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RESEARCH ARTICLE

Effect of mycosynthesized silver nanoparticles from filtrate of *Trichoderma harzianum* against larvae and pupa of dengue vector *Aedes aegypti* L

Chandran Sundaravadivelan · Madanagopal Nalini Padmanabhan

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Abstract Mosquitoes transmit dreadful diseases, causing millions of deaths every year. Therefore, screening for larvicidal and pupicidal activity of microbial extracts attributes could lead to development of new and improved mosquito control methods that are economical and safe for nontarget organisms and are ecofriendly. Synthetic chemical insecticides occupy predominant position in control strategies. These hazardous chemicals exert unwarranted toxicity and lethal effects on nontarget organisms, develop physiological resistance in target, and cause adverse environmental effect. For vector control, fungal-mediated natural products have been a priority in this area at present. In the current study, effective larvicidal and pupicidal effect of mycosynthesized silver nanoparticles (Ag NPs) using an entomopathogenic fungi *Trichoderma harzianum* against developmental stages of the dengue vector *Aedes aegypti* was investigated. An attractive possibility of green nanotechnology is to use microorganisms in the synthesis of nanosilver especially Ag NPs. The mycosynthesized Ag NPs were characterized to find their unique properties through UV-visible spectrophotometer, X-ray diffraction analysis, Fourier transform infrared, and surface characteristics by scanning electron microscopy. To analyze the bioefficacy, different test concentrations for extracellular filtrate (0.2, 0.4, 0.6, 0.8, and 1.0 %) and Ag NPs (0.05, 0.10, 0.15, 0.20, and 0.25 %) were prepared to a final volume of 200 mL using deionized water; 20 larvae of each instars (I–IV) and pupa were exposed to each test concentration separately which included a set of control (distilled water) group with five replicates. Characterization of the synthesized Ag NPs were about 10–20 nm without aggregation. Susceptibility of larval instars to synthesized Ag NPs was higher than the extracellular filtrate of *T. harzianum* alone after 24-h exposure, where the highest mortality was recorded as 92 and 96 % for first and second instars and 100 % for third, fourth instars, and pupa. Lethal concentration 50 values of 0.079, 0.084, 0.087, 0.068, and 0.026 % were recorded for I–IV instars and pupa, respectively, when exposed to Ag NPs at 0.25 % concentration. Toxicity was exhibited against first (1.076 %), second (0.912 %), third (0.770 %), fourth (0.914 %) instars larvae, and pupa (0.387 %) with extracellular filtrate at a concentration of 1 % that was three- to fourfold higher compared to Ag NPs; no mortality was observed in the control. The present study is the first report on effective larvicidal and pupicidal activity of Ag NPs synthesized from an entomopathogenic fungi *T. harzianum* extracellular filtrate and could be an ideal ecofriendly, single-step, and inexpensive approach for the control of *A. aegypti*.

Keywords *Trichoderma harzianum* · *Aedes aegypti* · Bioefficacy · Mycosynthesis · Ecofriendly approach

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Introduction

Mosquitoes are the most important group of insects, which transmit numerous dreadful diseases such as dengue fever, yellow fever, chikungunya, malaria, encephalitis, and filariasis. They have been a perennial problem in many parts of the world, particularly in countries with tropical and subtropical regions; however, no part of the world is free from these vector-borne diseases (Fradin and Day 2002), and hence,

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mosquitoes have been declared as "public enemy number one" (World Health Organization 1996). To date, dengue fever is endemic in Southeast Asia (Akram and Ahmed 2005). Two main species namely, the Asian tiger mosquito (*Aedes albopictus*) and the Yellow fever mosquito (*Aedes aegypti*), are responsible for causing dengue fever in China (Liu et al. 2012). In particular, *A. aegypti* is the vector of arbovirus responsible for causing yellow fever, chikungunya, and dengue in more severe forms, like dengue hemorrhagic fever and dengue shock syndrome. Over 30 million people have been infected worldwide exclusively by these diseases (Chakravarti and Kumaria 2005).

Therefore, control of mosquito vectors is a public health concern. Since mosquitoes are holometabolous and their developmental stages restricted to aquatic habitat, the larval and pupal stages were attractive target for controlling these dreadful vectors. In this aspect, control of mosquito larvae is dependent on chemical methods using synthetic insecticides like organophosphates such as temephos, fenthion, and insect growth regulators such as diflubenzuron, methoprene (Yang et al. 2002), and pyrethroides (Ranson et al. 2010). Although effective, repeated use of these synthetic insecticides for mosquito control has resulted in lower efficacy of such insecticides due to development of resistance in the mosquito population (Harris et al. 2010; Polson et al. 2011), bioaccumulation, ecological imbalance, harmful to mammals as well as nontarget organisms, and is also costlier. Constant application of these synthetic insecticides in ponds, wells, and other water bodies for the control of mosquito population may cause severe health hazards to human beings and larvivorous fishes.

These problems have highlighted the constant need for developing biologically active natural larvicides using plants and entomopathogenic microbes. In plants, solvent extracts of leaves of *Eugenia jambolana* (Raghavendra et al. 2011), *Mimosa pudica* (Aarthi et al. 2011), *Leucas aspera* (Maheswaran et al. 2008), *Ocimum canum*, *Ocimum sanctum*, and *Rauvolfia serpentine* (Kamaraj et al. 2008; Das and Chandra 2012); *Cleistanthus collinus*, *Murraya koenigii*, and aerial part of *Sphaeranthus indicus* (Kovendan et al. 2012), *Jatropha curcas*, *Pedilanthus tithymaloides*, *Phyllanthus amarus*, *Euphorbia hirta*; and stem bark of *Euphorbia tirucalli* (Tomass et al. 2011; Abdul Rahuman et al. 2008), sea grass *Syringodium isoetifolium*, *Cymodocea serrulata*, and *Halophila beecarii* (Syed Ali et al. 2012); and aril and kernel of *Knema attenuata* (Vinayachandra et al. 2011) have been reported to possess larvicidal and pupicidal effects against *A. aegypti*, *A. albopictus*, *Anopheles arabiensis*, *Anopheles stephensi*, and *Culex quinquefasciatus*. Microbial control with entomopathogenic fungi like *Aspergillus flavus*, *Aspergillus parasiticus*, *Penicillium falicum*, *Fusarium vasinfectum*, and *Trichoderma viride* were most effective against *C. quinquefasciatus* (Govindarajan et al. 2005) and bacterial isolates *Bacillus thuringiensis* was found to possess potent larvicidal activity against *A. stephensi* and *Culex pipiens* (Sur et al. 2003). However, 2.78-fold increase in tolerance to *B. thuringiensis* as a result of 20 generations of selection in larvae of *C. pipiens* were reported (Saleh et al. 2003).

