ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 4, Issue, 10, pp.1481-1487, October, 2013

International Journal of Recent Scientific Research

RESEARCH ARTICLE

FUNGUS GENERATED NOVEL NANOPARTICLES: A NEW PROSPECTIVE FOR MOSQUITO CONTROL

Namita Soni and Soam Prakash*

Environmental and Advanced Parasitology and Vector Control Biotechnology Laboratories, Department of Zoology, Faculty of Science, Dayalbagh Educational Institute, Dayalbagh, Agra, India-282 110

ARTICLE INFO

Article History:

Received 16th, September, 2013
Received in revised form 26th, September, 2013
Accepted 12th, October, 2013
Published online 28th October, 2013

Key words:
Fusarium oxysporum f.sp. pisi, nanoparticles, mosquito control

ABSTRACT

This work was to evaluate the efficacies of the nanoparticles (NPs) of silver (Ag) and Gold (Au) against the major tropical mosquitoes. In the present study, the Ag and Au NPs were synthesized by using the cell free extract of *Fusarium oxysporum* f.sp. pisi fungus. The bioreduction of AgNPs and AuNPs was monitored by ultraviolet-visible spectroscopy, and the AgNPs and AuNPs obtained were characterized by transmission electron microscopy and scanning electron microscopy. The synthesized AgNPs and AuNPs were spherical particles ranging in size from 20-40 nm (AgNPs) and 2-10 nm (AuNPs). Further, these synthesized NPs were also tasted as larvicides and pupicides against the larvae and pupae of *Cx. quinquefasciatus, An. stephensi* and *Ae. aegypti*. The efficacy test was performed at different concentrations for a period of different hours by the probit analysis. The maximum efficacy was observed in synthesized AgNPs against the larvae of *Ae. aegypti* (LC_{50} 8, 6, 4, LC_{90} 12.30, 12.58, 11.48, LC_{99} 15.48, 13.48, 12.88 ppm, respectively for first, second and fourth in stars) after 1 h and pupae (LC_{50}, 2, LC_{90} 11 and LC_{99} 13 ppm) after 2 h. The maximum efficacy was observed in AuNPs against the larvae of *Cx. quinquefasciatus* (LC_{50} 12.58, LC_{90} 30.00 and LC_{99} 42.65 ppm) after 48 h. This suggest that the synthesized Ag and Au NPs could be an environmentally safer, greener and better approach for mosquito control than current approach.

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 *Phytopthora infestans* (Thirumurugan *et al.*, 2009), *Trichoderma reesei* (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), *Rhizopus* (Das *et al.*, 2012a, b), *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

* Corresponding author: **Soam Prakash***
Department of Zoology, Faculty of Science, Dayalbagh Educational Institute, Dayalbagh, Agra, India-282 110

irradiation has been evaluated as larvicide against the *Ae. aegypti* (Sap-Iam *et al.*, 2010). Larvicidal activity of silver and gold nanoparticles synthesized by *Chrysosporium 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 Sauboraud's Dextrose Agar (SDA) medium at 25^0C. 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 25^0C 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 25^0C. After then, the aqueous solution components were separated by filtration using Whatman-1 filter paper. To this solution (liquid fungal), $AgNO_3$ and $HAuCl_4$ (10^{-3} M) was added and kept for 72 h at 25^0C. Simultaneously, control with fungal liquid of *F. oxysporum* f.sp. pisi without $AgNO_3$ and $HAuCl_4$ 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^oC, 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).

