RESEARCH ARTICLE

FUNGUS GENERATED NOVEL NANO PARTICLES: A NEW PROSPECTIVE FOR MOSQUITO CONTROL

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INTRODUCTION

Diseases are spread like malaria, filariasis, dengue and chikungunya etc. by mosquitoes. Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected mosquitoes. Anopheles species are the most important species as they are capable vector for malaria parasites. According to the latest estimates, there were about 219 million cases of malaria in 2010 (with an uncertainty range of 154 million to 289 million) and an estimated 660 000 deaths (with an uncertainty range of 490 000 to 836 000). Malaria mortality rates have fallen by more than 25% globally since 2000 and by 33% in the WHO African Region. Most deaths occur among children living in Africa where a child dies every minute from malaria. Country-level burden estimates available for 2010 show that an estimated 80% of malaria deaths occur in just 14 countries and about 80% of cases occur in 17 countries. Together, the Democratic Republic of the Congo and Nigeria account for over 40% of the estimated total of malaria deaths globally (World Health Organization 2013a). Moreover, Culex mosquitoes are painful and persistent biters and are responsible for filariasis. Lymphatic filariasis is a neglected tropical disease. Nearly 1.4 billion people in 73 countries worldwide are threatened by lymphatic filariasis, commonly known as elephantiasis. Over 120 million people are currently infected, with about 40 million disfigured and incapacitated by the disease (World Health Organization 2013b). Aedes mosquitoes on the other hand are also painful and persistent biters. Aedes aegypti could also be responsible for spreading Dengue. The incidence of dengue has grown dramatically around the world in recent decades. Over 2.5 billion people – over 40% of the world’s population – are now at risk from dengue. WHO currently estimates there may be 50–100 million dengue infections worldwide every year (World Health Organization 2012)?

There is a need to control mosquito population so that people can be protected from mosquito borne diseases. Fungi and fungus–derived products are highly toxic to mosquitoes, yet have low toxicity to non-target organisms (Govindrajan et al., 2005). But there is a problem with fungi because fungal metabolites have the slow reaction on the target organisms. There is an urgent need to develop new insecticides for controlling mosquitoes which are more environmentally safe and also biodegradable and target specific against parasites. Fungi are currently been used for nanoparticles synthesis. Many of the fungi like Phytophthora infestans (Thirumurugan et al., 2009), Trichoderma reesi (Vahabi et al., 2011), Aspergillus (Bharathidasan and Panneerselvam, 2012; Moharrer et al., 2012; Alexandre et al., 2012; Kumar et al., 2012; Raliya and Tarafdar, 2012; Soni and Prakash, 2011; Saha et al., 2012; Gupta and Bector, 2013), Schizophyllum (Chan and Don, 2012) and Epicoccum nigrum (Quian et al., 2013) have been used for synthesis of silver and gold nanoparticles. Polymethacrylate (PMA) stabilized silver nanoparticles synthesized by UV...
irradiation has been evaluated. Larvicidal activity of silver and gold nanoparticles synthesized by Chrysosporum tropicum has been screened against the An. stephensi, Cx. quinquefasciatus and Ae. aegypti larvae (Soni and Prakash, 2012a, d). The adulticidal efficacies of C. keratinophilum, F. oxysporum f.sp. pisi and V. lecanii have been determined against the adults of Cx. quinquefasciatus (Soni and Prakash, 2012c). The larvicidal potential of silver nanoparticles synthesized by using fungus C. lunatus against Ae. aegypti and An. stephensi have been observed (Salunkhe et al., 2011). In the present study we have synthesized of silver and gold nanoparticles by using fungus F. oxysporum f.sp. pisi. Further, these synthesized AgNPs and AuNPs have also been tested as larvicides and pupicides against the larvae and pupae of An. stephensi, Cx. quinquefasciatus and Ae. aegypti. This could be a rapid way to avoid resistance problem effectively minimized while using new fungal based nanolarvicide and nanopupicide.