The collected information in the current situations paved the way to develop an alternative ecofriendly biomolecule as a potential biocontrol agent. In this regard, development of reliable processes for the synthesis of nanoparticles with unique properties is an important aspect of nanotechnology. During synthesis, physical and chemical procedures could be followed for metallic nanoparticles; however, these method posses many problems including the use of toxic solvents, generation of hazardous by-products, high energy consumption, pressure, and is also expensive. Therefore, biological methods for synthesis of nanoparticles using plants and microorganisms have been investigated as possible ecofriendly alternatives to physical and chemical methods (Mohanpuria et al. 2008).

In addition, synthesis of nanoparticles using plant leaves of *E. hirta* (Agalya Priyadarshini et al. 2012), *Nelumbo nucifera* (Santhoshkumar et al. 2011), *Calotropis gigantean* (Vaseeharan et al. 2012), and *Vitex negundo* (Zargar et al. 2011); fungus *Pycnoporus sanguineus*, *Schizophyllum commune*, *Lentinus sajor caju*, *Trametes feei*, *Trametes pocas* (Chan and Mat Don 2013), and *A. flavus* (Vigneshwaran et al. 2007); and bacteria *Bacillus flexus* (Priyadarshini et al. 2013), *Bacillus cereus* (Ganesh Babu and Gunasekaran 2013), and *Streptomyces* sp. (Vinay Gopal et al. 2013) have been found to be a simple, rapid, cost-effective, and a novel method to ecofriendly approach. These biologically synthesized nanoparticles play an important role in medical, pharmaceutical, industrial, and biotechnological applications (Sintubin et al. 2012). In recent progress of nanotechnology, many laboratories around the world have investigated silver nanoparticles to utilize it in various applications. Specifically, silver nanoparticles are proved to have potential antimicrobial (Fayaz et al. 2010), antifungal (Ales Pana et al. 2009), antibacterial (Krishnaraj et al. 2010), antiviral (Lara et al. 2010), antiplasmodial (Ponarulselvam et al. 2012), drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants, and tissue engineering (Morons et al. 2005) as well as larvicidal and pupicidal (Sundaravadivelan et al. 2013) activities reported towards medically challenged pathogens and dreadful vectors. Therefore, synthesis of silver nanoparticles (Ag NPs) using microorganisms could be potentially biocompatible.

In the present investigation, we performed mycosynthesis of Ag NPs using entomopathogenic fungi *Trichoderma harzianum*, which is a fungus present in nearly all soils and diverse habitats. The genus *Trichoderma* comprises 89 species in which *T. harzianum* belongs to family Hypocreaceae and has potential to be involved in the following functions such as mycoparasitism, antibiosis, tolerance to stress through enhanced root and plant development, solubilization and sequestration of inorganic nutrients, inactivation of the

pathogens enzymes, and act as an effective biocontrol agent (Benítez et al. 2004) due to its production of volatile and nonvolatile toxic metabolites. It has been reported that it produces enzymes and metabolites for its own survival, which are involved in breaking of silver nitrate a complex hazardous chemical into Ag^+ ions and NO_3^- due to the potential action of hydrolytic/nitrate reductase enzymes. In this process, the toxic Ag^+ ions are further reduced to nontoxic (Ag^0=biosilver) metallic nanoparticles through the catalytic effect of extracellular fungal secondary metabolites; mechanism involved in this synthesis is illustrated in Fig. 1. Therefore, we selected this entomopathogenic fungus, *T. harzianum*, for their progressive reduction of silver nitrate to produce a potential deliverable Ag NPs with effective larvicidal and pupicidal activity against dengue vector *A. aegypti*.

Taxonomy
Kingdom Fungi
Division Ascomycota
Subdivision Pezizomycotina
Class Sordariomycetes
Order Hypocreales
Family Hypocreaceae
Genus *Trichoderma*
Species *harzianum* (Rifai 1969)

Experimental design materials

Fungal strain and biomass production

The fungal strain of *T. harzianum* obtained from Tamil Nadu Agriculture University, Coimbatore, was maintained in our laboratory on Sabouraud's dextrose agar medium at 25±2 °C and slant culture was stored at 4 °C as stock culture for further use up to 1 week. From the plate culture (Fig. 2), a well (10 mm diameter) was cut and inoculated in 250 mL Erlenmeyer flask containing 100 mL of Sabouraud's dextrose broth which was incubated at 27 °C up to 5 days for biomass production.

Methods

Synthesis of silver nanoparticles (Ag NPs)

The 5-day-old fungal biomass was washed thrice with Milli-Q-deionized water by pouring through fine mesh in order to completely eliminate nutrient broth. The washed mycelium was transferred to 100 mL deionized sterile water which was incubated at RT for 48 h. The culture was filtered through sterile mesh line cloth (100 μm) representing the aqueous filtrate (=extracellular filtrate) which was further used for synthesis. Silver nitrate (1 mM) was dissolved in extracellular filtrate and incubated in dark at RT. The reduction of silver ions was routinely monitored visually for color change at regular intervals.

Characterization of synthesized Ag NPs

An aliquot (1 mL) was drawn from the reaction mixture at every 6 h and the absorbance was measured using UV-visible spectrophotometer (Shimadzu UV-2450, Japan) operated at a resolution of 1 nm with scan range between 200 and 800 nm continuously up to 24 h. Subsequently, the reaction mixture

