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Biocontrol of *Aedes aegypti* using *Talaromyces islandicus* Synthesized Silver Nanoparticles

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Aedes aegypti is the vector that spreads the dengue virus, causing dengue fever and dengue hemorrhagic fever. With more than half the world's population at the risk of acquiring this infection, controlling the *Aedes* mosquitoes is the only path to limit the spread of the fatal disease. The emergence of insect resistance in mosquitoes raised the need for developing novel insecticides. Present research is focused on using fungus (*Talaromyces islandicus*) as the biosystem in the synthesis of nanoparticles. Myco-synthesized silver nanoparticles were characterized using UV-visible spectrometry that exhibited a peak at 429 nm. The XRD spectral peaks were in the range of 27.83º, 32.27º, 38.23º and 65.01º. The FTIR spectrum showed peaks corresponding to O-H, N-O, S=O, *etc.* representing the silver nanoparticles. SEM and EDAX represent the formation of silver ions that are spherical in shape with a size range of 23 to 26 nm. The antioxidant activity of silver nanoparticles and the extract of *Talaromyces islandicus* were assessed by DPPH assay, reducing power assay and hydrogen peroxide assay. The nanoparticles studied for its bio efficacy against the larval stages of *Aedes aegypti* indicated the LC_{50} value of 352.03, 389.86, 397.72 and 443.50 when tested against first, second, third and fourth instar larvae. respectively. The LC_{50} value of 540.41 was determined against the pupae of *Aedes.* The predatory efficiency of *P. reticulata* indicated the positive feeding behaviour of the fish when exposed to the silver nanoparticles. The cell toxicity assay was conducted against C6/36 insect cell lines and the cell viability inhibition was calculated. A toxic free, environmentally acceptable approach for controlling the mosquito vector by utilizing fungal nanoparticles was assessed and their efficacy in vector control was analyzed in this study.

Keywords: ***Aedes aegypti*, Silver nanoparticles, Antilarval, *Talaromyces islandicus*, Antioxidant.**

INTRODUCTION

Rapid fluctuations in climatic conditions and environmental changes have led to an increase in the unnoticeable sites for mosquito development. Mosquitoes are the vectors that utilize moist, damp and stagnated water areas of the land for completion of its lifecycle. Various species of mosquito inhabits different viruses, among which *Aedes aegypti* (Diptera: Culicidae) is a species of mosquito that provides ambient conditions for the inhabitation of the dengue virus (Flavivirus) [1]. *Aedes aegypti* inhabits four different serotypes of dengue virus such as DENV1, DENV2, DENV3 and DENV4. All four serotypes are reported to cause dengue and dengue hemorrhagic fever with slight variations in its severity [2]. Center for disease control and prevention (CDC) reported that the incidence of dengue transmission has increased to 30-folds in the last fifty years.

The World Health Organization (WHO) confirms the spread of dengue and severe dengue fever in regions that are sub-tropical and tropical all throughout the world [3]. In spite of the under-reporting of dengue virus transmission in most of the countries due to misdiagnosis and incidence of non symptomatic infections worldwide infection rate peaks to 400 million infection every year. Misdiagnosis occurs mainly due to the common symptoms such as rashness, mild fever, *etc.* that has been misdiagnosed with the entry of the dengue virus into the human bloodstream [4]. Displacement of less virulent strain with the lethal strains capable of possessing higher vectorial capacity is a challenge in controlling disease transmission. Factors such as (i) infection by one serotype does not conferring immune tolerance to subsequent infection by other serotypes; (ii) immunopathogenesis related to antibody dependent enhancement; (iii) non-availability of vaccines; (iv) easy transmission through the human-mosquito-human cycle; and (v) wider global distribution of *Aedes aegypti* had initiated research in developing vector control of strategies for efficient mosquito abatement.

