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Research Article

Combinatorial Efficacy of Silver Nanoparticles against Mosquito Larvae

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Keywords Chrysosporium tropicum; Chrysosporium keratinophilum; Fusarium oxysporum; Aspergillusnige; Verticillium lecanii; Silver nanoparticles, Combinations; Larvicides; Culex quinquefasciatu; Anopheles stephensi

Abstract

Background: We have synthesized silver nanoparticles using fungi. The larvicidal efficacy was noted when performed against all instars of Cx. quinquefasciatus and An. stephensi at six different log concentrations after 24 h of exposures using the probit analysis.

Methods: The nanoparticles have been characterized through Micro-scan reader, X-ray diffractometer, transmission electron microscopy, and further confirmed by scanning electron microscopy. After characterization, these aqueous silver nanoparticles have been tested in 1:1 combinations (Chrysosporium tropicum Ag nanoparticles: Chrysosporium keratinophilum Ag nanoparticles, Chrysosporium tropicum Ag nanoparticles: Fusarium oxysporum Ag nanoparticles, Chrysosporium. tropicum Ag nanoparticles: Aspergillus niger Ag nanoparticles, Chrysosporium tropicum Ag nanoparticles: Verticillium lecanii Ag nanoparticles, Fusarium oxysporum Ag nanoparticles: Chrysosporium keratinophilum Ag nanoparticles, Fusarium oxysporum Ag nanoparticles: Aspergillus niger Ag nanoparticles, Fusarium oxysporum Ag nanoparticles: Verticillium lecanii Ag nanoparticles, Aspergillus niger Ag nanoparticles: Verticillium lecanii Ag nanoparticles, Aspergillus niger Ag nanoparticles: Chrysosporium keratinophilum Ag nanoparticles, and Verticillium lecanii Ag nanoparticles: Chrysosporium keratinophilum Ag nanoparticles, respectively). These combinations have also been tested as larvicides against the larvae of Culex quinquefasciatus and Anopheles stephensi.

Results: The all larval stages of Cx. quinquefasciatus were found more susceptible to the combinations than the An. stephensi.

Conclusion: The results suggest that this could be a useful tool for mosquito control.

Introduction

Mosquitoes are the vectors of many diseases, including malaria, filariasis, chikunguniya, and dengue. Control and eradication of the mosquito population could significantly restrict the spread of disease. Synthesizing nanoparticles using fungi can eliminate this problem by making the nanoparticles more biocompatible. Silver is a soft, white, lustrous transition metal, it has the highest electrical conductivity of any element and the highest thermal conductivity of any metal. Nanotechnology is involving the production, manipulation and use of materials managing in size less than a micron to an individual atom. Although nano materials can also be synthesized using chemical approaches. The biological method preferred for various reasons. It is now possible to include the use of fungi, bacteria and other biological materials.

Presently, fungi are also been used in nanotechnology for producing nanoparticles. Therefore, present green synthesis has shown that the environmentally benign and renewable source of fungi used as an effective reducing agent for the synthesis of silver nanoparticles. This biological reduction of metal would be boon for the development of clean, nontoxic and environmentally acceptable "green approach" to produce metal nanoparticles. It is well known that some microbes such as bacteria [1] yeast [2] and fungi [3] are potentially useful in the preparation of metal nanoparticles under normal air pressure and at room temperature. Many of the species of fungi like *Fusarium oxysporum* [4, 5], *Aspergillus fumigates* [6] and *Verticillium species* [7], used in nanotechnology for nanoparticles production. The silver nanoparticles have been synthesized within 10 min from potato plant pathogenic fungus *Phytophthora infestans* and their anti-bacterial activity was investigated by disc diffusion method and MIC [8]. Anti parasitic activities to determine the efficacies of synthesized silver nanoparticles using aqueous leaf extract of *Mimosa pudica* against the larvae of malaria vector, *An. subpictus*, filariasis vector *Cx. quinquefasciatus* and *Rhipicephalusmicroplus* have been evaluated [9]. *Nelumbonucifera* synthesized silver nanoparticles using aqueous leaf extract against larvae of *An. subpictus* and *Cx.quinquefasciatus* has been observed [10]. Recently, the larvicidal potential of silver nanoparticles synthesized using fungus *Cochlioboluslunatus* against *Ae. aegyptiand An. stephensi* have been observed [11]. larvicidal activity of Synthesized Silver Nanoparticles (AgNPs) utilizing aqueous extract from *Ecliptaprostrata*, a member of the *Asteraceae* has been investigated against fourth instar larvae of filariasis vector, *Culex quinquefasciatus* say and malaria vector, *Anopheles subpictus* [12].The efficacy of fungus mediated silver and gold nanoparticles has been

evaluated against the *Ae. Aegypti* larvae (Soni and Prakash 2011).The present communication describes the larvicidal effect of extracellular synthesized and combinatorial silver nanoparticles with the fungi. We have chosen the species of those fungi because of their chitinolytic activity and good source of reducing agents.