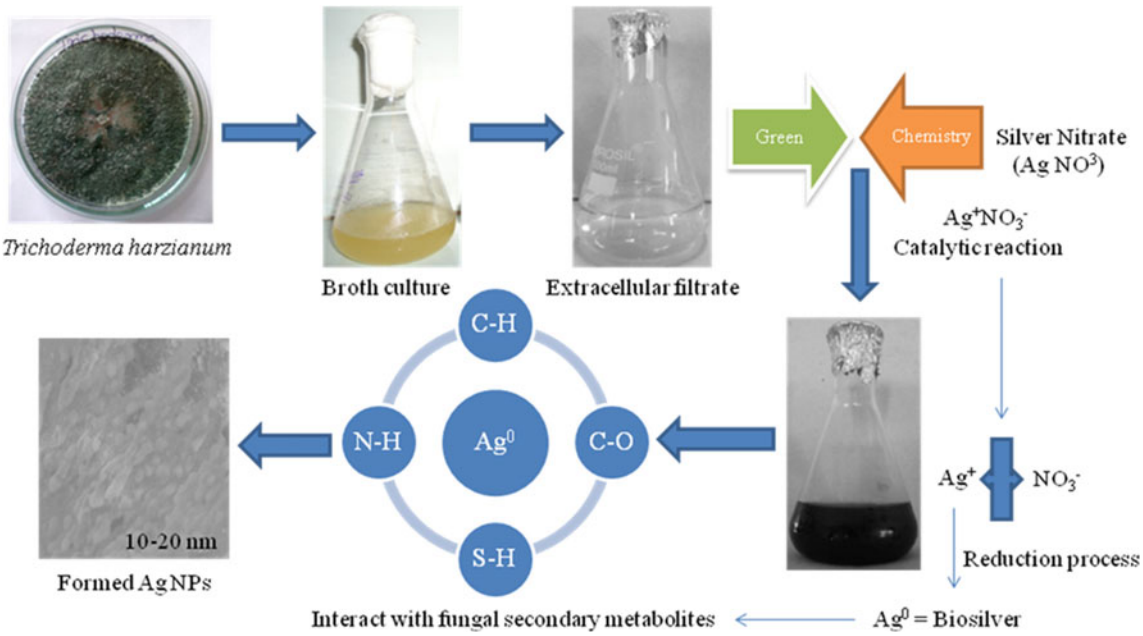

Fig. 1 Mechanisms involved in mycosynthesis of silver nanoparticles using *T. harzianum* extracellular filtrate

Fig. 2 *Trichoderma harzianum*

was subjected to centrifugation (20,000×*g*, 4 °C, 20 min); the resulting pellet was suspended in deionized water and washed thrice by centrifugation. The dried powder form of the pellet was mixed with KBr and pelleted by pressing with pellet-making machine which was exposed to infrared (FTIR, IRAffinity-1, Shimadzu) where the wave number ranged from 4,000 to 400 cm^{-1}. The observed peak value indicated the responsible functional groups for synthesized Ag NPs. The average grain size of the produced Ag NPs was measured through X-ray diffraction analysis (XRD; X'Pert PRO, PANalytical) at a voltage of 40 kV and a current of 30 mA with Cu Kα1 radiation, where the scan range was 10–90° at 2θ angle and nature of particle size and shape was observed under an advanced scanning electron microscopy (SEM; FEI Quanta 200, Icon Analytical).

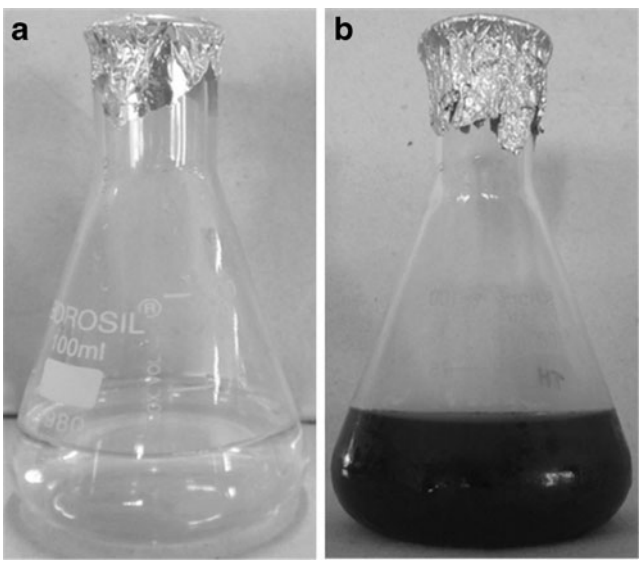

Fig. 3 **a** *T. harzianum* extracellular filtrate. **b** Synthesized Ag NPs after adding $AgNO_3$ (1 mM)

Assessment of the larvicidal and pupicidal activity

To verify, the larvicidal and pupicidal effect of produced Ag NPs from extracellular filtrate of *T. harzianum* against developmental stages of the dengue vector *A. aegypti* was evaluated by WHO method (World Health Organization 1996) with slight modification as per the method of Sundaravadivelan et al. (2013) to substantiate the efficacy of test materials. To analyze the bioefficacy, different test concentrations for extracellular filtrate (0.2, 0.4, 0.6, 0.8, and 1.0 %) and Ag NPs (0.05, 0.10, 0.15, 0.20, and 0.25 %) were prepared to final volume of 200 mL deionized water in 250-mL capacity autoclaved glass bottles. Twenty larvae of each instar (I–IV) and pupa were exposed to each test concentration separately which included a set of control (distilled water) with five replicates. No food was provided during the period of experiment. The number of dead larvae and pupa were recorded after 24 h of exposure.

Statistical analysis

Mean percent mortality for larvae and pupa treated with extracellular filtrate and Ag NPs were subjected to variance analysis of variance and probit analysis for calculating lethal concentration 50 (LC_{50}) with their associated confidence intervals (lower and upper confidence limit) at 95 % was performed using SPSS, a statistical software package 13.0 version (SPSS 2007). Results with $p < 0.05$ were considered to be statistically significant.

Results and discussion

Use of plants and microbial-derived products are safe, well-contained, and readily decomposable with no or minimal pollutants in ecosystem would be a promising approach for biological control of mosquitoes unlike the inherent dangers of synthetic insecticides. In this respect, we intended to develop an alternative biocontrol agent from natural products which are generally preferred because of their less harmful nature on nontarget organism and ecofriendly approach (Abdul Rahuman et al. 2008) to prevent the increasing resistance to pesticides (Ranson et al. 2001). In addition, fungi secretes large quantities of enzymes such as nitrate reductase and hydrogenase or electron shuttle quinones, proteins, active metabolites, and easier large-scale production as well as recovery of silver nanoparticles (Chan and Mat Don 2013; Salunkhe et al. 2011). Thus, the present study revealed for the synthesis of Ag NPs from extracellular filtrate of *T. harzianum* with unique characteristic features displaying larvicidal and pupicidal effect against the fourth instar larva and pupa of the dengue vector *A. aegypti*.

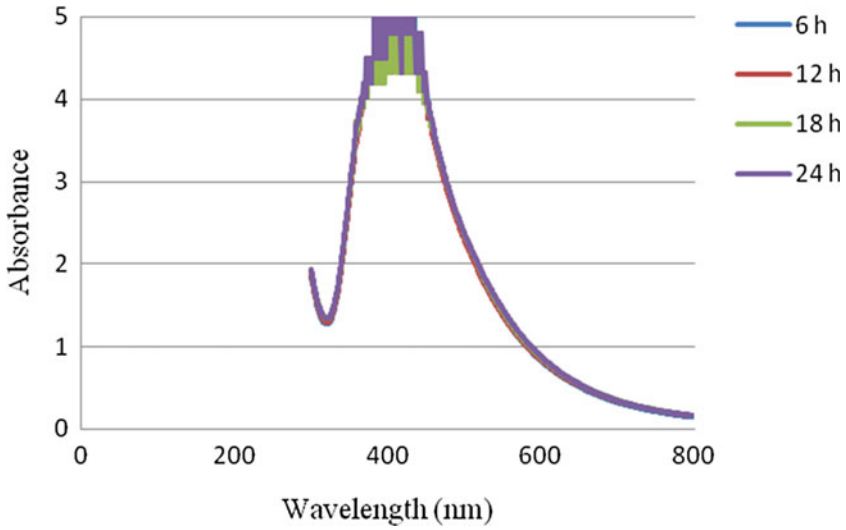

Fig. 4 UV-visible spectrum of mycosynthesized Ag NPs using *T. harzianum* extracellular filtrate