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Some of the methods of mosquito control included controlling the stagnation sites to prevent breeding, genetic modifications of the mosquito genome to bring population depression in breeding, mechanical methods of setting insect traps, chemicals methods of using insecticides, *etc.* [5]. Most of the methods have not reported for complete success due to the enormous reproductive capacity and the acquired resistance for overused insecticides. Major drawbacks related to the extensive usage of insecticides includes causing harm to non-target organisms and to the environment [6]. Strategies such as sterile insect technique still need to be reviewed for its applicability in the field due to the higher genomic flexibility of the vectors. Alternative search for control measures led the field of research in utilizing biological sources for the synthesis and preparation of insecticide that would not cause negative effects on humans and the environment are urgently required.

Owing to the possibility of developing nanoparticles of desired sizes and shape, biological routes as controlled sources are gaining remarkable interest. Green synthesis has been evaluated to be an easier and cost-effective method compared to the chemical and physical mode of nanoparticle synthesis [7-10]. Microorganisms release secondary metabolites that act as reducing or capping agent during the synthesis of nanoparticles [11]. Fungal species *viz. Penicillium*, *Aspergillus* and *Neurospora* are reported to act as nano factories. Nano pesticides are gaining attention in recent years due to its efficiency in controlling the mosquito species [12,13]. Fungi produces secondary metabolites such as alkenes, phenols and proteins that act as mosquitocidal agents. Kamradgi *et al.* [14] reported the synthesis of silver nanoparticles using filamentous fungi *Talaromyces islandicus*, the fungal filtrate was reported to act as reducing agent that aided in the synthesis of silver nanoparticles. Filamentous fungi possess a larger surface area for enabling the interaction between the silver ions and the fungal metabolite to yield silver nanoparticles [15]. Presence of protein and S-H groups of secretory compounds interact with the silver ions during synthesis [16]. Fayaz *et al.* [17] report the possible mechanism that involves the utilization of NADPH-dependent reductase enzymes that aids in the reduction of the Ag^+ to Ag^0.

Fungal nanoparticles are receiving more applications due to the excessive resistance conferred by the fungal mycelial biomass to metals and the manipulating characteristic features offered by the fungal nanoparticles. Control of *A. aegypti* egg hatchability using silver nanoparticles at a concentration of 25 ppm resulted in zero hatchability against the arbo vector [18]. Silver nanoparticles have the capability to act as adulticidal compounds against the *A. aegypti* adult mosquitoes. Lower concentrations of silver nanoparticles caused a LD_{50} value of 44.85 ppm against the adult species of *A. aegypti* [19]. Similar studies against adult species of *A. albopictus* were conducted and found to show lesser LD_{50} of 14 ppm on the adulticidal activity and 13 ppm on larvicidal activity was reported using green synthesized silver nanoparticles [19,20]. *Acacia caesia* synthesized silver nanoparticles exhibited potential ovicidal (75 µg/mL), larvicidal (11.31 µg/mL) and adulticidal (20.34 µg/mL) activity against *Aedes albopictus* [21]. The silver nanoparticles have also positively affected the predatory behaviour of various non-targeted species of animals when exposed to the predation against mosquito larvae [22]. Present study focuses on employing *Talaromyces islandicus* (MT123786) as a potential larvicidal biomaterial and elucidated the properties of the synthesized silver nanoparticles and its functionality in control of the larval and pupal stages of *A. aegypti.*

EXPERIMENTAL

Metal tolerability of *Talaromyces islandicus* (MT123786) against silver nitrate: The metal tolerability of *Talaromyces islandicus* (MT123786) was tested by subjecting the fungi on the potato dextrose media supplemented with various concentrations of silver nitrate. Following the methods of Iram *et al.* [23] with modifications, silver nitrate metal solution was diluted to make concentrations of 200, 400, 600, 800 and 1000 µg/L and the plates were cultured with the fungal spore solution. The culture plates were incubated for a period of 7 days for fungal growth. The spores were counted to calculate the number of cells per milliliter of inoculation [23].