Materials and Methods

Fungal strains

The fungal strains of *C. tropicum* (MTCC 2828), *C. keratinophilium* (MTCC 2827), *F. oxysporum* (MTCC 2480), *A. niger* (MTCC 2587) and *V. lecanii* (MTCC 3692) were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology Chandigarh India. These fungi were routinely maintained in our laboratory on different medium at 25^{0}C.

Preparation of broth and culture of fungi

The broths were prepared for culture of fungi by the method [13]. *C. tropicum* and *C. keratinophilum* were grown in Sauboraud's Dextrose Broth (SDB). F. oxysporum and V. lecanii were grown in Potato Dextrose Broth (PDB).A. nigerwas grown on CzapekDox Broth (CDB). Five 250 ml conical flask, each containing 100 ml Sauboraud's dextrose broth (Dextrose 40 g, peptone 10 g, deionized water 1000 ml), Potato dextrose broth (Infusion of potatoes 200 g, Dextrose 20g and deionized water 1000 ml) and Czapekdox broth (Sucrose 30 g, Sodium nitrate 3 g, Dipotassium phosphate 1 g, Magnesiumsulphate 0.05 g, Potassium chloride 0.05 g, Ferrous sulphate 0.01 g, deionized water 1000 ml) were autoclaved at 20 psi for 20 min. The broths were supplemented 50 µg/ml chloramphenicol as a bacteriostatic agent. *C. tropicum* and *C. keratinophilum* colonies were grown on the Sauboraud's Dextrose Agar, *F. oxysporum* and *V. lecanii* colonies were grown on Potato Dextrose Agar, and A. niger colonies were grown on Czapek Dextrose Agar plates, were transferred to each flask using the Inoculation needle. The conical flasks inoculated with *C. tropicum*, *C. keratinophilum*, *A. niger*, *F. oxysporum* and *V. lecanii* were incubated 25^{0}C for 15 days.

Collection and maintenance of mosquito larvae in laboratory

Mosquito larvae were collected from various localities, including urban, rural and semi-urban regions of Agra (27^0, 10'N, 78^{0}05'E), India and reared in deionized water containing glucose and yeast power. The colonies of *Cx. quinquefasciatus* and *An. stephensi* were maintained in the laboratory at a temperature of 25^{0}C, with a relative humidity of 75±5% and 14h photoperiod. The larvae of *Cx. Quinquefasciatus* and *An. stephensi* were maintained in separate enamel containers as per the standard method [14].

Synthesis of silver nanoparticles

After incubation the fungal biomass of all selected fungi was separated from the medium by filtration through whatman-1 filter paper and washed thrice in sterile distilled water to remove any nutrient media that might interact with the silver ions. Approximately 10g of fungal wet biomass of all selected fungi was transferred to a 250 ml conical flask containing 100 ml of distilled water and incubated for 72 h at 25^{0}C and then the aqueous solution components were separated by filtration using Whatman-1 filter paper. To these solutions (liquid fungal), AgNO3 (10^{-3} M) was added and kept for 72 h at 25^{0}C.Simultaneously, control with fungal liquid of all selected fungi without silver nitrate was maintained under same conditions, separately.

Characterization of silver nanoparticles

Periodically, aliquots of the reaction solutions were removed and their absorption was measured in a Micro-Scan reader model no. MICROSCAN MS5608A and X-rays diffractometer model no. Bruker AXS D_8 Advance. The micrographs of silver nanoparticles were obtained by Philips CM-10 Transmission electron microscope and confirmed by Scanning electron microscope. Elemental analysis on single particle was carried out by EDX analysis.