Visual analysis and UV-visible spectrum of Ag NPs

Bioreduction of silver ions due to active reducing ingredients in the extracellular filtrate of *T. harzianum* was observed due to development of dark brown color (Fig. 3; Vahabi et al. 2011). The rate of reduction of silver ions was evaluated by UV-visible spectrophotometer and a maximum absorption at 433.5 nm with an optical density of 5.0 was obtained after 6 h of incubation period. This reduction of silver ions is attributed to the excitation of surface plasmon resonance, essentially the vibration of group conduction electrons exhibiting an increase in absorbance with an increase in reducing agents during the reaction time. The observations were recorded at different time intervals (6, 12, 18, and 24 h; Fig. 4) indicating that the fungal metabolites actively reduced the silver ions and confirmed the production of colloidal silver in the reaction mixture (Santhoshkumar et al. 2012). In contrast, fairly broad absorption band at 480 nm due to presence of slight aggregated structure of silver particles in the fungal liquid *Chrysporium tropicum* has been reported (Soni and Prakash 2012); *A. flavus*-mediated Ag NPs were highly stable for 3 months, confirmed by microscan at 420 nm (Vigneshwaran et al. 2007) which could be attributed to a higher concentration of Ag NPs synthesized extracellular than the culture-free supernatant synthesis of *P. sanguineus* (Chan and Mat Don 2013).

FTIR: involved fungal metabolites

FTIR is a powerful tool for identifying responsible functional groups involved in synthesis process of Ag NPs using extracellular filtrate of *T. harzianum*. The types of chemical bonds

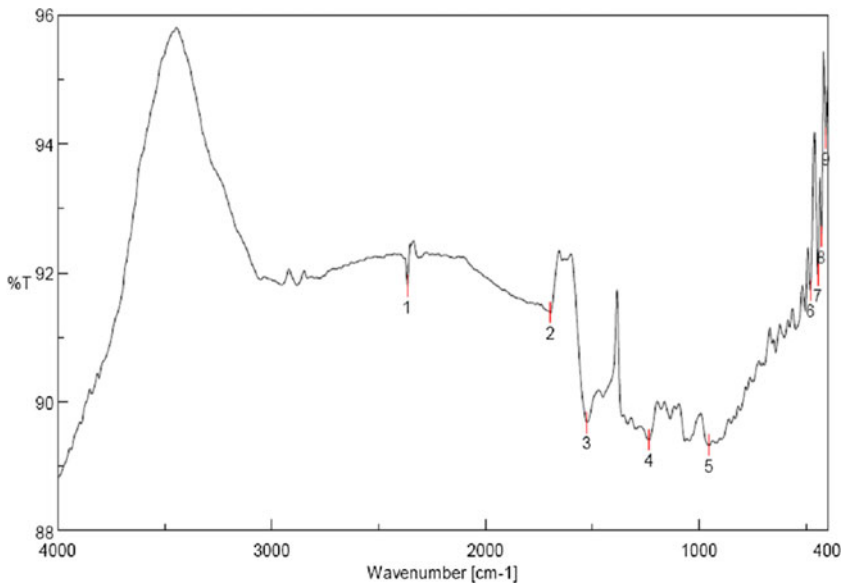

Fig. 5 FTIR spectrum of Ag NPs produced from *T. harzianum* extracellular filtrate

Table 1 FTIR spectrum absorption bands with their respective functional groups of *Trichoderma harzianum* extra cellular filtrate involved in synthesis of Ag NPs

S. no.	Characteristic absorption (cm^{-1})	Types of vibration(s)	Functional group(s)
1	2,364.3	S–H stretch	Mercaptans
2	1,694.16	C–C triple bond stretch	Alkynes
3	1,524.45	N–H bend	Amides
4	1,234.22	C–C (O)–C stretch (acetates)	Esters
5	953.627	C–O stretch	Anhydrides
6	479.224	C–H bend	Alkenes
7	445.476	C–H bend	Alkenes
8	428.12	C–H bend	Alkenes
9	407.871	C–H bend	Alkenes

in a molecule can be determined by infrared absorption using this annotated spectrum (Fig. 5), where the strength of absorption is proportional to concentration of active metabolites present in the reaction mixture. The absorption bands due to vibration of chemical molecules at 2,364.30, 1,694.16, 1, 524.45, 1,234.22, 953.62, 479.22, 445.47, 428.12, and 407.87 cm^{-1} were recorded in the whole spectrum of wave number between 4,000 and 400 cm^{-1}. The absorption bands with their functional groups due to vibration of chemical bonds were shown in Table 1. Among them, the amide group by vibration of N–H bend indicates the linkage between amino acid residues in polypeptides and proteins which rise to well-known signatures for myco-synthesis observed in infrared region of the electromagnetic spectrum. Similarly, the position of amide I and II bands representing proteins is a sensitive indicator of conformational changes in protein secondary structure (Mukherjee et al. 2001; Oksanen et al. 2000). Involvement of other possible functional biomolecules like mercaptans, alkynes, alkenes, esters, anhydrides, and enzymes in cell wall of mycelia for synthesis of Ag NPs is in accordance with Udayasoorian et al. (2011) and Sastry et al. (2003). Janerio et al. (2005) reported the bioreduction of Ag^{+} ions to Ag^{0} especially the formation of biosilver which could be due to tentative mechanism of oxidation. The overall observation confirms the presence of potential metabolites/biomolecule(s) with stronger ability to bind reduced metal, thus forming a layer covering on metal nanoparticles (i.e., capping of silver nanoparticles) to prevent aggregation and thereby stabilize the medium.