MATERIALS AND METHODS
Microorganism and their culture on broth for biomass production
The fungal strain of F. oxysporum f.sp. pisi (MTCC 2480) was procured from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology Chandigarh India. This strain was routinely maintained in our laboratory on Saubouraud’s Dextrose Agar (SDA) medium at 25°C. For fungal culture, broth was prepared by the suggested method (Gardner and Pillai, 1987). F. oxysporum f.sp. pisi was grown on Potato Dextrose Broth (PDB). Five 250 ml conical flask, each containing 100 ml PDB (Infusion of potatoes 200 g, Dextrose 20 g and deionized water 1000 mL) were autoclaved at 20 psi for 20 min. The broth was supplemented 50 µg/ml chloramphenicol as a bacteriostatic agent. F. oxysporum f.sp. pisi colonies grown on Potato Dextrose Agar plates were transferred to each flask using the inoculation needle. The conical flasks inoculated with F. oxysporum f.sp. pisi were incubated 25°C for 15 days. After 15 days of incubation, the fungal biomass was separated from the culture media by filtration through Whatman-1 filter paper and washed three times to remove nutrient media from the fungal biomass.

Synthesis and characterization of AgNPs and AuNPs
The 10 g of wet biomass of F. oxysporum f.sp. pisi was placed into a 250 mL of conical flask containing 100 mL of deionized water and incubated for 72 h at 25°C. After then, the aqueous solution components were separated by filtration using Whatman-1 filter paper. To this solution (liquid fungal), AgNO₃ and HAuCl₄ (10⁻³ M) was added and kept for 72 h at 25°C. Simultaneously, control with fungal liquid of F. oxysporum f.sp. pisi without AgNO₃ and HAuCl₄ was maintained under same conditions, separately. Periodically, aliquots of the reaction solutions were removed and their absorption was measured in a UV-3600 Shimadzu spectrophotometer. The micrographs of silver and gold nanoparticles were obtained by Philips CM-10 Transmission electron microscope and conformed by Scanning electron microscope.

Rearing of mosquitoes
The mosquito larvae of An. stephensi, Cx. quinquefasciatus and Ae. aegypti were collected from the botanical garden of Dayalbagh Educational Institute, Agra, campus and local area of Agra. These mosquito colonies were reared in the laboratory in separate enamel container containing deionized water and supplemented with glucose and yeast powder at 25°C, with a relative humidity of 75±5% and 14 h photoperiod as per standard method (Geberg et al., 1994).

Data management, statistical analysis of mosquito larvicidal and pupicidal bioassays
F. oxysporum f.sp. pisi synthesized AgNPs and AuNPs larvicidal and pupicidal tests were performed against the larvae and pupae of An. stephensi, Cx. quinquefasciatus and Ae. aegypti as per WHO method (World Health Organization, 2005). All mosquito larvae of An. stephensi, Cx. quinquefasciatus and Ae. aegypti were separated and placed in a container in microbe free deionized water. After that different test concentrations of Ag and Au NPs in 100 mL deionized were prepared in 250 mL beakers. Bioassays were conducted separately for each instar at six different test log concentrations (0.30, 0.60, 0.77, 0.90, 1, and 1.08 ppm and 0.77, 1.07, 1.25, 1.38, 1.47, and 1.55 ppm) of aqueous Ag and Au NPs. To test the larvicidal and pupicidal activity of Ag and Au NPs, 20 larvae of each stage were separately exposed to 100 ml of test concentration. Similarly, the control (without Ag and Au NPs) was run to test the natural mortality. Thereafter, we could further examine the mortality which was determined after different hours of the treatment, the experiment time. No food was offered to the larvae and pupae during the experiments. Experiments were replicated thrice to validate the results. The data on the efficacy were subjected to probit analysis (Finney, 1971). The control mortality was corrected by Abbott’s formula (Abbott, 1925).