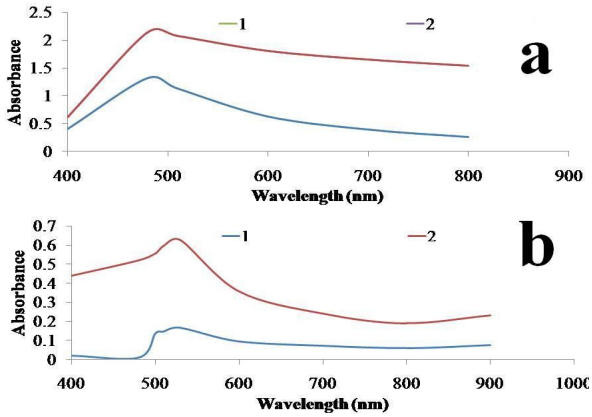

Fig. 1 UV-Visible spectra of silver (a) and gold (b) nanoparticles synthesized by using the *F. oxysporum* f.sp. pisi.

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.*

oxysporum f.sp. pisi recorded form the reaction medium before (curve 1) and after immersion of $AgNO_3$ and $HAuCl_4$ (curve 2) after 72 h. Absorption spectra of Ag and Au NPs formed in the reaction media has a broad absorption band centered at ca. 480 and 530 nm. The presence of broad resonance indicated an aggregated structure of the Ag and Au NPs in the solution.

Electron microscopic analysis of NPs

After reduction, Ag and Au NPs were precipitated at the bottom of conical flask. This precipitate was washed out twice with double distilled water and then analyzed by employing Philips CM-10 Transmission Electron Microscope. The samples of Ag and Au NPs synthesized using fungal liquid were prepared by placing a drop of reaction mixture over copper grid and allowing water to evaporate. Fig. 2a shows typical TEM micrographs of AuNPs of *F. oxysporum* f.sp. pisi. The different (2-10 nm) sized and spherical shaped AuNPs were observed. The SEM images are showing distinctly the high density AuNPs synthesized by *F. oxysporum* f.sp. pisi (Fig. 2b) fungal species further confirmed the development of Au nanostructures. The fig. 3a is showing the TEM micrographs of AgNPs of *F. oxysporum* f.sp. pisi. The different (20-40 nm) sized and spherical shaped AgNPs were observed. The SEM images are showing distinctly the high density AgNPs synthesized by *F. oxysporum* f.sp. pisi (Fig. 3b) fungal species further confirmed the development of Ag nanostructures.

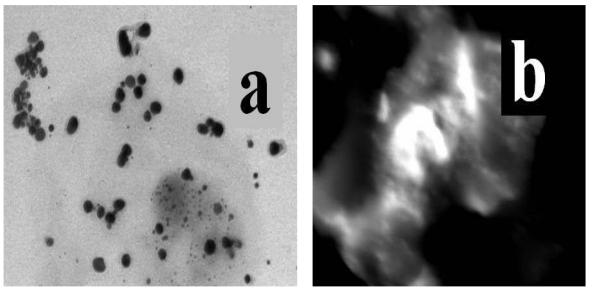

Fig. 2 (a) TEM and (b) SEM images of gold nanoparticles synthesized by using the *F. oxysporum* f.sp. pisi.

Efficacies of AgNPs and AuNPs against An. stephensi

The larvae of *An. stephensi* were found highly susceptible to the AgNPs than the AuNPs synthesized by *F. oxysporum* f.sp. pisi. 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 and LC_{50} 2, LC_{90} 12.30, LC_{99} 13.18 ppm, respectively) to the silver nanoparticles synthesized by *F. oxysporum* f.sp. pisi. Whereas, for third in stars (LC_{50} 6 ppm, LC_{90} 14.12 ppm, 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*.

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 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 48 h (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.