XRD: purity and crystalline nature of Ag NPs

Figure 6 depicts the XRD pattern of Ag NPs synthesized from *T. harzianum* extracellular filtrate. These myco-synthesized particles showed intense peaks at 2θ values of 27.89°, 32.37°, 38.29°, 46.41°, 64.69°, and 77.46° which indexed the full-width half maximum of (98), (101), (111), (200), (220), and (311) lattice planes of face-centered cubic structure of silver, respectively. The average grain size of Ag NPs formed by mycoreduction process was determined using Debye–Scherr's

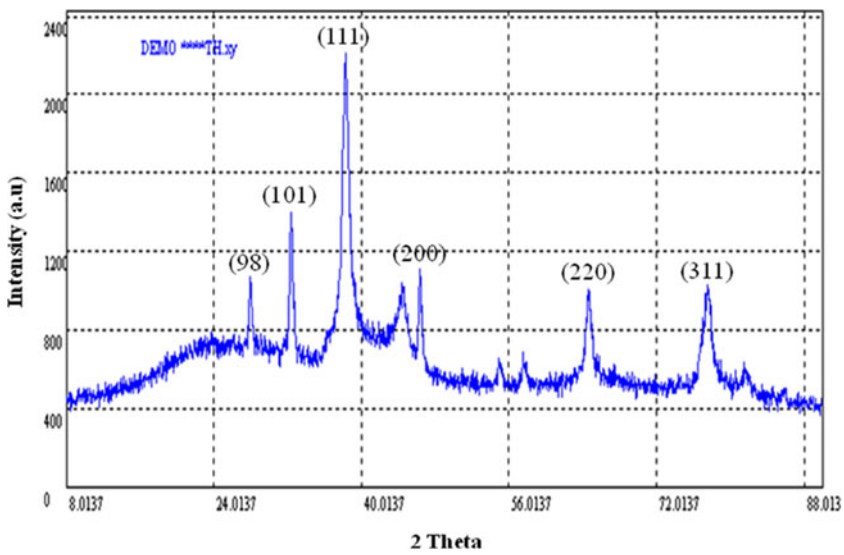

Fig. 6 XRD pattern analysis of mycosynthesized Ag NPs using *T. harzianum* extracellular filtrate

Fig. 7 Scanning electron microscopy of Ag NPs from *T. harzianum* extracellular filtrate

equation, $D = (0.9\lambda \times 180°)/\beta \cos\theta\,\pi$, of observed peaks at 2θ diffraction angle and was calculated to be 6.78 nm. The analysis confirmed that the mycosynthesized Ag NPs are oval shaped, crystalline in nature, and monodispersed in colloidal form and no corresponding peaks to Ag was observed from XRD pattern which showed that formed Ag NPs has a high purity (Ganesh Babu and Gunasekaran 2013). The line broadening of peaks exhibits the presence of small particles in the medium. These findings revealed that the produced Ag NPs are viable, stable, potential, and biomineralized form of crystalline nature due to capping of extracellular filtrate containing bioactive metabolites.

SEM: particle size confirmation

Scanning electron microscopy of produced Ag NPs is shown in Fig. 7, where the nanoparticles are not in direct contact even within aggregates indicating stabilization of Ag NPs by a capping agent. The majority of synthesized Ag NPs might be present on cell surface, which could be due to the absorption of silver ions and interaction with the mycelial surface functional groups and the size measured about 10–20 nm. In addition, synthesis of similar-sized particles from fungus *Aspergillus ochraceus* (20 nm), *Neurospora crassa* (11 nm), *Penicillium fellutanum* (5–25 nm), *T. viride* (2–4 and 10–40 nm), *Verticillium* sp. (25 nm), and *Volvariella volvacea* (15 nm) were reported (Sintubin et al. 2012).

Advantages and applications of mycosynthesized Ag NPs

The mycosynthesized biogenic Ag NPs from extracellular filtrate of *T. harzianum* has an alternative and additional advantage over the physical and chemical methods such as liquid-phase synthesis, high-temperature reduction, vapor-phase condensation, laser ablation, photo reduction, and electrolysis which require high pressure, energy, chemical precursors, and high cost. The ecofriendly mycosynthesized Ag NPs possess potential action in medical, pharmaceutical, industrial, and biotechnological applications and is probably the most commercialized nanomaterials as an antimicrobial agent and has been applied in textiles, home water purification system, medical devices, cosmetics, electronics, and household

Table 2 Larvicidal and pupicidal activity of Ag NPs from extracellular filtrate of *Trichoderma harzianum* against *Aedes aegypti*

Developmental stages of *A. aegypti*	Extracts	Percent mortality (%)±SE					LC_{50}	95 % Confidence limit		χ^2 (*df*=3)*
		0.2	0.4	0.6	0.8	1.0		LCL	UCL	
I instar	Extracellular filtrate	12±1.294	22±1.294	24±1.510	34±1.510	48±1.294	1.076	0.941	1.319	1.368
II instar		18±1.480	28±1.294	34±1.337	42±1.294	56±1.742	0.912	0.803	1.091	0.590
III instar		20±1.189	24±1.742	38±1.294	52±0.946	66±1.047	0.770	0.697	0.863	1.072
IV instar		14±1.047	26±1.510	36±1.510	42±1.294	54±1.510	0.914	0.813	1.075	1.091
Pupa		40±1.778	50±1.778	62±1.615	72±1.294	80±1.934	0.387	0.262	0.475	0.038
Concentrations (%)		0.05	0.10	0.15	0.20	0.25				
I instar	Synthesized Ag NPs	46±1.510	54±1.742	64±2.195	78±1.294	92±1.294	0.079	0.052	0.099	3.495
II instar		40±1.414	58±1.722	66±1.337	82±1.615	96±1.047	0.084	0.063	0.099	4.104
III instar		38±1.615	54±1.047	70±1.189	88±0.946	100±0.000	0.087	0.040	0.115	6.617
IV instar		50±1.414	58±1.615	70±1.934	82±1.294	100±0.000	0.068	0.135	0.117	12.234
Pupa		76±1.510	82±2.082	90±1.189	96±1.047	100±0.000	0.026	0.099	0.011	2.866

Values are mean of five replicates with±SE

LC_{50} Lethal concentration 50, *LCL* lower confidence limit, *UCL* upper confidence limit, χ^2 Chi-square value, *df* degrees of freedom

*$p < 0.05$, significant level

appliances (Maynard 2007; Wijnhoven et al. 2009). Interestingly, the use of mycomediated Ag NPs as larvicidal and pupicidal agent against mosquito vector is relatively new.

Larvicidal and pupicidal effect of Ag NPs

In this respect, potential larvicidal and pupicidal activity of mycosynthesized Ag NPs from extracellular filtrate of *T. harzianum* has been reported using a novel method. Considerable mortality was evident at different concentrations after treatment with Ag NPs (0.05, 0.10, 0.15, 0.20, and 0.25 %) and extracellular filtrate (0.2, 0.4, 0.6, 0.8, and 1.0 %) alone. The mortality increased as the concentration increased (Table 2). The notable mortality of 92 and 96 % were observed for first and second instar and 100 % for third; fourth instar and pupa were observed at 0.25 % concentration treated with Ag NPs after 24 h of exposure, their LC_{50} (LCL/UCL at 95 % confidence) were recorded for I–IV instars and pupa to be 0.079 % (0.052:0.099), 0.084 % (0.063:0.099), 0.087 % (0.040:0.115), 0.068 % (0.135:0.117), and 0.026 % (0.099:0.011), respectively. Likewise, LC_{50} for second (1.0 %), third (0.87 %), and fourth (0.82 %) instar larvae of *A. aegypti* was observed when exposed to Ag NPs produced from *Cochliobolus lunatus* (Salunkhe et al. 2011) and interestingly, 100 % mortality was recorded in early second instar after 1 h exposure of Ag NPs derived from *Chrysosporium tropicum* (Soni and Prakash 2012).