RESULTS
UV-Visible analysis of NPs
By the mixing the fungus liquid with the aqueous solution of Ag and Au ions, the color of the fungal liquids changed from yellow to ruby red and dark brown after 72 h. The colour change is therefore a signal for the formation of Ag and Au nanoparticles. Because without treating with the Ag and Au ions there was no change in the colour of cell free extract of F. oxysporium f.sp. pisi while, after addition the Ag and Au ions the colourless solution change into coloured solution which has been described in the previous study (Salunkhe et al., 2011; Du et al., 2011).

[Fig. 1 UV-Visible spectra of silver (a) and gold (b) nanoparticles synthesized by using the F. oxysporum f.sp. pisi.]

The color of the solution is due to the excitation of surface plasmon vibrations (essentially the vibration of the group conduction electrons) in the Ag and Au NPs. Fig. 1a, b shows the Micro-scan spectra of Ag and Au NPs synthesized with F.
Efficacies of AgNPs and AuNPs against Ae. aegypti

The larval stages of Cx. quinquefasciatus were found more susceptible to AgNPs than the AuNPs synthesized by F. oxysporum f.sp. pisi. The first and second instar larvae were found highly susceptible to the silver nanoparticles than the other instars. The mortality was recorded after 24 h and 48 h exposure of silver and gold. The first and second instars of Cx. quinquefasciatus have been shown 100% mortality to the AgNPs synthesized by F. oxysporum f.sp. pisi. Whereas, for third instars (LC$_{50}$ 6 ppm, LC$_{90}$ 10.71 ppm, LC$_{99}$ 15.84 ppm) and fourth instars (LC$_{50}$ 10 ppm, LC$_{90}$ 11.22 ppm, and LC$_{99}$ 16.98 ppm), while, the pupa have shown the efficacy (LC$_{50}$ 4, LC$_{90}$ 13 and LC$_{99}$ 15 ppm) after 20 h were observed with their probit equations, confidential limits, mortality rate and chi-square values after 24h (Table 1). Chi-square values for third and fourth instars of Cx. quinquefasciatus were found higher than the critical value of chi-square at 0.05 significance level. In control group no mortality could be observed. The observed LC values have shown the degree of susceptibility of fungal AgNPs amongst the four larval stages of Cx. quinquefasciatus in order of first instar > second instar > third instar > fourth instar. The first and second instars of Cx. quinquefasciatus have been shown 100% mortality to the AuNPs synthesized by F. oxysporum f.sp. pisi. Whereas, for third instars (LC$_{50}$ 12.58 ppm, LC$_{90}$ 30 ppm, LC$_{99}$ 42.65 ppm) and fourth instars (LC$_{50}$ 30 ppm, LC$_{90}$ 46.77 ppm, and LC$_{99}$ 91.20 ppm) were observed with their probit equations, confidential limits, mortality rate and chi-square values after 48h (Table 1). Chi-square values for third and fourth instars of Cx. quinquefasciatus were found higher than the critical value of chi-square at 0.05 significance level. In control group no mortality could be observed. The observed LC values have shown the degree of susceptibility of fungal AuNPs amongst the four larval stages of Cx. quinquefasciatus in order of first instar > second instar > third instar > fourth instar. However, no adverse effect could be observed against the pupa.