Silver nanoparticle synthesis by *Talaromyces islandicus* (MT123786): *Talaromyces islandicus* (MT123786) was subcultured in compost media consisting of KH_2PO_4, K_2HPO_4, $MgSO_4{\cdot}7H_2O$, $(NH_4)_2SO_4$, yeast extract and glucose. The media inoculated with the fungal spore was incubated on an orbital shaker at 175 rpm at 26 ºC for 7 days. The culture harvested was filtered using Whatman filter paper grade No. 1. Biomass was washed thrice and more until all the media particles were completely removed. The biomass (10 g) was weighed and transferred to 100 mL of sterile distilled water and incubated for 72 h at 175 rpm at 26 ºC [14]. After the incubation for 72 h, the biomass was filtered and the filtrate was collected in a sterile Erlenmeyer flask. The filtrate obtained after incubation was measured and transferred to a sterile Erlenmeyer flask and equal volume of 1 mM $AgNO_3$ solution was added and covered with aluminium foil to maintain the dark conditions. The setup was maintained on a closed shaker incubator and maintained for 48-72 h at 125 rpm at room temperature. The change in colour was observed after the incubation was subjected to UV visible spectroscopy. The solution was centrifuged at 10,000 rpm for 12 min, washed thrice with water and ethanol. The obtained sample was dried for further evaluation.

Antioxidant activity of *Talaromyces islandicus* synthesized silver nanoparticles

DPPH assay: The scavenging capacity of free radicals by the myco-synthesized silver nanoparticles were determined using DPPH (1,1-diphenyl-2-picryl-hydrazil). Following the methods of Hamelian *et al.* [24] and Paari *et al.* [25] with slight modifications, silver nanoparticles were aliquoted into different concentrations such as 200, 400, 600, 800 and 1000 µg/L and was made up to 2 mL. Then, 2 mL of ethanol solution of DPPH (0.1 mM) was added to the aliquoted samples and incubated in dark conditions for 30 min and the absorbance was read at 517 nm [24]. Ascorbic acid was used as standard. The free radical scavenging percentage was calculated as follows:

$$\text{Free radical scavenging by DPPH} = \frac{A_c - A_s}{A_c} \times 100$$

where A_c is the absorbance of the DPPH solution and A_s is the absorbance of the test samples.

Reducing power assay: The silver nanoparticles were tested for its capability in the reduction of the free radicals. Following the methodology of Akintola *et al.* [26] with slight modifications, various concentrations of the test samples such as 200, 400, 600, 800 and 1000 µg/L were aliquoted into different vials. To the vials containing 500 µL of test samples, 1.5 mL of phosphate buffer (200 mM, pH 6.6) along with 1.5 mL of 1% potassium ferricyanide was added. The vials were incubated for 20 min at 55 ºC. 1.5 mL of trichloroacetic acid (10%), was added and centrifuged at 5000 rpm for 5 min. To the supernatant, equal volume of distilled water was added along with 1 mL of 0.1% $FeCl_3$ and checked for its absorbance at 700 nm with ascorbic acid as standard. Increase in absorbance indicated the increased reducing activity of the myco-synthesized silver nanoparticles [26].

Hydrogen peroxide assay: The hydrogen peroxide scavenging assay was conducted following the methods of Keshari *et al.* [27] with slight modifications. Concentrations of the test sample were taken as 200, 400, 600, 800 and 1000 µg/L. The final concentration was made up to 1 mL using a phosphate buffer (50 mM pH 7.4). To this solution, 500 µL of H_2O_2 (5mM made up using phosphate buffer 50mM and final pH set to 7.4) was added. The samples were mixed well and allowed to rest at room temperature for 20 min and the absorbance was measured at 230 nm [27]. Ascorbic acid was used as standard. Scavenging percentage was calculated as follows:

$$\text{Hydrogen peroxide scavenging (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

where A_c is the absorbance of the hydrogen peroxide aliquoted with phosphate buffer solution and A_s is the absorbance of the test samples.

Characterization: The size, morphology and properties of the myco-synthesized *Talaromyces islandicus* synthesized silver nanoparticles were analyzed. The colour change was observed for peak ranging between 200-800 nm using UV-visible spectrometry (UV-1800 Shimadzu UV-spectrophotometer). The groups present in the green synthesized silver nanoparticles were analyzed by performing Fourier transform infrared spectroscopy using Shimadzu IR spirit with single reflection ATR accessory. The nanostructures were further analyzed by XRD using Rigaku-miniflex 600 having a fine focal point of the Cu-anode working in 40 kV and 15 mA. The nanoparticle topology and atomic composition were studied by SEM (model JEOL JSM-6390) and EDX (EDX Oxford Instrument, INCA Penta FETX3).