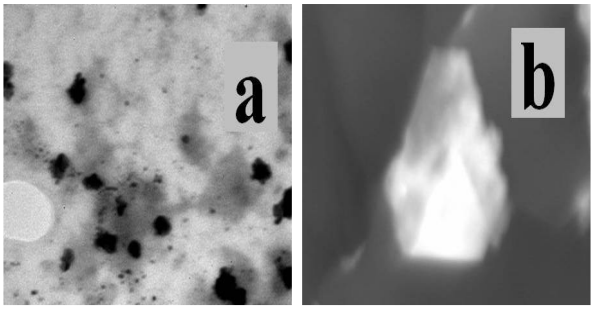

Fig. 3(a) TEM and (b) SEM images of silver nanoparticles synthesized by using the *F.oxysporum*f.sp.pisi

Efficacies of AgNPs and AuNPs against Ae. aegypti

The *Ae. aegypti* larvae were found highly susceptible to AgNPs than the AuNPs synthesized by *F. oxysporum* f.sp. pisi. The third instar larvae of *Ae. aegypti* were found more effective to the AgNPs than the other instars. The mortality was scored after 1 h of exposure. The first and second instar larvae were found highly susceptible to the gold nanoparticles than the other instars. The mortality was recorded after 48h of exposure. However, the third instars of *Ae. aegypti* have been shown 100% mortality to the AgNPs synthesized by *F. oxysporum* f.sp. pisi.

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

Nanoparticles	Time (Hours)	Instar	Probit equation	LC_{50} (95% CL)	LC_{90} (95% CL)	LC_{99} (95% CL)	χ^2	r^2
An stephensi								
Ag	24	1st	y=0.59+6.52x	1.77 (0.60-2.94)	12.30 (11.18-13.42)	13.18 (12.04-14.32)	47.15	0.99
		2nd	y=0.5+6.56x	2 (0.83-3.17)	12.30 (11.18-13.42)	13.18 (12.04-14.32)	46.17	0.98
		3rd	y=0.46+5.91x	6 (4.96-7.04)	14.12 (12.95-15.29)	14.45 (13.28-15.62)	37.66	0.98
		4th	y=0.45+6.46x	4 (2.93-5.07)	12 (10.88-13.12)	12.58 (11.44-13.72)	43.22	0.86
		Pupa	--	--	--	--	--	--
Au			--	--	--	--	--	--
Cx. Quinquefasciatus								
Ag								
	24	1st	**	**	**	**	**	**
		2nd	**	**	**	**	**	**
		3rd	y=0.34+5.78x	6 (4.96-7.04)	10.71 (9.62-11.8)	15.84 (14.7-16.98)	28.74	0.97
		4th	y=0.35+5.62x	10 (8.88-11.12)	11.22 (10.1-12.34)	16.98 (15.78-18.18)	37.42	0.98
	20	pupa	y=0.45+6.52x	4 (2.86-5.14)	13 (11.86-14.14)	15 (13.86-16.14)	45.26	0.92
Au	48	1st	**	**	**	**	**	**
		2nd	**	**	**	**	**	**
		3rd	y=0.13+4.40x	12.58 (11.49-13.67)	30 (28.88-31.12)	42.6 (41.48-43.82)	19.54	0.93
		4th	y=0.17+3.64x	30 (28.86-31.14)	46.77 (45.57-47.97)	91.20 (88.86-92.54)	12.27	0.95
		Pupa	--	--	--	--	--	--
Ae. aegypti								
Ag	1	1st	y=0.45+5.67x	8 (6.91-9.09)	12.30 (11.16-13.44)	15.48 (14.34-16.65)	35.74	0.96
		2nd	y=0.43+6.07x	6 (4.96-7.04)	12.58 (11.46-13.7)	13.48 (12.36-14.6)	39.20	0.95
		3rd	**	**	**	**	**	**
		4th	y=0.44+6.07x	4 (2.93-5.07)	11.48 (10.39-12.57)	12.88 (11.76-14.00)	47.32	0.95
	2	pupa	y=0.45+6.76x	2 (0.86-3.14)	11 (9.86-12.14)	13 (11.86-14.14)	48.30	0.79
Au	48	1st	**	**	**	**	**	**
		2nd	y=0.16+4.01x	18 (16.91-18.09)	37.15 (36.01-38.29)	60.25 (59.02-61.48)	16.36	0.92
		3rd	**	**	**	**	**	**
		4th	y=0.22+4.12x	6 (4.83-7.17)	38.01 (36.87-39.15)	52.48 (51.28-53.68)	18.63	0.91
		Pupa	--	--	--	--	--	--