Efficacies of AgNPs and AuNPs against Cx. quinquefasciatus

The larval stages of Cx. quinquefasciatus were found more susceptible to AgNPs than the AuNPs synthesized by F. oxysporum f.sp. pisi. The first and second instar larvae of Cx. quinquefasciatus were found highly susceptible to AgNPs than the other instars. The mortality was recorded after 24 h of exposure only. The first and second instar larvae of An. stephensi have shown the efficacy (LC$_{50}$ 1.77, LC$_{90}$ 12.30, LC$_{99}$ 13.18 ppm, respectively) to the silver nanoparticles synthesized by F. oxysporum f.sp. pisi. Whereas, for third instars (LC$_{50}$ 6 ppm, LC$_{90}$ 14.12 ppm, and LC$_{99}$ 14.45 ppm) and fourth in stars (LC$_{50}$ 4 ppm, LC$_{90}$ 12 ppm, and LC$_{99}$ 12.58 ppm) were observed with their probit equations, confidential limits, mortality rate and chi-square values after 24 h (Table 1). Chi-square values for first, second, third and fourth instars of An. stephensi were found higher than the critical value of chi-square at 0.05 significance level. In control group no mortality could be observed. The observed LC values have shown the degree of susceptibility of fungal AgNPs amongst the four larval stages of An. stephensi in order of first instar > second instar > third instar > fourth instar. The AgNPs synthesized by using the F. oxysporum f.sp. pisi were found least effective against the pupae of An. stephensi. However, no adverse effects could be observed for AuNPs synthesized by F. oxysporum f.sp. pisi against the larvae and pupae of An. stephensi.
Table 1  Efficacies of silver and gold nanoparticles synthesized by using cell free extract of *F. oxysporum* f.sp. pisi against the larvae and pupae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* after different time (hours) of exposure

<table>
<thead>
<tr>
<th>Nanoparticles</th>
<th>Time (Hours)</th>
<th>Instar</th>
<th>Probit equation</th>
<th>LC95 (95% CL)</th>
<th>LC90 (95% CL)</th>
<th>LC80 (95% CL)</th>
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<th>( r^2 )</th>
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<tbody>
<tr>
<td><em>An. stephensi</em></td>
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<tr>
<td>Ag</td>
<td>24</td>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>y=0.59+6.52x</td>
<td>1.77 (0.60-2.94)</td>
<td>12.30 (11.18-13.42)</td>
<td>13.18 (12.04-14.32)</td>
<td>47.15</td>
<td>0.99</td>
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<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>y=0.58+5.66x</td>
<td>2.0 (0.83-3.17)</td>
<td>12.30 (11.18-13.42)</td>
<td>13.18 (12.04-14.32)</td>
<td>46.17</td>
<td>0.99</td>
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<td></td>
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<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>y=0.46+5.91x</td>
<td>6.49 (4.96-7.04)</td>
<td>14.12 (12.95-15.29)</td>
<td>14.45 (13.28-16.52)</td>
<td>37.66</td>
<td>0.98</td>
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<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>y=0.45+6.46x</td>
<td>4.29 (3.53-5.07)</td>
<td>12.10 (10.88-13.12)</td>
<td>12.58 (11.44-13.72)</td>
<td>43.22</td>
<td>0.86</td>
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<td>Pupa</td>
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<td><em>Cx. Quinquefasciatus</em></td>
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<tr>
<td>Ag</td>
<td>24</td>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>y=0.54+5.78x</td>
<td>6.49 (4.96-7.04)</td>
<td>10.71 (9.62-11.88)</td>
<td>15.84 (14.76-16.98)</td>
<td>28.74</td>
<td>0.97</td>
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<td></td>
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<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>y=0.55+6.62x</td>
<td>10.88 (11.12)</td>
<td>11.22 (10.13-12.34)</td>
<td>16.98 (15.78-18.18)</td>
<td>37.42</td>
<td>0.98</td>
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<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>y=0.45+5.62x</td>
<td>4.28 (6.54)</td>
<td>13.11 (11.86-14.14)</td>
<td>15 (13.36-16.14)</td>
<td>45.26</td>
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<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>y=0.13+4.40x</td>
<td>12.58 (11.49-13.67)</td>
<td>30 (28.88-31.12)</td>
<td>42.6 (41.46-43.82)</td>
<td>19.54</td>
<td>0.93</td>
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<td>5&lt;sup&gt;th&lt;/sup&gt;</td>
<td>y=0.17+3.64x</td>
<td>30 (28.36-31.14)</td>
<td>46.77 (45.57-47.97)</td>
<td>91.20 (88.86-92.54)</td>
<td>12.27</td>
<td>0.95</td>
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<td><em>Ae. aegypti</em></td>
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<tr>
<td>Ag</td>
<td>1</td>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>y=0.45+5.67x</td>
<td>8.61 (9.09)</td>
<td>12.30 (11.16-13.44)</td>
<td>15.48 (14.36-16.65)</td>
<td>35.74</td>
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<td></td>
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<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>y=0.43+6.07x</td>
<td>6.49 (7.04)</td>
<td>12.58 (11.46-13.7)</td>
<td>13.48 (12.36-14.6)</td>
<td>39.20</td>
<td>0.95</td>
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<tr>
<td></td>
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<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>y=0.44+6.07x</td>
<td>4.23 (5.07)</td>
<td>11.48 (10.39-12.57)</td>
<td>12.88 (11.76-14.0)</td>
<td>47.32</td>
<td>0.95</td>
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<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>y=0.16+4.01x</td>
<td>18 (16.99-8.09)</td>
<td>37.15 (36.01-38.29)</td>
<td>60.25 (59.02-61.48)</td>
<td>16.36</td>
<td>0.92</td>
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<tr>
<td></td>
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<td>5&lt;sup&gt;th&lt;/sup&gt;</td>
<td>y=0.22+4.12x</td>
<td>6.48 (4.33-7.17)</td>
<td>38.01 (36.87-39.15)</td>
<td>52.48 (51.28-53.68)</td>
<td>18.63</td>
<td>0.91</td>
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<td>Pupa</td>
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** 100% mortality  
-- no mortality