Larvicidal activity: Following the standard protocol of the World Health Organization (2005), the mosquito larvae were subjected to mortality using the myco-synthesized silver nanoparticles. *Aedes aegypti* larval stages were procured from National Centre for Disease Control (NCDC), New Delhi, India. *Talaromyces islandicus* (MT123786) synthesized silver nanoparticles were prepared in five concentrations (200, 400, 600, 800 and 1000 µg/L) and tested against the first, second, third, fourth larval stages of *Aedes aegypti*. Hundred larvae were transferred into the vials containing 250 mL of distilled water to which the various concentrations of the silver nanoparticles are added and observed for mortality at every 15 min of time interval up to 24 h. The percentage mortality was calculated using the following formula:

$$\text{Total mean mortality (\%)} = \frac{N_d}{N_t} \times 100$$

where, N_d is the number of larvae dead and N_t is the total number of larvae treated. With the percentage mortality, the lethal concentration of 50% and 90% was calculated using probit analysis [1].

Pupicidal activity: The larvae were grown up to its pupal stage in distilled water provided with the diet that included yeast extract and other requirements as per the WHO (2005) standards. The pupae were then transferred into vials containing the silver nanoparticles in concentrations (200, 400, 600, 800 and 1000 µg/L) to study the pupicidal activity. The mortality rate was evaluated at every 15 min interval and the pupal mortality was observed and calculated for 24 h.

Predatory efficiency of *Poecilia reticulata*: Following the protocols of Murugan *et al.* [1], the predatory efficiency of *P. reticulata* was determined against the third and fourth instar larvae of *Aedes aegypti*. Fifty larvae were transferred into a bowl of 250 mL distilled water along with single *P. reticulata.* Experiment was tested to analyze the variations in the predation with the exposure of the myco-synthesized silver nanoparticles in two intervals of 12 h (day-time and night-time) for 24 h at concentrations such as 200, 400, 600, 800 and 1000 µg/L. Control group was observed for the predation efficiency without the exposure to nanoparticles.

Cytotoxicity of silver nanoparticles against C6/36 cell lines: The cytotoxic effects of the myco-synthesized silver nanoparticles were tested against the C6/36 insect cell line. Trypsinized monolayer cell culture was cultured in the presence of 10% FBS. Diluted cell suspension was added into 96 well plates (100 µL each). After incubation for 24 h, test concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 ppm myco-synthesized silver nanoparticles were added. The plates were incubated in a 5% CO_2 incubator at 28 ºC for 24 h. MTT was added to the wells after discarding the supernatants. The plates were incubated for 4 h in a 5% CO_2 atmosphere at 28 ºC. DMSO (100 µL) was added to solubilize the formazan formed. The absorbance was measured at 590 nm using Spectramax I3X plate reader. The concentration of silver nanoparticles required to inhibit the growth of C6/36 cell lines (IC_{50}) was calculated using the dose response curve. The percentage inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

where A_c is the absorbance of the control group without nanoparticle exposure and A_s is the absorbance of the cells after exposure to the nanoparticles.

Statistical analysis: The lethal concentration was calculated using SPSS 22.0 software, using the probit analysis for the development of regression equations in analysis of the LC_{50}

and LC_{90}. The predatory efficiency was calculated for its significance using one-way ANOVA. The cell toxicity was analyzed using GraphPad Prism version 9. To plot the logarithmic concentration of the X-axis and to normalize the inhibition percentage. The IC_{50} was calculated using the non-linear regression fit curve. Metal tolerance and antioxidant assays were estimated for their significance using one-way ANOVA in SPSS software.

