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Scientific Research

Possible Mosquito Control by Silver Nanoparticles Synthesized by Soil Fungus (*Aspergillus niger* 2587)

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ABSTRACT

Here, we have synthesized the silver nanoparticles (AgNPs) by using the soil fungus *Aspergillus niger* 2587. The results recorded from UV-vis spectrophotometer and transmission electron microscopy (TEM) support the biosynthesis and characterization of AgNPs. The synthesized silver nanoparticles have also been tested against the larvae and pupae of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. The efficacy test was performed at different concentrations for a period of different hours by the probit analysis. The larvae of *Cx. quinquefasciatus* have shown the 100% mortality to the synthesized AgNPs after 1 h of exposure, while the larvae of *An. stephensi* and *Ae. aegypti* were found less susceptible to the synthesized AgNPs. The pupa of *Ae. aegypti* has shown the efficacy LC_{50} 4, LC_{90} 12 and LC_{99} 19 ppm after 2 h of exposure of the synthesized AgNPs, while, the pupae of *Cx. quinquefasciatus* and *An. stephensi* were found less susceptible to the synthesized AgNPs. By this approach, it is suggestive that this rapid synthesis of nanoparticles would be proper for developing a biological process for mosquito control.

Keywords: Soil Fungus; Silver Nanoparticles; Mosquito Control

1. Introduction

Mosquito vectors are solely responsible for transmitting diseases such as malaria, dengue, chikungunya, Japanese encephalitis, and lymphatic filariasis. *Anopheles* species are the most important species as they are capable vector for malaria parasites. About 3.3 billion people—half of the world's population—are at risk of malaria. In 2010, there were about 216 million malaria cases (with an uncertainty range of 149 million to 274 million) and an estimated 655,000 malaria deaths (with an uncertainty range of 537,000 to 907,000). Increased prevention and control measures have led to a reduction in malaria mortality rates by more than 25% globally since 2000 and by 33% in the WHO African Region [1].

Culex mosquitoes are painful and persistent biters and are responsible for filariasis. Lymphatic filariasis is a neglected tropical disease. More than 1.3 billion people in 72 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 [2].

Aedes mosquitoes on the other hand are also painful and persistent biters. *Ae. aegypti* is 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 [3]?

The problem has a complex face and it has to be handled carefully. It is essential to control mosquito population so that people can be protected from mosquito borne diseases. These diseases can be controlled by targeting the causative parasites and pathogens. It is easier to control vectors than parasites. The chemical control was one of the most widely used conventional methods for mosquito control since chemical pesticides are relatively inexpensive usually produces immediate control. Generally, the chemical control is carried out by the indoor residual spraying of insecticides such as dichloro diphenyl trichloro ethane, hexa chlorocyclo hexane, benzene hexa chloride, melathion and synthetic pyrothroid. But, the development of resistance against these chemicals in various mosquito populations has been reported.

It is known that larvicides play a vital role in control-

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ling mosquitoes in their breeding sites. Two insecticidal bacteria have been used as larvicides to control larvae of nuisance and vector mosquitoes in many countries, *Bascillus thuringienesis* ssp. *Israelensis* and *Bascillus sphaericus* [4]. Field studies have shown that both are effective, but serious resistance, as high as 50,000 fold, has evolved where *B. sphaericus* is used against *Culex* mosquitoes. Unfortunately, the development of resistance against the larvicide in various mosquito populations has also been reported.

Therefore, biological control can thus provide and effective and environmental friendly approach, which can be used as an alternative to minimize the mosquito population. Fungi and fungus derived products are highly toxic to mosquitoes, yet have low toxicity to non-target organisms [5]. The secondary metabolites of entomopathogenic fungi *Chrysosporium* [6], *Fusarium* [7], *Aspergillus* [8], and *Verticillium* [9] have been screened successfully as a potential larvicide.