Whereas, for first instars (LC90 = 8 ppm, LC95 = 12.30 ppm, LC80 = 15.48 ppm), second instars (LC90 = 6 ppm, LC95 = 12.58 ppm, and LC80 = 13.48 ppm), fourth instars (LC90 = 4 ppm, LC95 = 11.84 ppm, LC80 = 12.88 ppm) after 1 h and pupa (LC90 = 2, LC90 = 11 and LC80 = 13 ppm) after 2 h, were observed with their probit equations, confidential limits, mortality rate and chi-square values (Table 1). Chi-square values for first, second and fourth instars of *Ae. aegypti* were found higher than the critical value of chi-square at 0.05 significance level. In control group no mortality could be observed. The observed LC values have shown the degree of susceptibility of fungal AuNPs amongst the four larval stages of *Ae. aegypti* in order of first instar < second instar < third instar > fourth instar. Moreover, the first and third instars of *Ae. aegypti* have been shown 100% mortality to the AuNPs synthesized by *F. oxysporum* f.sp. pisi. Whereas, for second instars (LC90 = 18 ppm, LC90 = 37.15 ppm, LC90 = 60.25 ppm) and fourth instars (LC90 = 6 ppm, LC90 = 38.01 ppm, and LC90 = 18.63 ppm) were observed with their probit equations, confidential limits, mortality rate and chi-square values after 48 h (Table 1). While, no mortality could be observed against the pupa. Chi-square values for second and fourth instars of *Ae. aegypti* were found higher than the critical value of chi-square at 0.05 significance level. In control group no mortality could be observed. The observed LC values have shown the degree of susceptibility of fungal AuNPs amongst the four larval stages of *Ae. aegypti* in order of first instar > second instar > third instar < fourth instar.