Combinations of silver nanoparticles

After obtaining the micrographs of all fungus mediated nanoparticles through the TEM, the fungal liquids containing silver nanoparticles have been used in combination of 1:1 ratio. These combinations of silver nanoparticles synthesized with fungal liquid have been tested against the *Cx. Quinquefasciatus* and *An. stephensi* larvae. The combinations are as-

Combination 1 - *C. tropicum* Ag nanoparticles: *C. keratinophilum* Ag nanoparticles

Combination 2 - *C. tropicum* Ag nanoparticles: *F. oxysporum* Ag nanoparticles

Combination 3 - *C. tropicum* Ag nanoparticles: *A. niger* Ag nanoparticles

Combination 4 - *C. tropicum* Ag nanoparticles: *V. lecanii* Ag nanoparticles

Combination 5 - *F. oxysporum* Ag nanoparticles: *C. keratinophilum* Ag nanoparticles

Combination 6 - *F. oxysporum* Ag nanoparticles: *A. niger* Ag nanoparticles

Combination 7 - *F. oxysporum* Ag nanoparticles: *V. lecanii* Ag nanoparticles

Combination 8 - *A. niger* Ag nanoparticles: *V. lecanii* Ag nanoparticles

Combination 9 - *A. niger* Ag nanoparticles: *C. keratinophilum* Ag nanoparticles

Combination 10 - *V. lecanii* Ag nanoparticles: *C. keratinophilum* Ag nanoparticles

Bioassays

Larvicidal activity of *Cx. Quinquefasciatus* and *An. stephensi* were assessed by using the standard method [15]. All larvae of *Cx. Quinquefasciatus* and *An. stephensi* were separated and placed in a container in microbe free deionized water. After that different test concentrations of formulated silver nanoparticles in 100 ml deionized were prepared in 250-ml beakers. Bioassays were conducted separately for each instar at six different log test concentrations (0.30, 0.60, 0.77, 0.90, 1, and 1.08 ppm) of aqueous silver nanoparticles. To test the larvicidal activity of all combination, 20 larvae of each stage

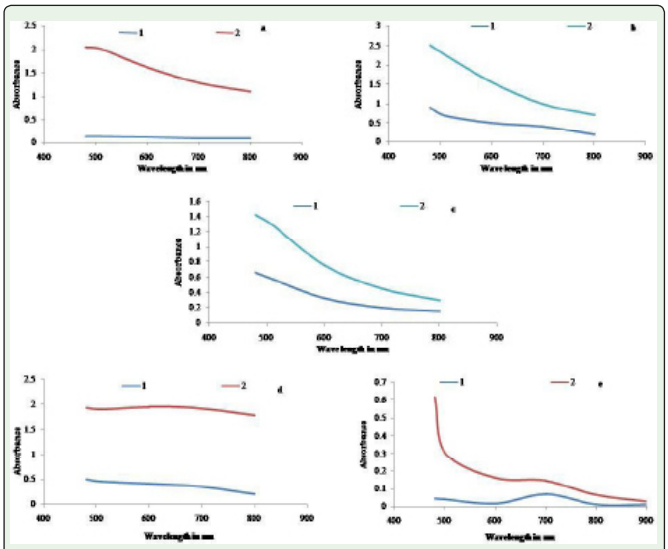

Figure 1: Micro-scan spectrum of fungal liquid (1) before and (2) after synthesis silver nanoparticles of C. tropicum, C. keratinophilum, F. oxysporum, A. nigerand V. lecanii (a-e).

were separately exposed to 100 ml of test concentration. Similarly, the control (without silver nanoparticles) was run to test the natural mortality. Thereafter, we could further examine the mortality which was determined after 24h of the treatment, the experiment time. No food was offered to the larvae during the experiments. Experiments were replicated thrice to validate the Results.

Data management and statistically analysis

The data on the efficacy was subjected to probit analysis [16]. The control mortality was corrected by Abbott's formula [17]. The relationship between probit and log concentrations were established as probit equations and probit regression lines were drown for each of larval stage.

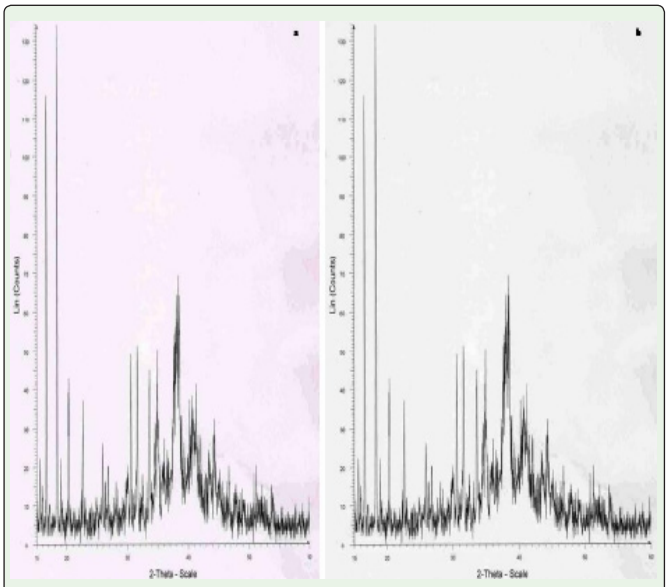

Figure 2: XRD pattern of biosynthesized silver nanoparticles (a) C. tropicum and C. keratinophilum (b) F. oxysporum, A. nigerand V. lecanii.