**** 100% mortality**
-- no mortality

Whereas, for first instars (LC_{50} 8 ppm, LC_{90} 12.30 ppm, LC_{99} 15.48 ppm), second instars (LC_{50} 6 ppm, LC_{90} 12.58 ppm, and LC_{99} 13.48 ppm), fourth instars (LC_{50} 4 ppm, LC_{90} 11.48 ppm, LC_{99} 12.88 ppm) after 1 h and pupa (LC_{50} 2, LC_{90} 11 and LC_{99} 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 AgNPs amongst the four larval stages of *Ae. aegypti* in order of first instar < second instar < third instar > fourth instar. Morever, 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 (LC_{50} 18 ppm, LC_{90} 37.15 ppm, LC_{99} 60.25 ppm) and fourth instars (LC_{50} 6 ppm, LC_{90} 38.01 ppm, and LC_{99} 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 larvicidal 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 Reesei* (Vahabi *et al.,* 2011).

Similarly, biosynthesis of silver nanoparticles using *Trichosporon beigelii* 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, chloroform, 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 prostrate* 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. lunntus* 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 LC_{50} and LC_{90} 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 larvicidal 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. Biolarvicidal 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 larvicidal 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* (LC_{50} 12.47 and 16.84 mg/mL and LC_{90} 36.33 and 68.62 mg/ mL) on 48 and 72 h of exposure and against *Cx. quinquefasciatus* (LC_{50} 43.80 mg/mL and LC_{90} 120.54 mg/mL) on 72-h exposure, and aqueous extract showed 100 % mortality against *An. stephensi* and *Cx. quinquefasciatus* (LC_{50} 78.62 and 55.21 mg/mL and LC_{90} 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 inhibitor 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* > *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 larvicidal activity against the larvae of *Haemaphysalis bispinosa* Neumann (Acarina: Ixodidae) and larvae of hematophagous fly *Hippobosca maculate* Leach (Diptera: Hippoboscidae) 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* f.sp. 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.

Acknowledgements

We sincerely thank to acknowledge Prof P. S. Satsangi Sahab, Chairman, of Advisory Committee on Education, Dayalbagh Educational Institute. We thank to Prof. V. G. Das, Director, Dayalbagh Educational Institute for providing support and encouragements for the work. We thank to UGC-MRP (39-599/2012) for financial support. We also thanks to Dr. Shashi Wadhawa for TEM (AIIMS, Delhi) and Dr. M. Shayam Prasad for SEM (NIO, Goa) for their generous help.

References

World Health Organization. 2013a Malaria. http:// www. who. int/mediacentre/factsheets/fs094/en/index.html

World Health Organization. 2013b. Lymphatic filariasis. http:// www.who.int/mediacentre/factsheets/fs102/en/

World Health Organization. 2012. Dengue and severe dengue. http://www.who.int/mediacemtre/factsheets/fs117/en/

Govindrajan, M., Jebamesan, A., Reetha, D. 2005. Larvicidal effect of extracellular secondary metabolites of different fungi against the mosquito, *Culex quinquefasciatus* Say. Trop Biomed., 22: 1-3.

Thirumurugan, G., Shaheedha, S.M., Dhanaraju, M.D. 2009. In vitro evaluation of anti-bacterial activity of silver nanoparticles synthesized by using *Phytopthora infestans.* Int J Chem Tech Res., 1: 714-716.

Vahabi, K., Mansoori, G.A., Karimi, S. 2011. Biosynthesis of silver nanoparticles by fungus *Trichoderma ressei*. Insciences J., 1: 65-79.