**DISCUSSION**

The selected fungal species like *F. oxysporum* f.sp. pisi is a keratinophilic fungus. It is being used for the first time to evaluate the larvicial and pupicidal effect of AgNPs and AuNPs against the larvae and pupae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* larvae. The extracellular biosynthesis of silver nanoparticles (AgNPs) by using a fungus named *Trichoderma Rees* (Vahabi et al., 2011). Similarly, biosynthesis of silver nanoparticles using *Trichosporon beigelli* NCIM 3326 and their antimicrobial activity has been evaluated (Ghodake et al., 2011). Consensus has emerged that reduction of the aqueous silver ions occurs by an enzymatic process thus showing a possibility of development of an eco-friendly, fungal-based nanomaterial synthesis. Unlike other mosquito control agents, the entomopathogenic fungi synthesized AgNPs and AuNPs unique. Fungal synthesized AgNPs and AuNPs have the ability to directly infect the host insect by penetrating into the cuticle and do not need to ingest by the insect to cause disease. There are preferential advantages when we use fungal AuNPs as biocontrol agent for mosquitoes. The fungal AuNPs have very narrow range. Considerable progress has been made in recent years in development of environmentally benign spores and mycelium-based biocontrol agent for the mosquito population. Fungal biocontrol agents have reduced inputs of harmful synthetic chemical pesticide in agriculture, horticultural, and forest system. The potential of the hexane, chlorofom, ethyl acetate, acetone, methanol, and aqueous leaf extracts of *Nelumbo nucifera* and synthesized silver nanoparticles using aqueous leaf extract against fourth instar larvae of *An. subpictus* and *Cx. quinquefasciatus* have already been tested (Santhoshkumar et al., 2011). Larvae were exposed to varying concentrations of plant extracts and synthesized silver nanoparticles for 24 h. Recently, the larvicidal activity of synthesized silver nanoparticles using *Eclipta prostrata* leaf extract against filariasis and malaria vector has been evaluated (Rajkumar and Rahuman, 2011). These results were based on plant synthesized silver nanoparticles and have been tested against filariasis and malaria vectors. Whereas, in our work we have synthesized silver and gold nanoparticles using keratinophilic fungus *F. oxysporum* f.sp. pisi. These nanoparticles have also been tested against filariasis and dengue vector larvae and pupae, showing potential for enhanced efficacy. The larvicidal potential of silver nanoparticles synthesized using fungus *Cochliobolus lunatus* against *Ae. aegypti* and *An. stephensi*
has already been tested (Salunkhe et al., 2011). They have also tested the potential of C. lunatus silver nanoparticles against non-target fish species Poecilia reticulata, the most common organism in the habitats of A. aegypti and A. stephensi showed no toxicity at LC₅₀ and LC₅₀ doses of the AgNPs. The gold nanoparticles synthesized with A. niger has been tested against the mosquito larvae (Soni and Prakash 2012b). The silver nanoparticles have also been tested as adulticide against the Cx. quinquefasciatus mosquito (Soni and Prakash, 2012c). The nanoparticles synthesized with the help of fungus have been tested against the larvae of Cx. quinquefasciatus and An. stephensi (Soni and Prakash, 2012d). The previous results were based on the larvicultural efficacy of nanoparticles synthesized by fungi. While, in our study we have also synthesized the Ag and Au NPs with the help of fungus. The fungus synthesized NPs not only tested as larvicides but as a pupicides also. Biolarvicultural effect of phyto-synthesized silver nanoparticles using Pedilanthus tithymaloides (L.) Poit stem extract against the dengue vector Ae. aegypti has been tested (Sundaravadivelan and Nalini, 2012). Green synthesis of silver nanoparticles for the control of mosquito vectors of malaria, filariasis and dengue has been evaluated (Arjunan et al., 2012). The larvicultural activities to determine the efficacies of synthesized silver nanoparticles using aqueous leaf extract of V. rosea against the larvae of malaria vector An. stephensi Liston and filariasis vector Cx. quinquefasciatus Say has been tested (Subarani et al., 2012). Their results showed that the maximum efficacy was observed in synthesized AgNPs against the fourth instar larvae of An. stephensi (LC₅₀ 12.47 and 16.84 mg/mL and LC₉₀ 36.33 and 68.62 mg/ mL) on 48 and 72 h of exposure and against Cx. quinquefasciatus (LC₅₀ 43.80 mg/mL and LC₉₀ 120.54 mg/mL) on 72-h exposure, and aqueous extract showed 100 % mortality against An. stephensi and Cx. quinquefasciatus (LC₅₀ 78.62 and 55.21 mg/mL and LC₉₀ 184.85 and 112.72 mg/ mL) on 72-h exposure at concentrations of 50 mg/mL, respectively. The AgNPs did not exhibit any noticeable toxicity on Poecilia reticulata after 24, 48, and 72 h of exposure. These results suggest that the synthesized AgNPs have the potential to be used as an ideal eco-friendly approach for the control of the An. stephensi and Cx. quinquefasciatus. Here, the results showed that the efficacies after a long time of exposure. Whereas, in our study the synthesized NPs have shown the efficacies after short time of exposure.

