote the cross-linking of the PMA polymer (Spadaro *et al*., 2009). The optical properties of purple color and SPR band at 525 nm of UV-induced silver nanoparticles gained from this study differed from those of silver nanoparticles prepared using chemical reduction method which were yellow with SPR band around 400-420 nm (Solomon *et al*., 2007). The findings were not unexpected since the excess PMA stabilizer was able to form Ag-PMA coordination reaction leading to dielectric change of the medium surround the particles and a red shift in absorbance (Dubas and Pimpan, 2008; Spadaro *et al*., 2009). The estimated size (5-10 nm) of silver nanoparticles from TEM study was similar to the size of 9.2 nm, obtained from a relationship between the full-width of half-maximum of the absorption peak (FWHM, in nm) and the particle diameter (D, in nm), which is FWHM = 50 + (230/D) (Choi *et al*., 2008).

For the larvicidal bioassay, the dose-dependent effect of silver nanoparticles on the mortality of the larvae was seen. The silver nanoparticles at concentrations of 0.01, 0.1 and 1 ppm were unlikely to act as larvicidal agent toward *A. aegypti*. The anionic PMA stabilizer was previously reported to be safe (Li and Lee, 2009) which was in agreement with our study in that none of the larvae were killed by the PMA solution. The larvicidal effect of PMA-capped silver nanoparticles was more obvious at higher dose (5 ppm) than that of silver nitrate solution (1 ppm). The similar finding was observed for antimicrobial activity in that silver nitrate had a lower inhibitory concentration than that of silver nanoparticles (Choi *et al*., 2008; Kvítek *et al*., 2008).

For PMA-capped nanoparticles, it seemed not the case that nanoparticles markedly interacted with the biological membrane which is negatively charged. The antibacterial activity of silver nanoparticles previously reported was possibly due to a high surface area of the nanoparticles especially those in a range of 10-20 nm which provided a better contact between nanoparticles and cell (Sondi and Salopek-Sondi, 2004; Panáček *et al*., 2006). According to the average of 5-10 nm PMA-capped silver nanoparticles, we then speculate that the mechanism which causes the death of larva is the ability of the nanoparticles to penetrate through the larval membrane. The dark spots inside the larval body as seen from a microscope could be presumed to be the silver nanoparticles that agglomerated to form the clusters since they were not found in the larvae untreated with nanoparticles.

The silver nanoparticles in the intracellular space can bind to sulfur-containing proteins or phosphorous containing compound like DNA, leading to the denaturation of some organelles and enzymes (Sondi and Salopek-Sondi, 2004; Choi *et al*., 2008; Rai *et al*., 2009). Thereafter, the dropping of membrane permeability and disturbance in proton motive force required for ATP construction are induced which cause the lost of cellular function and finally cell death (Lok *et al*., 2006). Although, 1 ppm temephos solution exhibited the similar effect to 5 ppm silver nanoparticles in which it could get rid of all the mosquito larvae within 3 h, its mechanism of action is different. The temephos is an organophosphate pesticide which inhibits the hydrolysis of acetylcholine in neuron. The accumulation of acetylcholine in neuromuscular synapse causes the death of larvae by depression of respiratory system (Aiub *et al*., 2002; Braga *et al*., 2004).

For silver ion ($AgNO_3$), the larvicidal mechanism is proposed to be the same as the nanoparticles but more focus on penetration promoted by the attraction of positive silver ion and the cell membrane (Choi *et al*., 2008; Kvítek *et al*., 2008). In spite of more toxicity to living cells of silver ion than silver nanoparticles, the drawback that limits the use of silver ion is the cause of argyria (Fung and Bowen, 1996). When used as antibiotics, few incidences of bacterial resistance to silver nitrate were seldom reported while none were found for silver nanoparticles (Kvítek *et al*., 2008). Additionally, silver nanoparticles had no inhibitory effect on hatchability of mosquito eggs of *A. aegypti*. It might arise from the fact that the mosquito egg shell contains chitinized serosal cuticle which can resist penetration of silver nanoparticles (Rezende *et al*., 2008).

CONCLUSION

Silver nanoparticles could be environmental friendly synthesized by UV irradiation method. The PMA-capped silver nanoparticles showed the potential as an alternative to organophosphate larvicidal agent for *A. aegypti*. An ability to penetrate through the living organism of silver nanoparticles leading to loss of cell function is considered as a mechanistic action for death of larvae. The advantageous point of silver nanoparticles as larvicides is that the drug resistance due to the overuse of pesticides can be overcome.

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