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. Biosynthesis of silver nanoparticles (AgNPs) by using a fungus *Trichoderma* [10,11], *Aspergillus* [12,13], and *Fusarium* [14,15] have been reported.

The larvicidal activities of mycosynthesized silver nanoparticles against vectors: *Ae. aegypti* and *An. stephensi* responsible for diseases of public health importance have been evaluated [16]. The silver and gold nanoparticles synthesized with *C. tropicum* have been tested as a larvicide against the mosquito larvae [17,18]. The silver nanoparticles synthesized by using the fungi have also been tested against adult mosquito [19].

The present communication describes the larvicidal and pupicidal effect of extracellular synthesized silver nanoparticles by using the soil fungi *A. niger* 2587 against the *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes. *A. niger* is filamentous keratinophilic fungi with compact white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads. This fungus secret some reducing agents which convert silver nitrate into silver nanoparticles. Therefore, it can be a useful green exercise to invent and discover new fungal nanolarvicide for respective ecology and environmental management system.

2. Experimental

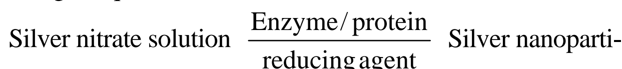
2.1. Fungal Strain, Preparation of Broth and Culture of *A. niger*

The fungal strain of *A. niger* (MTCC 2587) was obtained from the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India, and was routinely maintained in the laboratory on Czapek-Dox Agar (CDA) at 25˚C.

The broth was prepared for culture of *A. niger* following the method [20]. Five 250 ml conical flasks, each containing 100 ml of Czapek-Dox Broth (sucrose 30 g, sodium nitrate 3 g, dipotassium phosphate 1 g, magnesium sulphate 0.05 g, potassium chloride 0.05 g, ferrous sulphate 0.01 g, and deionized water 1000 mL), were autoclaved at 20 psi for 20 minutes. The broth was supplemented with chloramphenicol (50 µg/mL) as a bacteriostatic agent. *A. niger* colonies grown on CDA plates were transferred to each flask by inoculation needle. The conical flasks inoculated with *A. niger* were incubated at 25˚C for 15 days.

2.2. Synthesis and Characterization of AgNPs

The fungal colonies of *A. niger* was grown on CDA. After 7 days incubation of fungal colonies on CDA plates were further transferred CDB containing conical flask by inoculation needle. The conical flasks inoculated with *A. niger* were incubated at 25˚C for 15 days. After 15 days incubation the fungal biomass was separated from the medium by filtration through whatman-1 filter paper. The biomass was washed thrice in sterile distilled water to remove any nutrient media that might interact with silver ions. Approximately 10 g of fungal wet biomass of fungus was transferred to 250 ml conical flask containing 100 ml of distilled water and incubated for 72 h at 25˚C. After then the aqueous solution component was separated by filtration using whatman-1 filter paper. To this solution (aqueous solution component of *A. niger*), $AgNO_3$ (10^3 M) solution was added and kept for 72 h at 25˚C in BOD incubator. Simultaneously, the control without adding $AgNO_3$ was maintained under the same conditions, separately. The protein, enzyme and other compound present in the fungal liquid work as reducing agents and are responsible for conversion of silver nitrate to silver nanoparticles. The reaction may be written as *A. niger* (fungal liquid) +



$$\text{Silver nitrate solution} \xrightarrow[\text{reducing agent}]{\text{Enzyme/protein}} \text{Silver nanoparti-}$$

cles. Periodically, aliquot of the reaction solution was removed and their absorption was measured in UV-vis spectrophotometer. The micrograph of AgNPs was obtained by Philips CM-10 Transmission Electron Microscope.