The activity of silver nanoparticles (AgNPs) synthesized using P. rubra plant latex against second and fourth larval instar of Ae. aegypti and An. stephensi has been determined (Patil et al., 2012). They found that the synthesized AgNPs from P. rubra latex were highly toxic than crude latex extract in both mosquito species. The study on the activity of silver nanoparticles (AgNPs) synthesized using E. hirta plant leaf extract against malarial vector An. stephensi has been determined (Priyadarshini et al., 2012). Three types of nanosilica, namely lipophilic, hydrophilic and hydrophobic, to assess their larvicidal, pupicidal and growth inhibitory properties and also their influence on oviposition behaviour (attraction/deterrence) of mosquito species that transmit human diseases, namely malaria (Anopheles), yellow fever, chickungunya and dengue (Aedes), lymphatic filariasis and encephalitis (Culex and Aedes) have been tested (Barik et al., 2012). They found that the application of hydrophobic nanosilica at 112.5 ppm was found effective against mosquito species tested. The larvicidal effect of hydrophobic nanosilica on mosquito species tested was in the order of An. stephensi > Cx. quinquefasciatus > Ae. aegypti > Cx. quinquefasciatus, and the pupicidal effect was in the order of An. stephensi > Cx. quinquefasciatus > Ae. aegypti. The larvicidal activity of synthesized silver nanoparticles (AgNPs) using leaf extract of Nerium oleander (Apocynaceae) against the first to fourth instar larvae and pupae of malaria vector. An. stephensi (Diptera: Culicidae) has been determined (Roni et al., 2012). The acaricidal and larvicultural activity against the larvae of Haemaphysalis bispinosa Neumann (Acari: Ixodidae) and larvae of hematophagous fly Hippobosca maculate Leach (Diptera: Hippoboscoidea) and against the fourth-instar larvae of malaria vector, An. stephensi Liston, Japanese encephalitis vector, Cx. tritaeniorhynchus Giles (Diptera: Culicidae) of synthesized silver nanoparticles (AgNPs) utilizing aqueous leaf extract from Musa paradisiaca L. (Musaceae) has been investigated (Jayaseelan et al., 2011). The above results of efficacies of silver nanoparticles were based on the plant synthesized nanoparticles. Whereas, in the present study we could test the F. oxysporum fsp. pisi synthesized silver and gold nanoparticles against the larvae and pupae of An. stephensi, Cx. quinquefasciatus and Ae. aegypti.

**CONCLUSION**

The present investigation is probably the first report with synthesized silver and gold nanoparticles using keratinophilic fungus F. oxysporum f.sp. pisi and can be a successful candidate for mosquito control of vectors. The synthesized silver and gold nanoparticles have also been tested as a larvicide and pupicide agents against major mosquito larvae and pupae via: An. stephensi, Cx. quinquefasciatus and Ae. aegypti all major vectors of diseases in tropical world. The fungus mediated silver and gold nanoparticles have rapid impact on vector mosquitoes population. We can thus propose a new conclude that the fungus synthesized silver and gold nanoparticles could be a better, environmentally safer and greener approach for vector control strategy.

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