Results

Micro-scan reader analysis of silver nanoparticles

Figure 1 shows the Micro-scan spectra of silver nanoparticles synthesized by (a) *C. tropicum* (b) *C. keratinophilum* (c) *F. oxysporum* (d) *A. niger* and (e) *V. lecanii* recorded form the reaction medium before (curve 1) and after immersion of AgNO3 (curve 2) after 72h. Absorption spectra of silver nanoparticles formed in the reaction media has a broad absorption band centered at ca. 480 nm. The presence of broad resonance indicated an aggregated structure of the silver nanoparticles in the solution.

XRD analysis of silver nanoparticles

Figure 2 depicts the XRD pattern of (a) *C. tropicum* and *C. tropicum* powered silver nanoparticles in the 2θ range 15^0-60^0. It exhibits a broad peak at 38.4^0. The broadening of the peaks clearly indicates that the particles are in the nano regime. Apart from these, many unidentified peaks 22^0, 26^0, 30^0, 32^0, 34^0, 36^0 and 44^0 arises, possibly due to other chemical reactions or organic impurities presents in the sample. Figure 3 (b) depicts the XRD pattern of *F. oxysporum*, *A. niger* and *V. lecanii* powered silver nanoparticles in the 2θ range 20^0-60^0. It exhibits a broad peak at 38.4^0 and 44.4^0. The broadening of the peaks clearly indicates that the particles are in the nano regime. Apart from these, many unidentified peaks at 16^0, 18^0, 21^0, 26^0, 30^0, 32^0, 35^0, 43^0, 45^0 and 52^0 arises, possibly due to other chemical reactions or organic impurities presents in the sample.

TEM analysis of silver nanoparticles

Figure 4 showing the biosynthesized silver nanoparticles further characterized by Transmission Electron Microscopy for the micro structural analysis. TEM analysis of silver nanoparticles synthesized with (a) *C. tropicum* (b) *C. keratinophilum* (d) *F. oxysporum* (e) *A. niger* and (e) *V. lecanii* liquid indicated formation of spherical shaped structure with different edge lengths (Table 1).

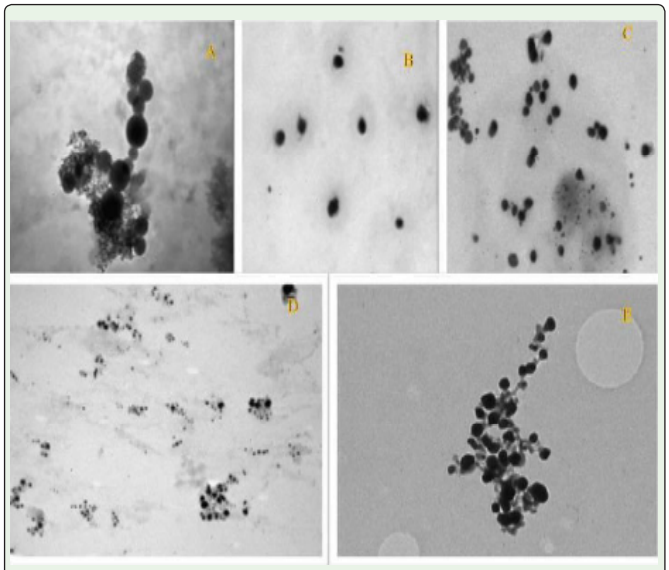

Figure 3: TEM images of biosynthesized silver nanoparticles using fungal liquid of C. tropicum, C. keratinophilum, F. oxysporum, A. nigerand V. lecanii (a-e).