RESULTS AND DISCUSSION

Metal tolerance: Metal tolerability of the microorganisms marks the potential of the microorganism to utilize the metal to reduce and synthesize nanoparticles. The concentration range of 200, 400, 600, 800 and 1000 µg/L was added to the PDA plates to determine the maximum metal tolerable potential of *Talaromyces islandicus*. The spore count was found to decline with the increased concentration of silver nitrate (Fig. 1). The study found a correlation between metal tolerance and nanoparticle synthesis. The number of spores was estimated after diluting the spores at 1:5 times of dilution. The control group had 4.95×10^{-7} cells, which gradually decreased to 2.56×10^{-7} cells at 2000 µg/L silver nitrate concentration. Berdy [28] reported the availability of more than thousand bioactive metabolites that are secreted by the filamentous fungi which can be utilized as reducing agents for heavy metal tolerance. Present study revealed the ability of the fungus to tolerate the heavy metal at an optimum concentration of 2000 µg/L. The metal tolerance could be due to the metal internalization and bioaccumulation ability of the filamentous fungi [29]. Similar studies by Iram *et al.* [23] indicated a decrease in the tolerability of the fungus with increased exposure to various metals.

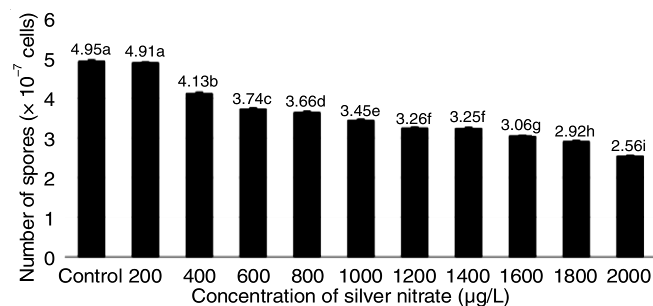

Fig. 1. Indicates the number of spores formed with the exposure of silver nitrate at various concentrations

Antioxidant assays: The antioxidant capability of the silver nanoparticles was studied to evaluate their ability to act as antioxidant agents when synthesized from biological sources. Three standard methods used for estimation are DPPH scavenging assay, Hydrogen peroxide scavenging assay and reducing power assay (Table-1). The study determines the capability of the fungal extracts to be capable of acting as a reducing agent for the synthesis of nanoparticles. The DPPH assay showed activity of 40.02%, 48.13%, 55.29%, 62.37% and 72.05%. whereas the ascorbic acid standard showed 40.23%, 48.77%, 57.58%, 63.39% and 75.48% at 200, 400, 600, 800, 1000 µg/L, respectively. The antioxidant activity tested by the methods of hydrogen peroxide scavenging assay indicated that there is an increase in the H_2O_2 antioxidant scavenging activity of the myco synthesized silver nanoparticles with the increase in the concentration (Table-1). The percentage activity was found to be 77.11%, 85.27%, 88.65%, 90.82% and 92.89% for the silver nanoparticles, 78.43%, 85.66%, 89.37%, 94.49% and 96.48% activity was reported for ascorbic acid at the concentration of 200, 400, 600, 800, 1000 µg/L, respectively. Further, the reducing power assay was performed to determine the capability of the myco-synthesized silver nanoparticle to form Fe^{2+} from the exposed Fe^{3+} indicating its reducing power. Reducing power absorbance values of 0.24, 0.27, 0.49, 0.53 and 0.66 nm were observed for silver nanoparticles and 0.35, 0.44, 0.55, 0.63 and 0.74 nm were observed for ascorbic acid at the concentrations of 200, 400, 600, 800, 1000 µg/L, respectively. An increase in the concentration of nanoparticle exhibited an increase in the absorbance values, which confirms the high reducing activity of the silver nanoparticles.