Bharathidasan, R., Panneerselvam, A. 2012. Biosynthesis and characterization of silver nanoparticles using endophytic fungi *Aspergillus concius*, *Panicillium janthinellum* and *Phomosis* sp. Int J Pharma Sci Res., 3: 3163-3169.

Moharrer, S., Mohammadi, B., Gharamohammadi, R.A., Yargoli, M. 2012. Biological synthesis of silver nanoparticles by *Aspergillus flavus*, isolated from soil of ahar copper mine. Indian J Sci Technol., 5: 2443-2444.

Alexandre, G.R., Ping, L.Y., Marcato, P.D., Alves, O.L., Silva, M.C.P., Ruiz, R.C., Melo, I.S., Tasic, L., Souza, A.D. 2012. Biogenic antimicrobial silver nanoparticles produced by fungi. App Microbiol Biotechnol., DOI 10.1007/s00253-012-4209-7

Kumar, R.R., Priyadarshini, K.P., Thamaraiselvi, K. 2012. Mycogenic synthesis of silver nanoparticles by the Japanese environmental isolate *Aspergillus tamarii*. J Nanopart Res., 14: 860.

Raliya, R., Tarafdar, J.C. 2012. Novel approach for silver nanoparticles synthesis using *Aspergillus terreus* CZR-1 mechanism perspective. J Bionanosci., 6: 12-16.

Soni, N., Prakash, S. 2011. Factors affecting the geometry of silver nanoparticles synthesis in *Chrysosporium tropicum* and *Fusarium oxysporum*. American J Nanotechnol., 2: 112-121.

Saha, P., Rajkumar, K., Abraham, J. 2012. Comparative study on antimicrobial property of silver nanoparticles synthesized by *Fusarium equiseti* and *Fusarium solani*. J Bionanosci., 6: 28:32.

Gupta, S., Bector, S. 2013. Biosynthesis of extracellular and intracellular gold nanoparticles by *Aspergillus fumigatus* and *Aspergillus flavus*. Antonie van Leeuwenhoek., 103:1113–1123

Das, S.K., Dickinson, C., Lafir, F., Brougham, D.F., Marsili, E. 2012a. Synthesis, characterization and catalytic activity of gold nanoparticles biosynthesized with *Rhizopus oryze* protein extract. Green Chem., DOI: 10.1039/c2gc16676c

Das, S.K., Liang, J., Schmidt, M., Laffir, F., Marsili, E. 2012b. Biomineralization mechanism of gold by Zygomycete fungi *Rhizopus oryze*. ACS Nano., 6: 6165-6173.

Chan, S.Y., Don, M.M. 2012. Instantaneous biosynthesis of silver nanoparticles by selected macro fungi. Australian J Basic App Sci., 6: 222-226.

Quian, Y., Yu, H., He, D., Yang, H., Wang, W., Wan, X., Wang, L. 2013. Biosynthesis of silver nanoparticles by the endophytic fungus *Epicocuum nigrum* and their activity against pathogenic fungi. Biopro Biosys Eng., DOI 10.1007/s00449-013-0937-z

Sap-Iam, N., Homklichan, C., Larpudomlert, R., Warisnoicharoen, W., Sereemaspun, A., Dubas, S.T. 2010. UV irradiation-induced silver nanoparticles as mosquito larvicides. J App Sci., 10: 3132-3136.

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

Soni, N., Prakash, S., 2012d. Entomopathogenic fungus generated nanoparticles for enhancement of efficacy in *Culex quinquefasciatus* and *Anopheles stephensi*. Asian Pac J Trop Dis., S356-S361.

Soni, N., Prakash, S. 2012c. Fungal-mediated nano silver: an effective adulticide against mosquito. Parasitol Res., 111: 2091-2098.

Salunkhe, R.B., Patil, S.V., Patil, C.D., Salunke, B.K. 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.

Gardner, J., Pillai, J.S. 1987. *Tolypocladium cylindrosporum* (Deuteromycotina: Moniliales), a fungal pathogen of the mosquito *Aedes australis*. Mycopathol., 97: 77-82.