2.3. Bioassays and Statistical Analysis

The larvicidal and pupicidal activity of synthesized AgNPs against the *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* was assessed by using the standard method [21]. Bioassays were conducted separately at six different

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test concentrations (2, 4, 6, 8, 10, and 12 ppm) of aqueous AgNPs. To test the larvicidal and pupicidal activity of synthesized AgNPs, 20 larvae and pupae of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* were separately exposed to 100 ml of test concentrations. Similarly, the control (without AgNPs) was run to test the natural mortality. Thereafter, we could further examine the mortality which was determined after different hours of treatment, the experiment time. No food was offered to the larvae during the experiment. Experiments were replicated thrice to validate the results. The data on the efficacy was subjected to probit analysis [22]. The control mortality was corrected by Abbott's formula [23].

3. Results

3.1. UV-Vis Spectrophotometer and TEM Analysis of Synthesized AgNPs

By mixing the fungal liquid component of *A. niger* with the aqueous solution of Ag ions, the colour of fungal liquid changed from white to dark brown colour after 72 h of incubation. The change in colour is a signal for the formation of AgNPs. **Figure 1(a)** shows the UV-vis spectra of AgNPs synthesized by using the *A. niger* recorded from the reaction medium before (1) and after immersion of $AgNO_3$ (2) after 72 h. Absorption spectra of AgNPs formed in the reaction medium has a broad absorption band centred at ca. 480 nm. The presence of broad resonance indicated an aggregated structure of the AgNPs in the solution.

Figure 1(b) shows the TEM micrograph of *A. niger* synthesized AgNPs. The 20 - 70 nm sized and spherical shaped AgNPs were observed.

3.2. Efficacy Study of Synthesized AgNPs by Using the *A. niger* against the Larvae and Pupae of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*

The *A. niger* synthesized AgNPs were found effective against the larvae and pupae of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi.* The larvaes of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* were found highly susceptible to the synthesized AgNPs than the pupae at the same test concentrations. The mortality could be observed after different hours of exposure.

The larvae of *Cx. quinquefasciatus* were found highly susceptible to the synthesized AgNPs than the larvae of *Ae. aegypti* and *An. stephensi*. The mortality was scored after 1 h. The early three instars of *Cx. quinquefasciatus* were found more susceptible to the synthesized AgNPs and shown the 100% mortality after 1 h of exposure. While, the fourth instar larvae were less susceptible to the synthesized AgNPs.

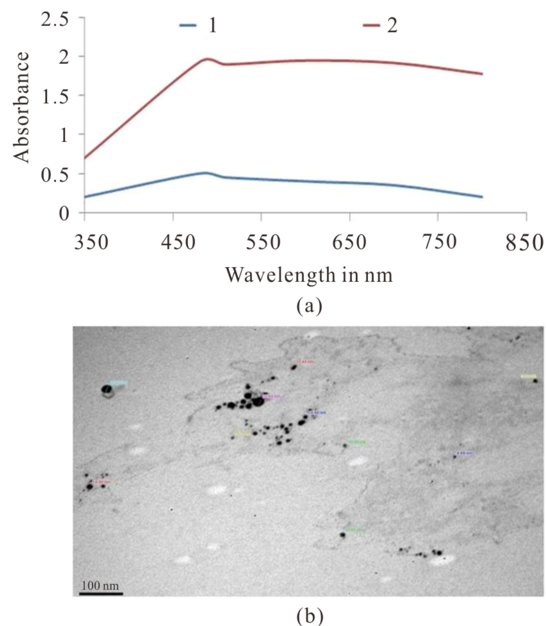

Figure 1. (a) UV-vis spectra of synthesized AgNPs from fungal liquid of *A. niger* before (1) and after immersion in 10^{-3} M aqueous $AgNO_3$ solution for 72 h (2); (b) TEM image of *A. niger* synthesized AgNPs.