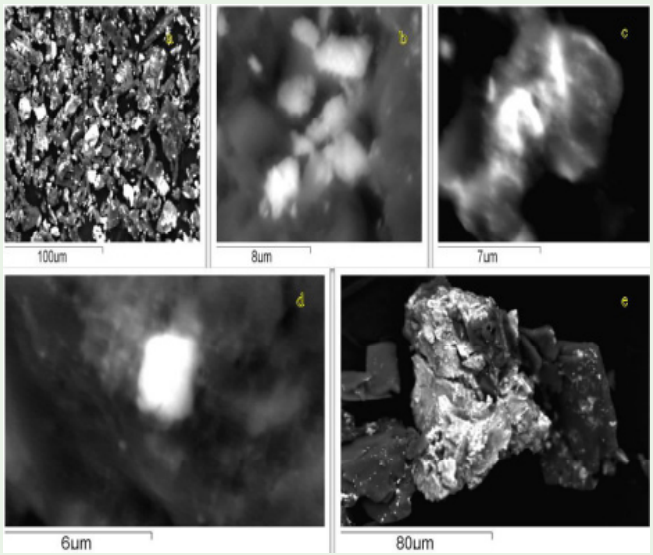

Figure 4: SEM images of biosynthesized silver nanoparticles using fungal liquid of C. tropicum, C. keratinophilum, F. oxysporum, A. nigerand V. lecanii (a-e).

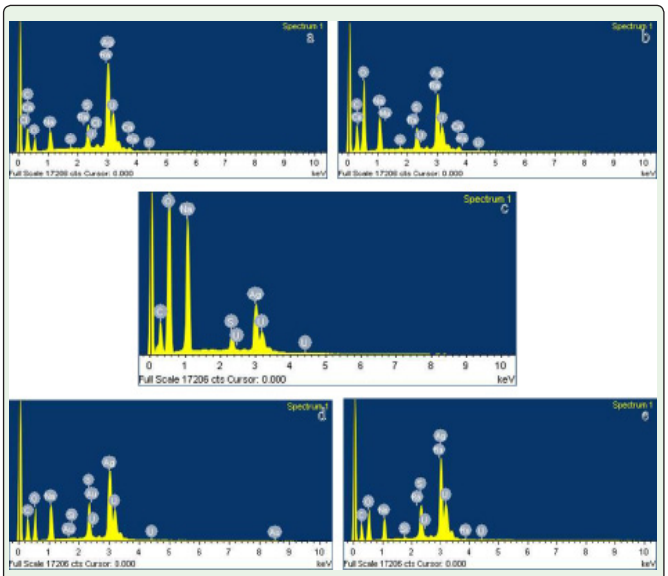

Figure 5: EDX spectrum of biosynthesized silver nanoparticles using fungal liquid of C. tropicum, C. keratinophilum, F. oxysporum, A. nigerand V. lecanii (a-e).

SEM and EDX analysis of silver nanoparticles

Figure 4 shows a scanning electron microscope picture of silver nanoparticles synthesized with (a) *C. tropicum* (b) *C. keratinophilum* (d) *F. oxysporum* (e) *A. niger* and (e) *V. lecanii* after 72h. The bright area clearly is seen in the image. The SEM images are showing distinctly the high density silver nanoparticles synthesized by *C. tropicum*, *C. keratinophilum, F. oxysporum, A. niger and V. lecanii* confirmed the development of silver nanostructures.

The particles were checked and were found to contain a great deal of Ag, using EDX analysis. Figure 5 shows the EDX spectrum (a) *C. tropicum* (b) *C. keratinophilum* (d) *F. oxysporum* (e) *A. niger* and (e) *V. lecanii* recorded in the spot- profile mode from one of the densely populated silver nanoparticles region on the surface of the film. Strong signals from the silver atoms in the nanoparticles while weaker signals from X-ray emission from proteins/enzymes present in the cell wall of the biomass were observed (Table 1).

Efficacy of different combinations against Cx. quinquefasciatus larvae

The first, second and third instars larvae of *Cx. quinquefasciatus* have shown 100% mortality for combination 1. Whereas, the LC_{50} 2 ppm, LC_{90} 12.30 ppm and LC_{99} 12.58 ppm values were evaluated for fourth instars with their probit equations and confidential limits after 24h.

The combinations 2, 3 and 4 have shown 100% mortality against the first, second and third instar larvae of *Cx. quinquefasciatus*. Whereas, the LC_{50} 4 ppm, LC_{90} 12.88 ppm and LC_{99} 13.18 ppm (Combination 2), the LC_{50} 1.58 ppm, LC_{90} 12.32 ppm and LC99 12.58 ppm (Combination 3) and the LC_{50} 2 ppm, LC_{90} 12.58 ppm and LC_{99} 12.88 ppm (Combination 4) values were evaluated for fourth instars with their probit equations and confidential limits after 24h.