Considerable percent mortality (LC_{50}, LCL/UCL at 95 %) was recorded when treated with extracellular filtrate, where the susceptible rate for first instar, 48 % (1.076, 0.941:1.319); second instar, 56 % (0.912, 0.803:1.091); third instar, 66 % (0.770, 0.697:0.863); fourth instar, 54 % (0.914, 0.813:1.075); and pupa, 80 % (0.387, 0.262:0.475) at 1.0 % concentration, which is three- to fourfold higher than the highest concentration treated using Ag NPs. Similar culture filtrates of fungi (LC_{50}), *A. flavus* (38.34 mg/L), *A. parasiticus* (40.39 mg/L), *P. falicum* (44.97 mg/L), *F. vasinfectum* (50.03 mg/L), and *T. viride* (54.16 mg/L) were moderately toxic to larvae of *C. quinquefasciatus* (Govindarajan et al. 2005). In all treatments, chi-square values were significant at $p < 0.05$.

Similar observations with Ag NPs from aqueous leaf and stem extract of *P. tithymaloides* against developmental stages of *A. aegypti* (Sundaravadivelan et al. 2013; Sundaravadivelan and Nalini 2012) were reported recently. In addition, larvicidal and pupicidal effect of Ag NPs produced from leaves extract of *M. pudica* (LC_{50}=13.90 and 11.73 mg/L), *N. nucifera* (LC_{50}=0.69 and 1.10 mg/L), and *Eclipta prostrata* (LC_{50}=5.14 and 4.56 mg/L) against *A. subpictus* and *C. quinquefasciatus* (Marimuthu et al. 2011; Santhoshkumar et al. 2011; Rajakumar and Rahuman 2011). Agalya Priyadarshini et al. (2012) found larval and pupal toxicity (LC_{50}=10.14, 16.82, 21.51, 27.89, and 34.52 ppm) of Ag NPs using *E. hirta* leaf extract against I–IV instars and pupa of *A. stephensi*. Jayaseelan et al. (2011) reported that pediculocidal and larvicidal activity of produced Ag NPs using leaf extract of *Tinospora cordifolia* and their LC_{50} for *Pediculus humanus capitis* (12.46 mg^{-1}), *Anopheles subpictus* (6.43 mg^{-1}), and *C. quinquefasciatus* (6.96 mg^{-1}) synthesized using *Catharanthus roseus* possessed antiplasmodial activity as well (Ponarulselvam et al. 2012).

Earlier findings related to mosquito control using various solvent extract of *L. aspera* (Maheswaran et al. 2008), *R. serpentine* (Das and Chandra 2012), *S. isoetifolium*, *C. serrulata*, and *Halophila beccarii* (Syed Ali et al. 2012), *J. curcas*, *P. tithymaloides*, *P. amarus*, *E. hirta*, and *E. tirucalli* (Abdul Rahuman et al. 2008); entomopathogenic fungi *Metarhizium anisopliae* (Murugan et al. 2012); and bacteria *B. thuringiensis* (Kovendan et al. 2011), *B. sphaericus* (Paily et al. 2012) against *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* were also reported. Simultaneously, possible development of resistance in insects treated with *B. thuringiensis* has been investigated (Saleh et al. 2003).

Possible mechanism(s)

The formation of Ag NPs due to reduction of Ag^+ ions to Ag^0 by *T. harzianum* filtrates containing active metabolites like extracellular proteins and enzymes in the colloidal solution may disrupt the structure of cell membrane due to interaction between Ag NPs and membrane molecule, thus interrupting the membrane permeability and leading to cell death. Like this, the participation of NADPH-dependent reductase in reduction mechanism has been investigated recently (Fayaz et al. 2010). Still, the exact mechanism of larvicidal action of mycosynthesized Ag NPs is unclear. Yamanaka et al. (2005) have also shown that biologically reduced silver ions primarily interact with cytoplasm in interior of the cell and denature the ribosome, finally suppress the expression of enzymes and proteins essential for ATP production and leads to disruption of cell. In addition, Raffi et al. (2008) observed the presence of Ag NPs in cell wall membrane and it inhibits the cell wall synthesis pathway in *Streptococcus aureus* (Song et al. 2006).

We conclude that the bioreduction of silver is based on the presence of respective functional groups in the extracellular filtrate of *T. harzianum* which is more efficient than other fungi and not harmful to humans. This filamentous fungus exhibited a higher surface area capable of binding $Ag^+ \rightarrow Ag^0$ by making this process easier, cheaper for large-scale production at industrial level, thereby being cost-effective. Thus this nanosized alternative biocontrol agent can be utilized for integrated mosquito control program towards public health.

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References

Aarthi N, Vasugi C, Panneerselvam C, Prasana Kumar K, Madhiyazhagan P, Murugan K (2011) Toxicity and smoke repellency effect of *Mimosa pudica* L. against the malarial vector *Anopheles stephensi* (Diptera: Culicidae). The Bioscan 6:211–214

Abdul Rahuman A, Gopalakrishnan G, Venkatesan P, Geetha K (2008) Larvicidal activity of some Euphorbiaceae plant extracts against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol Res 102:867–873

Agalya Priyadarshini K, Murugan K, Panneerselvam C, Ponarulselvam S, Hwang J-S, Nicoletti M (2012) Biolarvicidal and pupicidal potential of silver nanoparticles synthesized using *Euphorbia hirta* against *Anopheles stephensi* Liston (Diptera: Culicidae). Parasitol Res 111:997–1006

Akram DS, Ahmed S (2005) Dengue fever. Infect Dis J 14:124–125

Ales Pana C, Milan K, Renata V, Robert P, Jana S, Vladimir K, Petr H, Radek Z, Libor K (2009) Antifungal activity of silver nanoparticles against *Candida* spp. Biomater 30:6333–6340

Benítez T, Rincón AM, Limoń MC, Codón AC (2004) Biocontrol mechanisms of *Trichoderma* strains. Int Microbiol 7:249–260

Chakravarti A, Kumaria R (2005) Eco-epidemiological analysis of dengue infection during an outbreak of dengue fever, India. Virology J 2:32

Chan YS, Mat Don M (2013) Biosynthesis and structural characterization of Ag nanoparticles from white rot fungi. Mater Sci Eng:C 33:282–288