The third instar larvae of *Ae. aegypti* were found highly susceptible to the synthesized AgNPs and shown the 100% mortality after 1 h. However, the first and fourth instar larvae were less effective to the synthesized AgNPs and no adverse effects could be observed in the second instar larvae after same hour of exposure. The efficacy for first instars (LC_{50} 5.62, LC_{90} 12.50, LC_{99} 13.48 ppm) and for fourth instars (LC_{50} 4.67, LC_{90} 8, LC_{99} 12.58 ppm) were recorded with their probit equations and confidential limits (**Table 1**). The chi-square values calculated at 4 df were 39.17 and 48.80 for first and fourth instars. These chi-square values for first and fourth instars were found higher than the critical value of chi-square at 0.05 significance level. The relationship between the concentrations and % mortality were shown for each of larval stage of *Ae. aegypti* (**Figure 2**).

The AgNPs synthesized by using the *A. niger* were found effective against the larval stages of *An. stephensi*. The mortality was observed after 24 h of exposure. The first and second instar larvae of *An. stephensi* have shown 100% mortality after 24 h. While, the efficacy for third instars (LC_{50} 1.58, LC_{90} 8.91, LC_{99} 12.30 ppm) and for fourth instars (LC_{50} 2, LC_{90} 12, LC_{99} 13.18 ppm) were calculated with their probit equations and confidential limits **(Table 1).** The chi-square values calculated at 4 df were 56.10 and 46.70 for third and fourth instars. These chi-square values for third and fourth instars were higher than the critical value of chi-square at 0.05 significance level. The relationship between the concentrations and % mortality were shown for each of larval

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Table 1. Larvicidal efficacies of AgNPs synthesized by using the *A. niger* against the *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*.

Species	Instar	Exposure time (hrs)	Concentrations (ppm)	% mortality	Probit equations	LC_{50}	LC_{90}	LC_{99}	x^2
Cx. quinquefasciatus	1st	1	2	100	a	a	a	a	a
			4	100					
			6	100					
			8	100					
			10	100					
			12	100					
	2nd	1	2	10	a	a	a	a	a
			4	50					
			6	65					
			8	90					
			10	95					
			12	100					
	3rd	1	2	60	a	a	a	a	a
			4	70					
			6	80					
			8	90					
			10	100					
			12	100					
	3rd	1	2	00	b	b	b	b	b
			4	00					
			6	00					
			8	00					
			10	00					
			12	00					
Ae. aegypti	1st	1	2	20	y = 0.4 + 6.12x	5.62 (4.53 - 6.71)	12.50 (11.38 - 13.62)	13.48 (12.36 - 14.6)	39.17
			4	45					
			6	55					
			8	65					
			10	70					
			12	75					
	2nd	1	2	00	b	b	b	b	b
			4	00					
			6	00					
			8	00					
			10	00					
			12	00					
	3rd	1	2	60	a	a	a	a	a
			4	70					
			6	80					
			8	90					
			10	100					
			12	100					
	4th	1	2	20	y = 0.32 + 6.89x	4.67 (3.6 - 5.74)	8 (3.6 - 5.74)	12 58 (11.46 - 13.7)	48.80
			4	40					
			6	65					
			8	90					
			10	95					
			12	95					

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An. stephensi	1st	24	2	100	a	a	a	a	a
			4	100					
			6	100					
			8	100					
			10	100					
			12	100					
	2nd	24	2	10	a	a	a	a	a
			4	50					
			6	65					
			8	90					
			10	95					
			12	100					
	3rd	24	2	75	y = 0.66 + 7.21x	1.58 (0.41 - 2.75)	8.91 (7.87 - 9.95)	12.30 (11.18 - 13.42)	56.10
			4	80					
			6	85					
			8	90					
			10	90					
			12	95					
	4th	24	2	50	y = 0.48 + 6.61x	2 (0.83 - 3.17)	12 (10.88 - 13.12)	13.18 (12.04 - 14.32)	46.70
			4	60					
			6	65					
			8	70					
			10	85					
			12	90					

a, 100% mortality; b, no mortality.

Table 2. Pupicidal efficacies of AgNPs synthesized by using the *A. niger* against the *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi*.