Results obtained from the DPPH assay, showed a similar pattern of results compared to the studies conducted by Thakor *et al.* [30], which indicated steady increases in the scavenging activity of the *P. oxalicum* synthesized silver nanoparticles to increase up to 71. 33 µg/mL with the increased concentration. The studies conducted by Sharma *et al.* [31] revealed the capability of silver nanoparticles in reducing the Fe^{3+} ions resulted with an increase with the absorbance from 0.150 to 0.300 with the increase in the silver nanoparticles concentration from 0.5 to 2 mg/mL. Hydrogen peroxide assay exhibited an increase with the scavenging percentage of the silver nanoparticles compared to the ascorbic acid standard. Results of present

TABLE-1
EVALUATION OF ANTIOXIDANT ACTIVITY OF MYCO SYNTHESIZED SILVER NANOPARTICLES

Antioxidant assay	Absorbance (nm)	Concentration of silver nanoparticles (µg/L)				
		200	400	600	800	1000
DPPH Scavenging assay (%) - AgNp	517	40.02 ± 0.28	48.13 ± 1.64	55.29 ± 1.84	62.37 ± 0.74	72.05 ± 5.27
Talaromyces islandicus extract		25.22 ± 0.91	33.49 ± 1.69	42.16 ± 1.98	51.08 ± 1.00	62.06 ± 0.94
Ascorbic acid (%)		40.23 ± 0.10	48.77 ± 0.14	57.58 ± 1.67	63.39 ± 2.52	75.48 ± 0.74
H_2O_2 scavenging assay - AgNp	230	77.11 ± 0.63	85.27 ± 0.44	88.65 ± 0.89	90.82 ± 1.03	92.89 ± 0.48
Talaromyces islandicus extract		55.64 ± 1.14	62.81 ± 0.73	73.05 ± 1.01	77.74 ± 0.85	81.15 ± 0.94
Ascorbic acid (%)		78.43 ± 1.14	85.66 ± 0.33	89.37 ± 0.54	94.49 ± 1.29	96.48 ± 0.46
Reducing power assay - AgNp	700	0.24 ± 0.04	0.27 ± 0.03	0.49 ± 0.04	0.53 ± 0.03	0.66 ± 0.04
Talaromyces islandicus extract		0.14 ± 0.04	0.31 ± 0.02	0.43 ± 0.03	0.51 ± 0.01	0.62 ± 0.01
Ascorbic acid (nm)		0.35 ± 0.03	0.44 ± 0.03	0.55 ± 0.03	0.63 ± 0.03	0.74 ± 0.03

DPPH and H_2O_2 scavenging assay percentage is listed with ± SD for triplicate values. Reducing power assay values are listed with increasing absorbance

study were confirmed with the studies of Kesari *et al.* [27], which showed an increased scavenging percentage of 41.41% for the silver nanoparticles compared to 61.63% of vitamin C as standard.

Visible and spectrometric analysis of silver particles: The change in colour of the fungal filtrate before and after the exposure to 1 mM silver nitrate is shown in Fig. 2. The colour of fungal filtrate changed from pale gray to deep brown in colour with the exposure to silver nitrate. The result was further confirmed by spectrometric analysis using UV-visible spectrometry (UV-1800 Shimadzu UV-spectrophotometer), where the absorbance was measured in the range of 200-800 nm. The absorption band at 429.6 nm validated the formation of silver particles in the solution that was exposed to silver nitrate. Darker brown colour was observed during the synthesis of silver nanoparticles. The result was confirmed with similar studies by Govindappa *et al.* [32], where the UV-visible spectrometric results showed peaks at the range of 429-430 marking the formation of silver nanoparticles. Kamradgi *et al.* [14] has reported the similar range of UV spectral analysis of 400 nm range, which confirms the formation of silver particles in the solution [14]. The results are further confirmed by the studies conducted by Banu *et al.* [33], where the silver nanoparticles synthesized using the fungi *Isaria fumosorosea* indicated the incidence of peak at 410 nm for the formation of silver nanoparticles [33] (Fig. 2).