Gerberg, E.J., Barnard, D.R., Ward, R.A. 1994. Manual for mosquito rearing and experimental techniques. *Journal of American Mosquito Control Association* 5. American Mosquito Control Association, New Brunswick, NJ, USA.

World Health Organization. 2005. Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCDPP/13

Finney, D.J. 1971. Probit analysis, 3rd edition, Cambridge University Press, Cambridge, UK

Abbott, S.W. 1925. A method of computing the effectiveness of an insecticide. J Econom Entomol., 18: 265-266.

Ghodake, V.P., Kininge, P.T., Magdum, S.P., Dive, A.S., Pillai, M.M. 2011. Biosynthesis of silver nanoparticles using *Trichosporon beigelii* NCIM 3326 and evaluation of their antimicrobial activity. J Eng Res Stu., 2: 32-36.

Santhoshkumar, T., Rahuman, A.A., Rajakumar, G., Marimuthu, S., Bagavan, A., Jayaseelan, A., Zahir, A.A., 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.

Rajkumar, G., Rahuman, A.A. 2011. Larvicidal activity of synthesized silver nanoparticles using *Eclipta prostrata* leaf extract against filariasis and malaria vector. Acta Trop., 118: 196-203.

Soni, N., Prakash, S. 2012b. Synthesis of gold nanoparticles by the fungus *Aspergillus niger* and its efficacy against mosquito larvae. Reports Parasitol., 2: 1-7.

Sundaravadivelan, C., Nalini, M. 2012. Biolarvicidal effect of phyto-synthesized 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.

Arjunan, N.K., Murugan, K., Rejeeth, C., Madhiyazhagan, P., Barnard, D.R. 2012. Green synthesis of silver nanoparticles for the control of mosquito vectors of malaria, filariasis and dengue. Vec Borne Zoo Dis., 12: 262-268.

Subarani, S., Sabhanayakam, S., Kamaraj, C. 2012. Studies on the impact of biosynthesized silver nanoparticles (AgNPs) in relation to malaria and filariasis vector control against *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol Res., DOI 10.1007/s00436-012-3158-5

Patil, C.D., Patil, S.V., Borase, H.P., Salunke, B.K., Salunkhe, R.B. 2012. Larvicidal activity of silver nanoparticles synthesized using *Plumeria rubra* plant latex against *Aedes aegypti* and *Anopheles stephensi*. Parasitol Res., 110: 1815-1822.

Priyadarshini, K.A., Murugan, K., Panneerselvam, C., Ponarulselvam, S., Hwang, J.S., Nicoletti, M. 2012. Biolarvicidal and pupicidal potential of silver nanoparticles synthesize using *Euphorbia hitra* against *Anopheles stephensi* Liston (Diptera: Culicidae) Parasitol Res., DOI 10.1007/s00436-012-2924-8

Barik, T.K., Kamaraju, R., Gowswami, A. 2012. Silica nanoparticles: a potential new insecticide for mosquito vector control. Parasitol Res., 111: 1075-1083.

Roni, M., Murugan, K., Panneerselvam, C., Subramaniam, J., Hwang, J.S. 2012. Evaluation of leaf aqueous extract and synthesized silver nanoparticles using *Nerium oleander* against *Anopheles stephensi* (Diptera: Culicidae). Parasitol Res., DOI 10.1007/s00436-012-3220-3.

Jayaseelan, C., Rahuman, A.A., Rajakumar, G., Kirthi, A.V., Santhoshkumar, T., Marimuthu, S., Bagavan, A., Kamaraj, C., Zahir, A.A., Elango, G. 2011. Synthesis of pediculocidal and larvicidal silver nanoparticles by leaf extract from heartleaf moonseed plant, *Tinospora cordifoloia* Miers. Parasitol Res., 109: 185-194.

****