Species	Exposure time (hrs)	Concentrations (ppm)	% mortality	Probit equations	LC_{50}	LC_{90}	LC_{99}	x^2
Ae. aegypti	2	2	45	y = 0.44 + 6.51x	4 (2.77 - 5.23)	12 (11.77 - 13.23)	14 (12.77 - 15.23)	44.48
		4	50					
		6	55					
		8	70					
		10	80					
		12	90					
Cx. quinquefascitus	20	2	40	y = 0.40 + 6.25x	6 (4.77 - 6.23)	14 (12.77 - 15.23)	17 (15.17 - 17.23)	42.40
		4	45					
		6	50					
		8	65					
		10	70					
		12	80					
An. stephensi	24	2	00	b	b	b	b	b
		4	00					
		6	00					
		8	00					
		10	00					
		12	00					

b, no mortality.

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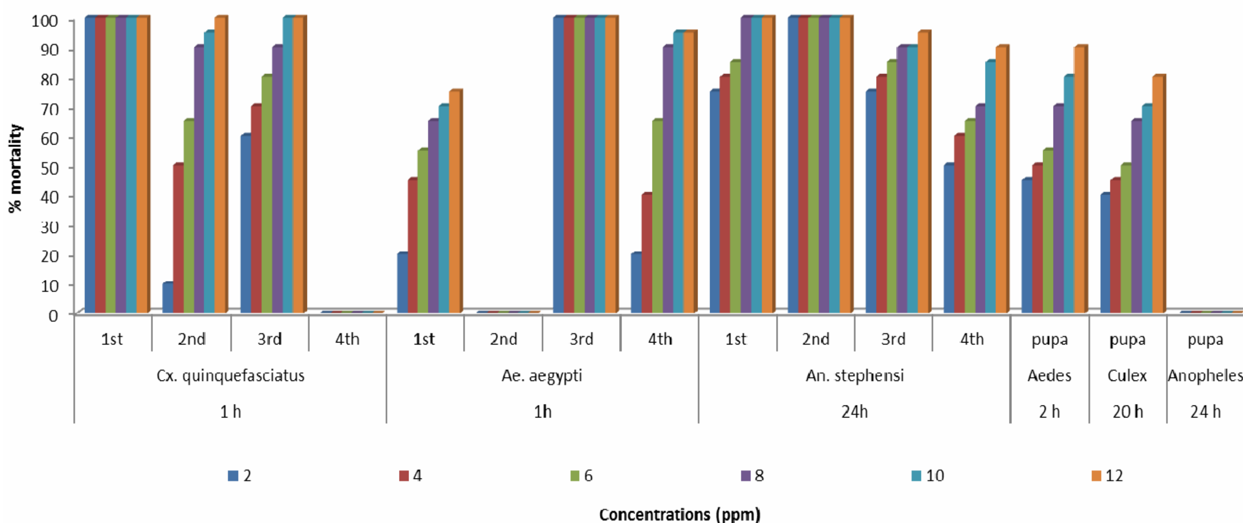

Figure 2. Relationship between the % mortality and concentrations of AgNPs synthesized by *A. niger* against the larvae and pupae of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* after different hours of exposure.

stage of *An. stephensi* (**Figure 2**).

The pupae of *Ae. aegypti* were found highly susceptible to the *A. niger* synthesized AgNPs than the pupae of *Cx. quinquefasciatus* and *An. stephensi*. The mortality was scored after different hours of exposure. The efficacy for the pupae of *Ae. aegypti* (LC_{50} 4, LC_{90} 12, LC_{99} 14 ppm) after 2 h, for *Cx. quinquefasciatus* (LC_{50} 6, LC_{90} 14, LC_{99} 17 ppm) after 20 h were observed with their probit equations and confidential limits and no mortality could be observed for the pupae of *An. stephensi* after 24 h (**Table 2**). The chi-square values calculated at 4 df were 44.84 and 42.40 for pupae of *Ae. aegypti* and *Cx. quinquefasciatus*. These chi-square values for pupae of *Ae. aegypti* and *Cx. quinquefasciatus* were higher than the critical value of chi-square at significance level. The relationship between the concentrations and % mortality were shown for pupae of *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* (**Figure 2**).