Das D, Chandra G (2012) Mosquito larvicidal activity of *Rauvolfia serpentine* L. seeds against *Culex quinquefasciatus* Say. Asian Pac J Trop Med 5(1):42–45

Fayaz AM, Balaji K, Girilal M, Yadav R, Kalaichelvan PT, Venketesan R (2010) Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria. Nanomedicine:NBM 6:103–109

Fradin MS, Day JF (2002) Comparative efficacy of insect repellent against mosquito bites. New Engl J Med 347:13–18

Ganesh Babu MM, Gunasekaran P (2013) Extracellular synthesis of crystalline silver nanoparticles and its characterization. Mater Lett 90:162–164

Govindarajan M, Jebanesan A, Reetha D (2005) Larvicidal effect of extracellular secondary metabolites of different fungi against the mosquito, *Culex quinquefasciatus* Say. Trop Biomed 22:1–3

Harris A, Rajatileka S, Ranson H (2010) Pyrethroid resistance in *Aedes aegypti* from Grand Cayman. Am J Trop Med Hyg 83:277–284

Janerio P, Corduneann O, Brett AMO (2005) Chrysin and (±)-taxifolin electrochemical oxidation mechanisms. Electroanal 17: 1059–1064

Jayaseelan C, Rahuman AA, Rajakumar G, Santhoshkumar T, Kirthi AV, Marimuthu S, Bagavan A, Kamaraj C, Zahir AA, Elango G, Velayutham K, Rao KV, Karthik L, Raveendran S (2011) Efficacy of plant-mediated synthesized silver nanoparticles against hematophagous parasites. Parasitol Res 11:2473–2476

Kamaraj C, Abdul Rahuman A, Bagavan A (2008) Antifeedant and larvicidal effects of plant extracts against *Spodoptera litura* (F.), *Aedes aegypti* L. and *Culex quinquefasciatus* Say. Parasitol Res 103:325–331

Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N (2010) Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf B 76:50–56

Kovendan K, Murugan K, Vincent S, Kamalakannan S (2011) Larvicidal efficacy of *Jatropha curcas* and bacterial insecticide, *Bacillus thuringiensis*, against lymphatic filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol Res 109: 1251–1257

Kovendan K, Arivoli S, Maheswaran R, Baskar K, Vincent S (2012) Larvicidal efficacy of *Sphaeranthus indicus*, *Cleistanthus collinus* and *Murraya koenigii* leaf extracts against filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol Res 111: 1025–1035

Lara HH, Ayala-Nuñez NV, Ixtepan-Turrent L, Rodriguez-Padilla C (2010) Mode of antiviral action of silver nanoparticles against HIV-1. J Nanobiotechnol 8:1–10

Liu ZL, He Q, Chu SS, Wang CF, Du SS, Deng ZW (2012) Essential oil composition and larvicidal activity of *Saussurea lappa* roots against the mosquito *Aedes albopictus* (Diptera: Culicidae). Parasitol Res 110:2125–2130

Maheswaran R, Sathish S, Ignacimuthu S (2008) Larvicidal activity of *Leucas aspera* (Willd.) against the larvae of *Culex quinquefasciatus* Say. and *Aedes aegypti* L. Int J Integr Biol 2:214–217

Marimuthu S, Rahuman AA, Rajakumar G, Santhoshkumar T, Vishnu Kirthi A, Jayaseelan C, Bagavan A, Abduz Zahir A, Elango G, Kamaraj C (2011) Evaluation of green synthesized silver nanoparticles against parasites. Parasitol Res 108:1541–1549

Maynard AD (2007) Nanotechnology: the next big thing, or much ado about nothing? Ann Occup Hyg 51:1–12

Mohanpuria P, Rana NK, Yadav SK (2008) Biosynthesis of nanoparticles: technological concepts and future applications. J Nanopart Res 10:507–517

Morons JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramfrez JT, Yacaman MJ (2005) The bactericidal effect of silver nanoparticles. Nanotechnology:NBM 16:2346–2353

Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Ramani R, Parischa R, Ajaykumar PV, Alam M, Sastry M, Kumar R (2001) Bioreduction of $AuCl_4$-ions by the fungus, *Verticillium sp*. and surface trapping of the gold nanoparticles formed. Angew Chem Int Edit 40:3585–3588

Murugan K, Kovendan K, Vincent S, Barnard DR (2012) Biolarvicidal and pupicidal activity of *Acalypha alnifolia* Klein ex Willd. (Family: Euphorbiaceae) leaf extract and microbial insecticide, *Metarhizium anisopliae* (Metsch.) against malaria fever mosquito, *Anopheles stephensi* Liston. (Diptera: Culicidae). Parasitol Res 110: 2263–2270

Oksanen T, Pere J, Paavilainen L, Buchert J, Viikari L (2000) Treatment of recycled kraft pulps with *Trichoderma reesei* hemicellulases and cellulases. J Biotechnol 78:39–44

Paily KP, Geetha I, Kumar BA, Balaraman K (2012) *Bacillus sphaericus* in the adults of *Culex quinquefasciatus* mosquitoes emerged from treated larvae and its effect on development of the filarial parasite, *Wuchereria bancrofti*. Parasitol Res 110:2229–2235

Polson KA, Brogdon WG, Rawlins SC, Chadee DD (2011) Characterization of insecticide resistance in Trinidadian strains of *Aedes aegypti* mosquitoes. Acta Trop 117:31–38

Ponarulselvam S, Panneerselvam C, Murugan K, Aarthi N, Kalimuthu K, Thangamani S (2012) Synthesis of silver nanoparticles using leaves of *Catharanthus roseus* Linn.G.Don and their antiplasmodial activities. Asian Pac J Trop Biomed 2(7):574–581

Priyadarshini S, Gopinath V, Meera Priyadharsshini N, Mubarak Ali D, Velusamy P (2013) Synthesis of anisotropic silver nanoparticles using novel strain, *Bacillus flexus* and its biomedical application. Colloid Surface B 102:232–237

Raffi M, Hussain F, Bhatti TM, Akhter JI, Hameed A, Hasan HM (2008) Antibacterial characterization of silver nanoparticles against *E. coli* ATCC-15224. J Mater Sci Technol 24:192–196

Raghavendra BS, Prathibha KP, Vijayan VA (2011) Larvicidal efficacy of *Eugenia jambolana* Linn. extracts in three mosquito species at Mysore. J Entomol 8:491–496

Rajakumar G, Rahuman AA (2011) Larvicidal activity of synthesized silver nanoparticles using *Eclipta prostrate* leaf extract against filariasis and malaria vectors. Acta Trop 118:196–203

