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Mosquitocidal potential of silver nanoparticles synthesized using local isolates of *Bacillus thuringiensis* subsp. *israelensis* and their synergistic effect with a commercial strain of *B. thuringiensis* subsp. *israelensis*

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ABSTRACT

Control of larval stages of *Aedes aegypti* is considered an effective approach for preventing outbreaks of dengue fever. In this work, silver nanoparticles (Ag NPs) were synthesized using the supernatant and insecticidal proteins from local isolates of *Bacillus thuringiensis* subsp. *israelensis* (Bti). Mosquitocidal activity assays against *A. aegypti* larvae revealed that the highest toxicity was obtained from the Ag NPs synthesized using supernatant of Bti K55 and the inclusion proteins of Bti K46 with a lethal concentration 50 (LC_{50}) of 0.001 and 0.008 µg/mL, respectively. The synthesized nanoparticles were characterized using UV-vis absorption spectrophotometry, scanning electron microscopy (SEM), SEM coupled with energy dispersive X-ray spectroscopy, X-ray diffraction and Fourier-transform infrared spectroscopy. The synergistic studies revealed that the Ag NPs synthesized using supernatant of Bti K55 were synergized with commercial Bti cells with a synergistic factor (SF) of 3.3 and 10.0 for LC_{50} and LC_{90}, respectively. In addition, the Ag NPs synthesized using inclusion proteins of Bti K46 were synergized with commercial Bti cells with a SF of 1.6 and 4.2 for LC_{50} and LC_{90}, respectively. This study provided the first report of the synergistic effect between Bti and Ag NPs. Such a combination could represent an effective approach for the control of the dengue vector and possibly reducing the likelihood of increased insect resistance to chemical control.

1. Introduction

Dengue is the most widespread vector-borne disease in the world that is primarily transmitted between people by the *Aedes aegypti* mosquito (Frentiu et al., 2014). The global burden of dengue outbreaks is large with a report estimating that there are approximately 390 million infections annually (Bhatt et al., 2013). The dengue vector, *A. aegypti,* is widely distributed across tropical and subtropical areas covering approximately 100 countries (Simmons et al., 2012). To date, the Dengvaxia vaccine developed by Sanofi Pasteur has been licensed in 11 countries as an initial stage of immunization (Aguiar et al., 2016). However, supportive care, especially in severe patients, is currently an effective way to reduce the death rate (Schwartz et al., 2015). Mosquito vector control using a combination of methods includes management of larval habitat and application of chemically and biologically based insecticides to reduce the infection rate (WHO, 2009). These methods have been only partially effective as there has been a 30-fold increase in incidence over the past half-century and this shows no sign of decreasing (Achee et al., 2015). However, a combination of the existing tools is the recommended approach under the World Health Organization (WHO) guidelines in parallel with developing novel approaches (WHO, 2009; Achee et al., 2015).

Bacillus thuringiensis subsp. *israelensis* (Bti), a Gram-positive rod shape bacterium, produces dipteran-specific insecticidal crystal proteins during the sporulation phase (Schnepf et al., 1998). Bti is the most powerful and environmentally friendly biological-based mosquitocide that has been recommended by the WHO for dengue vector control in drinking water (WHO, 2009). The larvicidal activity of Bti relies on at least four major mosquitocidal crystal toxins, Cry4Aa, Cry4Ba, Cry11Aa and Cyt1Aa (Berry et al., 2002). Currently, no resistance to Bti has been reported in field populations of the *Aedes* mosquito, even after decades of Bti treatment (Kamgang et al., 2011; Tetreau et al., 2013). The absence of insect resistance to Bti is mainly due to the synergism of Cry and Cyt toxins with their different mode of actions (Ben-Dov 2014). For example, Cyt1Aa was reported to synergize with Cry4Ba (Canton et al., 2011), Cry10Aa (Hernandez-Soto et al., 2009) and Cry11Aa (Perez et al., 2005) toxins. In addition, synergism among Cry toxins has been reported; for example, synergy of Cry4Ba with Cry11Aa against *A.*

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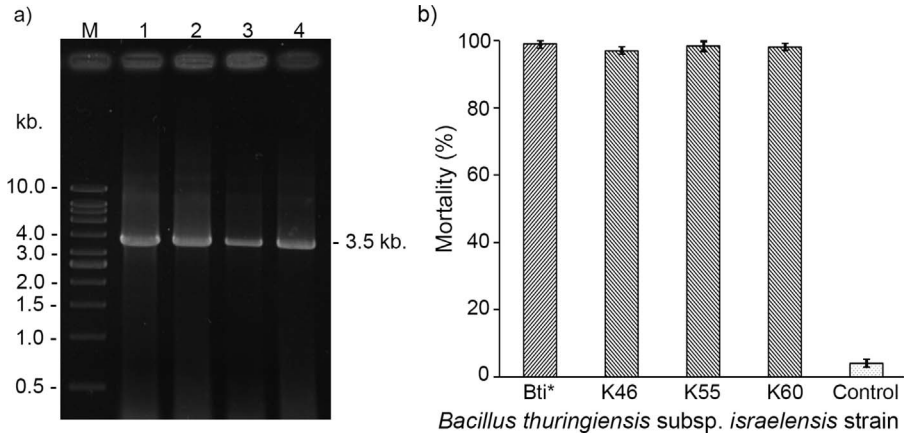

Fig. 1. a) PCR amplification products of 3.5-kb *cry* 4Ba gene in Bti; lane M: 1 kb DNA ladder; lane 1: commercial strain Bti; lane 2: isolate K46; lane 3: isolate K55; lane 4: isolate K60, b) Mosquitocidal activity of three local isolates of Bti (isolate K46, K55, and K60) and the commercial strain Bti (Bti*) against *Aedes aegypti* larvae. Error bars indicated SD of triplicate data from three independent experiments.