The all instar larvae of *Cx. quinquefasciatus* have shown 100% mortality for the combination 5 and 6. Whereas the combination7 has LC_{50} 6 ppm, LC_{90} 12.58 ppm and LC_{99} 14.12 ppm values for fourth instar larvae with their probit equations and confidential limits after 24h and 100% mortality for first, second and third instar larvae of *Cx. quinquefasciatus*. The combinations 8, 9 and 10 have produced 100% mortality for all larval stages of *Cx. quinquefasciatus* (Table 2).

Efficacy of different combinations against An. stephensi larvae

The larvae of *An. stephensi* have shown 100% mortality against the combination 1. Whereas, the combination 2 has LC_{50} 1.41 ppm, LC_{90} 8 ppm and LC_{99} 10 ppm values for fourth instar larvae with their

Table 1: Showing the Micro scan analysis, XRD patterns, particles size and EDX spectrum of biosynthesized silver nanoparticles.

S.NO	FUNGUS	SILVER NANO PARTICLES			
		Micro-scan analysis (Wavelength in nm)	XRD analysis (2θ)	TEM analysis (Particles size in nm)	EDX analysis
1	*C. tropicum*	480	38.4°	20-50	Ag, Na, Si, S, Cl, Ca, Ra and U
2	*C. keratinophilum*	480	38.4°	24-51	Ag, Na, Mg, Si, S, Ca, Ra and U
3	*F. oxysporum*	480	38.4°, 44.4°	20-40	Ag, Na, S, and Ra
4	*A. niger*	480	38.4°, 44.4°	20-70	Ag, Na, Si, S, Au, U and O
5	*V. lecanii*	480	38.4°, 44.4°	20-50	Ag, Na, Si, S, Ra, U and O

Table 2: Probit equations and susceptibility of Cx. quinquefasciatus larvae against silver nanoparticles of different combinations with probit equations and 95% confidential limits (C.L.) after 24 h.

Instars	Combinations	LC_{50} (ppm)	LC_{90} (ppm)	LC_{99} (ppm)
First in star	1	**	**	**
	2	**	**	**
	3	**	**	**
	4	**	**	**
	5	**	**	**
	6	**	**	**
	7	**	**	**
	8	**	**	**
	9	**	**	**
	10	**	**	**
Second in star	1	**	**	**
	2	**	**	**
	3	**	**	**
	4	**	**	**
	5	**	**	**
	6	**	**	**
	7	**	**	**
	8	**	**	**
	9	**	**	**
	10	**	**	**
Third in star	1	**	**	**
	2	**	**	**
	3	**	**	**
	4	**	**	**
	5	**	**	**
	6	**	**	**
	7	**	**	**
	8	**	**	**
	9	**	**	**
	10	**	**	**
Fourth in star	1	2 (0.83-3.17)	12.30 (11.13-13.47)	12.58 (11.41-13.75)
	2	4 (2.93-5.07)	12.88 (11.81-13.95)	13.18 (12.11-14.25)
	3	1.58 (0.38-2.78)	12.32 (11.18-13.44)	12.58 (11.46-13.7)
	4	2 (0.83-3.17)	12.58 (11.41-13.75)	12.88 (11.71-14.05)
	5	**	**	**
	6	**	**	**
	7	6 (4.98-7.04)	12.58 (11.52-13.62)	14.12 (13.08-15.16)
	8	**	**	**
	9	**	**	**
	10	**	**	**

** 100% mortality

probit equations and confidential limits after 24h and 100% mortality for first, second and third instar larvae of *An. stephensi*.

The first and second instar larvae of An. stephensi have shown 100% mortality against the combination 3 and 4. Whereas the LC_{50} 1.44 ppm, LC_{90} 12 ppm and LC_{99} 12.30 ppm values for third instar and the LC_{50} 12 ppm, LC_{90} 13.48 ppm and LC_{99} 17.78 ppm values were evaluated for fourth instars with their probit equations and confidential limits (combination 3). The LC_{50} 1.51 ppm, LC_{90} 12 ppm and LC_{99} 12.30 ppm values for third instar and the LC_{50} 2 ppm, LC_{90} 12.30 ppm and LC_{99} 12.58 ppm values were evaluated for fourth instars with their probit equations and confidential limits after 24h (combination 4).