4. Discussion

AgNPs synthesized by using the soil fungus *A. niger* 2587 possessed higher larvicidal and pupicidal efficacy against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* in the present study. The production of AgNPs synthesized by using the soil fungus *A. niger* 2587 was evaluated through the UV-vis spectrophotometer in a range of wavelength from 350 - 850 nm. This revealed a peak at 480 nm in the fungal liquid component of soil fungus *A. niger* 2587 indicating the productions of AgNPs. The result was similar to the previous study [24].

The larvicidal activity of silver nanoparticles synthesized by *N. nucifera* leaf extract has been evaluated against the malaria and filariasis vectors [25]. All extracts showed moderate larvicidal effects; however, the maximum efficacy was observed in crude methanol, aqueous, and synthesized silver nanoparticles against the larvae of *An. subpictus* (LC_{50} 8.89, 11.82, and 0.69 ppm, LC_{90} 28.65, 36.06, and 2.15 ppm) and against the larvae of *Cx. quinquefasciatus* (LC_{50} 9.51, 13.65 and 1.10 ppm, LC_{90} 28.13, 35.83 and 3.59 ppm), respectively. The antiparasitic activity efficacies of synthesized AgNPs using the aqueous leaf extract of *M. pudica* against the larvae of malaria vector *An. subpictus*, filariasis vector *Cx. quinquefasciatus* and *R. microplus* have been determined [26]. The maximum efficacy was observed in synthesized AgNPs against the larvae of *An. subpictus*, *Cx. quinquefasciatus* and *R. microplus* (LC_{50} 13.90, 11.73 and 8.98 mg/L and r^2 = 0.411, 0.286 and 0.479), respectively. The larvicidal activity of synthesized AgNPs utilizing aqueous extract from *E. prostrate*, a member of the Asteracae, has been investigated against fourth instar larvae of filariasis vector, *Cx. quinquefasciatus* and malaria vector, *An. subpictus* [27]. The maximum efficacy was observed in the crude aqueous and synthesized AgNPs against *Cx. quinquefasciatus* (LC_{50} 27.49 and 4.56 mg/L; LC_{90} 70.38 and 13.14 mg/L) and against *An. subpictus* (LC_{50} 27.85 and 5.14 mg/L; LC_{90} 71.45 and 25.68 mg/L), respectively. A biological method has been used to synthesize stable silver nanoparticles that were tested as mosquito larvicides against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* [28]. The median LC_{50} of silver nanoparticles that killed fourth instars of *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* were 0.30, 0.41 and 2.12 ppm, respectively. The biolarvicidal and pupicidal activity of silver nanoparticles synthesized using *E. hitra* plant leaf extract against malaria vector *An.*

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stephensi has been determined [29]. They found that the synthesized AgNPs were highly toxic than the methnolic crude extract against malaria vector. The larvicidal activity of silver nanoparticles synthesized using *P. daemia* plant latex against *Ae. aegypti*, *An. stephensi* and non-target fish *P. reticulate* has been evaluated [30]. The larvicidal activities to determine the efficacies of synthesized silver nanoparticles (AgNPs) using aqueous leaf extract of *V. rosea* against the larvae of malaria vector *An. stephensi* Liston and filariasis vector *Cx. quinquefasciatus* Say (Diptera: Culicidae) has been evaluated [31]. These results were based on plant mediated silver nanoparticles and have been tested against the larvae of mosquito and non-target organisms also, while, in the present study the AgNPs were synthesized by using the soil fungus and have also been tested against the larvae and pupae of mosquito.

5. Conclusion

The AgNPs synthesized by using the soil fungi *A. niger* has been tested against the larvae and pupae of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*. By this approach, it is suggestive that this rapid synthesis of nanoparticles would be proper for developing a biological process for mosquito control.

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