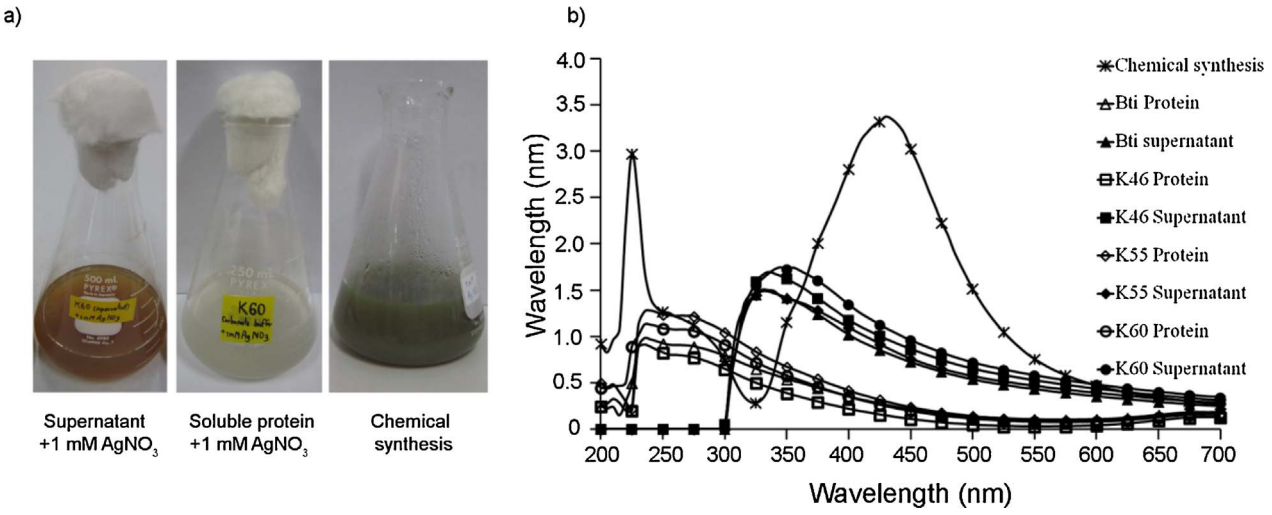

Fig. 2. a) Solution of supernatant and solubilized protein of Bti K60 with 1 mM $AgNO_3$ and the chemically synthesized Ag NPs, b) UV–vis spectra of synthesized Ag NPs.

Table 1
Larvicidal activity of Ag NPs synthesized using supernatant and inclusion proteins of commercial strain Bti and local isolates Bti, and chemically synthesized Ag NPs against *Aedes aegypti* larvae.

Bti isolate	Source of Ag NPs	LC_{50} (μg/mL) LCL-UCL)	LC90 (μg/mL) (LCL-UCL)	χ^2
K46	Supernatant	0.010 (0.006–0.015)	0.097 (0.073–0.138)	4.292
	Inclusion protein	0.008 (0.004–0.011)	0.050 (0.038–0.071)	2.643
K55	Supernatant	0.001 (0.000–0.001)	0.005 (0.004–0.007)	3.079
	Inclusion protein	0.027 (0.013–0.042)	0.188 (0.105–0.747)	5.945
K60	Supernatant	0.036 (0.010–0.080)	0.113 (0.055–1.677)	8.491
	Inclusion protein	0.032 (0.023–0.042)	0.343 (0.232–0.609)	2.367
Commercial Bti	Supernatant	0.055 (0.044–0.065)	0.370 (0.280–0.552)	2.576
	Inclusion protein	0.024 (0.020–0.045)	0.170 (0.091–1.472)	9.436
Chemical synthesis		0.916 (0.829–1.006)	2.788 (2.400–3.381)	6.760

aegypti larvae (Crickmore et al., 1995). Although no resistance of a field population of *A. aegypti* has been reported, increased resistance in laboratory strains to individual Bti toxins and commercial Bti has been observed (Paris et al., 2011).

Decreasing sensitivity to Bti requires developmental strategies for delaying the mosquito's resistance. Thus, the development of novel mosquitocidal agents has been attempted. Recently, silver nanoparticles (Ag NPs) have been considered suitable for the control of mosquito vectors, as the increased surface area of nano-size particles results in increased reactivity (Rai et al., 2012). There are different forms of silver nanomaterials, for example, metallic Ag NPs (Ag°), silver chloride particles (AgCl) and silver-titanium dioxide (Ag-TiO_2) have been synthesized and already used in various kinds of products (for a review see Marambio-Jones and Hoek 2010). Ag NPs exhibit a broad spectrum of antibacterial activity which has encouraged using these materials for bacterial growth inhibition. In addition, Ag NPs have been reported as toxic to other microorganisms, including virus, fungi and higher organisms (for a review see Marambio-Jones and Hoek 2010). Recently, Ag NPs synthesized using extract from plants and microorganisms have been intensively studied for their mosquitocidal activity. For example, Ag NPs were synthesized using *Plumeria rubra* plant latex (Patil et al., 2012), mangrove plant leaf extract from *Rhizophora mucronata* (Gnanadesigan et al., 2011) and microorganisms including fungi *Beauveria bassina* (Banu and Balasubramanian, 2014) and *Bacillus megaterium* bacterium (Banu and Balasubramanian, 2015) and reported as toxic against *Aedes aegypti* larvae.