The combination 5 have shown 100% mortality for first, second, third instars and LC_{50} 1.25 ppm, LC_{90} 8 ppm and LC_{99} 10 ppm values for fourth instars with their probit equations and confidential limits. The combination 6 have produced 100% mortality in first, second, fourth instars and LC_{50} 1.58 ppm, LC_{90} 12.32 ppm and LC_{99} 12.58 ppm values for third instars with their probit equations and confidential limits. Whereas, all larvae of *An. stephensi* have shown 100% mortality against combination 7.

However, the combinations 8 and 9 have produced 100% mortality in first, second and third instars while LC_{50} 1.58 ppm, LC_{90} 12.32 ppm and LC_{99} 12.58 ppm (combination 8) and LC_{50} 1.31 ppm, LC_{908} ppm and LC_{99} 10 ppm (combination 9) values for fourth instars with their probit equations and confidential limits. Moreover, the 100% mortality was recorded in larval stages of *An. stephensi* against the combination 10 (Table 3).

Discussion

C. tropicum, C. keratinophilum, F. oxysporum, A. niger and *V. lecanii* are the keratinophilic filamentous and entomopathogenic fungi. These fungi are effective against mosquito larvae. In the present investigation we have successfully been synthesized the silver nanoparticles and made different combinations for testing as a larvicide against the *Cx. quinquefasciatus* and *An. stephensi* larvae.

The microorganisms have been used to formation of metal nanoparticles [18]. There after the role of microorganisms and plants has been assessed in the synthesis of nanoparticles [19]. The metal nanoparticles have been synthesized by biological method [20]. The extracellular biosynthesis of silver nanoparticles (AgNPs) by using a

Table 3: Probit equations and susceptibility of An. stephensi larvae against silver nanoparticles of different combinations with probit equations and 95% confidential limits (C.L.) after 24 h.

Instars	Combinations	LC_{50} (ppm)	LC_{90} (ppm)	LC_{99} (ppm)
First in star	1	**	**	**
	2	**	**	**
	3	**	**	**
	4	**	**	**
	5	**	**	**
	6	**	**	**
	7	**	**	**
	8	**	**	**
	9	**	**	**
	10	**	**	**
Second in star	1	**	**	**
	2	**	**	**
	3	**	**	**
	4	**	**	**
	5	**	**	**
	6	**	**	**
	7	**	**	**
	8	**	**	**
	9	**	**	**
	10	**	**	**
Third in star	1	**	**	**
	2	**	**	**
	3	1.44 (0.24-2.64)	12 (10.8-12.2)	12.30 (11.10-13.50)
	4	1.51 (0.28-2.74)	12 (10.77-13.23)	12.30 (11.07-13.52)
	5	**	**	**
	6	1.58 (0.38-2.78)	12.32 (11.18-13.44)	12.58 (11.46-13.7)
	7	**	**	**
	8	**	**	**
	9	**	**	**
	10	**	**	**
Fourth instar	1	**	**	**
	2	1.41 (0.27-2.55)	8 (6.86-9.14)	10 (8.86-11.14)
	3	12 (10.86-13.14)	13.48 (12.34-14.62)	17.78 (16.64-18.92)
	4	2 (0.83-3.17)	12.30 (11.13-13.47)	
	5	1.25 (0.11-2.39)	8 (6.86-9.14)	12.58 (11.46-13.7)
	6	**	**	10 (8.86-11.14)
	7	**	**	**
	8	1.58 (0.38-2.78)	12.32 (11.18-13.44)	**
	9	1.31 (0.17-2.45)	8 (6.86-9.14)	12.58(11.46-13.7)
	10	**	**	10 (8.86-11.14)
				**

** 100% mortality

fungus named *TrichodermaReesei* has been recorded [21]. Similarly, biosynthesis of silver nanoparticles using *Trichosporonbeigelii* NCIM 3326 and their antimicrobial activity has been evaluated [22]. Extracellular biosynthesis and characterization of silver nanoparticles using *A. flavus* NJP08 has been evaluated . 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 unique because fungal AgNPs 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 AgNPs as biocontrol agent for mosquitoes. The fungal AgNPs have very narrow range, and considerable progress has been made in recent years in development of environmentally benign spores and mycelium-based bio control agent for the mosquito population. Fungal bio control agents have reduced inputs of harmful synthetic chemical pesticide in agriculture, horticultural, and forest system.