Ranson H, Rossiter L, Ortelli F, Jensen B, Wang XL (2001) Identification of a novel class of insect glutathione S-transferases involved in resistance to DDT in the malaria vector *Anopheles gambiae*. Biochem J 359:295–304

Ranson H, Burhani J, Lumjuan N, Black WC (2010) Insecticide resistance in dengue vectors. TropIKA.net:1–12

Rifai MA (1969) A revision of the genus *Trichoderma*. Commonw Mycol Inst Mycol Pap 116:1–56

Saleh MS, El Meniawi FA, Kelada NL, Zahran HM (2003) Resistance development in mosquito larvae *Culex pipiens* to the bacterial agent *Bacillus thuringiensis* var. *israelensis*. J Appl Entomol 127:29–32

Salunkhe RB, Patil SV, Patil CD, Salunke BK (2011) Larvicidal potential of silver nanoparticles synthesized using fungus *Cochliobolus lunatus* against *Aedes aegypti* (Linnaeus, 1762) and *Anopheles stephensi* Liston (Diptera: Culicidae). Parasitol Res 109:823–831

Santhoshkumar T, Abdul Rahuman A, Rajakumar G, Marimuthu S, Bagavan A, Jayaseelan C, Zahir AA, Elango G, Kamaraj C (2011) Synthesis of silver nanoparticles using *Nelumbo nucifera* leaf extract and its larvicidal activity against malaria and filariasis vectors. Parasitol Res 108:693–702

Santhoshkumar T, Abdul Rahuman A, Bagavan A, Marimuthu S, Jayaseelan C, Vishnu Kirthi A, Kamaraj C, Rajakumar G, Abduz Zahir A, Elango G, Velayutham K, Iyappan M, Siva C, Karthik L, Bhaskara Rao KV (2012) Evaluation of stem aqueous extract and synthesized silver nanoparticles using *Cissus quadrangularis* against *Hippobosca maculate* and *Rhipicephalus* (Boophilus) *microplus*. Exp Parasitol 132:156–165

Sastry M, Ahmad A, Khan MI, Kumar R (2003) Biosynthesis of metal nanoparticles using fungi and actinomycete. Curr Sci 85:162–170

Sintubin L, Verstraete W, Boon N (2012) Biologically produced nanosilver: current state and future perspectives. Biotechnol Bioeng 109:2422–2436

Song HY, Ko KK, Oh IH, Lee BT (2006) Fabrication of silver nanoparticles and their antimicrobial mechanisms. Eur Cell Mater 11:59

Soni N, Prakash S (2012) Efficacy of fungus mediated silver and gold nanoparticles against *Aedes aegypti* larvae. Parasitol Res 110:175–184

SPSS (2007) SPSS for windows. Version 13.0.SPSS, Chicago

Sundaravadivelan C, Nalini M (2012) Biolarvicidal effect of phytosynthesized silver nanoparticles using *Pedilanthus tithymaloides* (L.) Poit stem extract against the dengue vector *Aedes aegypti* L. (Diptera: Culicidae). Asian Pac J Trop Biomed 1–8

Sundaravadivelan C, Nalini M, Sivaprasath P, Kishmu L (2013) Biosynthesized silver nanoparticles from *Pedilanthus tithymaloides* leaf extract with anti-developmental activity against larval instars of *Aedes aegypti* L. (Diptera: Culicidae). Parasitol Res 112:303–311

Sur B, Nigam N, Joshi AK, Bihari V (2003) Characterization of mosquito larvicidal *Bacillus thuringiensis* isolated from soils of India. Indian J Biotechnol 2:268–270

Syed Ali M, Ravikumar S, Margaret Beula J (2012) Bioactivity of seagrass against the dengue fever mosquito *Aedes aegypti* larvae. Asian Pac J Trop Biomed 2(7):570–573

Tomass Z, Hadis M, Taye A, Mekonnen Y, Petros B (2011) Larvicidal effects of *Jatropha curcas* L. against *Anopheles arabiensis* (Diptera: Culicidea). MEJS 3:52–64

Udayasoorian C, Vinothkumar K, Jayabalakrishnan RM (2011) Extracellular synthesis of silver nanoparticles using leaf extract of *Cassia aurinculata*. Dig J Nanomater Bios 6:279–283

Vahabi K, Mansoori GA, Karimi S (2011) Biosynthesis of silver nanoparticles by fungus *Trichoderma reesei* (a route for large-scale production of AgNPs). Insciences J 1:65–79

Vaseeharan B, Clara Gunapoorani S, Lin YC, Chen JC (2012) Green synthesis of silver nanoparticles through *Calotropis gigantea* leaf extracts and evaluation of antibacterial activity against *Vibrio alginolyticus*. Nanotechnol Dev 2:12–16

Vigneshwaran N, Ashtaputre NM, Varadarajan PV, Nachane RP, Paralikar KM, Balasubramanya RH (2007) Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. Mater Lett 61:1413–1418

Vinay Gopal J, Thenmozhi M, Kannabiran K, Rajakumar G, Velayutham K, Abdul Rahuman A (2013) Actinobacteria mediated synthesis of gold nanoparticles using *Streptomyces* sp. VITDDK3 and its antifungal activity. Mater Lett 93:360–362

Vinayachandra, Shwetha R, Chandrashekar KR (2011) Larvicidal activities of *Knema attenuate* (Hook.f. & Thomson) Warb. (Myristicaceae) extracts against *Aedes albopictus* Skuse and *Anopheles stephensi* Liston. Parasitol Res 109:1671–1676

Wijnhoven SWP, Peijnenburg WJGM, Herberts CA, Hagens WI, Oomen AG, Heugens EHW, Roszek B, Bisschops J, Gosens I, Meent DVD, Dekkers S, Jong WHD, Zijverden MV, Sips AJAM, Geertsma RE (2009) Nano-silver—a review of available data and knowledge gaps in human and environmental risk assessment. Nanotox 3: 109–138

World Health Organization (1996) Report of the WHO informal consultation on the evaluation on the testing of insecticides. CTD/WHO PES/IC/96.l. WHO, Geneva:69

Yamanaka M, Hara K, Kudo J (2005) Bactericidal actions of a silver ion solution on *Escherichia coli*, studied by energy-filtering transmission electron microscopy and proteomic analysis. Appl Environ Microbiol 71:7589–7593

Yang YC, Lee SG, Lee HK, Kim MK, Lee SH, Lee HS (2002) A piperidine amide extracted from *Piper longum* L. fruit shows activity against *Aedes aegypti* mosquito larvae. J Agr Food Chem 50: 3765–3767

Zargar M, Hamid AA, Bakar FA, Shamsudin MN, Shameli K, Jahanshiri F, Farahani F (2011) Green synthesis and antibacterial effect of silver nanoparticles using *Vitex nugundo* L. Molecules 16:6667–6676