Toxicity of silver nanoparticles in zebra fish models have been recorded [23]. Using starch and bovine serum albumin (BSA) as capping agents, silver nanoparticles has been synthesized to study their deleterious effects and distribution pattern in zebra fish embryos (Daniorerio). Toxicological endpoints like mortality, hatching, pericardial edema and heart rate were recorded. A concentration-dependent increase in mortality and hatching delay was observed in Ag-np treated embryos. Additionally, nanoparticle treatments resulted in concentration-dependent toxicity, typified by phenotypes that had abnormal body axes, twisted notochord, slow blood flow, pericardial edema and cardiac arrhythmia. Ag+ ions and stabilizing agents showed no significant defects in developing embryos. The use of nanoparticles in insects and their potential for use in insect pest management have been focused [24]. These results were performed against the zebrafish and other pests. Whereas, in our study we have combined Ag nanoparticles using entomopathogenic fungi and performed against the mosquito larvae.

Crystallization of silver ions to nanosized particles by cell filtrates from live and dead cell filtrates *A.oryzaevar. Viridis* (*A. oryzaevar. Viridis*) through bio reduction process has been assessed [25]. The

synthesized AgNPs were tested for its bactericidal activity against the *Staphylococcus aureus* (*S. aureus*; KCCM 12256). The Minimum Bactericidal Concentration (MBC) for the *S. aureus* KCCM 12256 strain was found to be 40 mg/L. The potentiality of a phyto pathogenic fungus *Bipolarisnodulosa* to produce anisotropic silver nanoparticles using its Mycelia Free Media (MFM) has been reported [26]. The effects of these nanoparticles against different microorganisms have also been determined. Antimicrobial tests were performed against *Bacillus subtilis*, *B. cereus*, *Pseudomonas aeruginosa*, *Proteus vulgarius*, *Escherichia coli* and *Micrococcus luteus* on Petri plates by cup plate method. Silver nanoparticles at a concentration of 100 µg/ml showed a range of specificity towards its antimicrobial activity. Biosynthesis of stable silver nanoparticles has been done using Tulsi (*Ocimum sanctum*) leaf extract and their antimicrobial activity has been screened against both gram-negative and gram-positive microorganisms [27]. Silver nanoparticles has been synthesized extracellularly using *Streptomyces aureofaciens* and evaluated for its wound healing activity and antimicrobial by disc diffusion method [28]. The results suggested that the silver nanoparticles synthesized from *S. aureofaciens* possess effective wound healing activity when compared with silver nitrate. These results showed the effect of silver nanoparticles against the bacteria. Whereas, in the present investigation we have evaluated the effect of different combinations of silver nanoparticles against mosquito larvae.

The formulation of water dispersible nanopermethrin has been investigated for its larvicidal property [29]. The results extrapolated that nanopermethrin could serve selectively as a potential larvicide. The larvicidal potential of the hexane, chloroform, ethyl acetate, acetone, methanol, and aqueous leaf extracts of *Nelumbonucifera* and synthesized silver nanoparticles using aqueous leaf extract against fourth instar larvae of *Anopheles subpictus* and *Culex quinquefasciatus* have been tested [10]. 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 [12]. These results were based on plant synthesized silver nanoparticles and have been tested against filariasis and malaria vectors. Whereas, in our work we have tested the different combinations of silver nanoparticles using keratinophilic fungi.

The larvicidal potential of silver nanoparticles synthesized using fungus *Cochliobolus lunatus* against *Ae. aegypti* and *An. stephensi* has been tested [11]. They have also tested the potential of *C. lunntus* silver nanoparticles against non-target fish species *Poeciliareticulata*, the most common organism in the habitats of *A. aegypti* and *An. stephensi* showed no toxicity at LC_{50} and LC_{90} doses of the AgNPs. The rapid biological synthesis of silver nanoparticles from fungi isolated from marine mangrove soil sediment provides a simple and efficient route for the synthesis of nanoparticles with tunable optical properties directed by particle size. Investigation on the antibacterial effect of nanosized silver colloidal solution against *S. aureus* ATCC 29213, and *E. coli* ATCC 25922 reveals high efficacy of silver nanoparticles as a strong antimicrobial agent which can be useful in food industries, cosmetic industries and in Pharmaceuticals [30]. Whereas, in the present study we have tested the different combinations silver nanoparticles against the all larval instars of *Cx. quinquefasciatus* and *An. stephensi*.

Conclusion

In the present investigation the combinatorial efficacy of silver nanoparticles have been tested against mosquito larvae of *Cx. quinquefasciatus* and *An. stephensi*. These combinations have an immediate impact on mosquito control. These nano larvicides are environmentally safer, greener and rapid effective against mosquito vectors. We can therefore develop and can conclude that the fungus synthesized silver nanoparticles could be a better, environmentally safer and green approach for vector control.

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