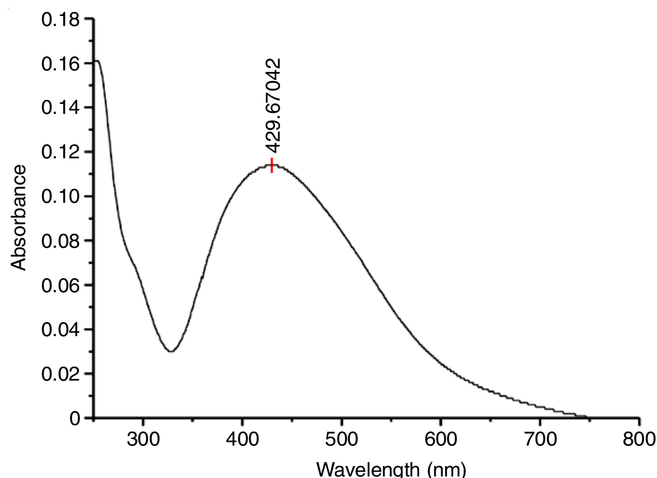

Fig. 2. UV-visible spectrum indicating the absorbance peak that resulted due to the colouration of silver nanoparticle formation

XRD studies: The X-ray diffraction patterns of the myco-synthesized silver nanoparticles is shown in Fig. 3. Using the JCPDS card no. 04-0783, the formation of silver nanoparticles was confirmed. The clear peaks at 27.83º, 32.27º, 38.23º, 44.62º, 46.42º, 54.81º, 57.76º and 65.01º correspond to the synthesized silver nanoparticles. The values corresponding to the crystalline structure of the nanoparticle was calculated for its size using Scherrer equation. The myco-synthesized silver nanoparticles were found to be at an average size of 25.58 nm. The XRD peaks obtained from the present studies were found similar to the peaks reported by Karthiga *et al.* [34] at 38.48º, 44.39º, 64.92º and 77.67º. The pattern obtained was compared with the JCPDS file number 04-0783, which confirms the peaks

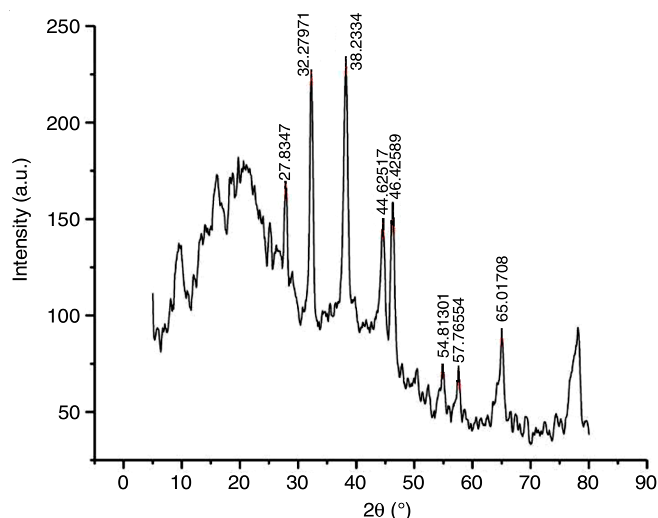

Fig. 3. XRD spectrum of 2θ scales indicating silver nanoparticles

formed was corresponding to the silver ions. Similar results of peaks at the 38.4º range confirm the silver nanoparticles formed by *Chrysosporium tropicum* [35].

FTIR studies: The FTIR spectrum was determined using Shimadzu IR spirit with single reflection ATR accessory, which indicated the occurrence of strong peaks at 3262.03 cm^{-1} (O-H group of alcohols), 2916.23 cm^{-1} (C-H group of alkanes), 1636.01 cm^{-1} (C=C double substituted group of alkenes), 1538.42 cm^{-1} (strong N-O stretching of nitro group), 1154.20 cm^{-1} (C-N stretching of medium amine group), 1384.73 cm^{-1} (O-H bending of medium phenol group), 1310.19 cm^{-1} (S=O stretching of strong sulfone group). The peaks represent the nanoparticles formation with the respective absorption range (Fig. 4). The peak stretching exhibited in this study is similar to the studies reported by Murugan *et al.* [36], where the bands stretched at 1045.1087, 1328.41, 1924.64, 2974.35, 3350.60 cm^{-1}. Other reports of O-H, NH and C-Br in the similar spectral range confirms the formation of silver nanoparticles [37].

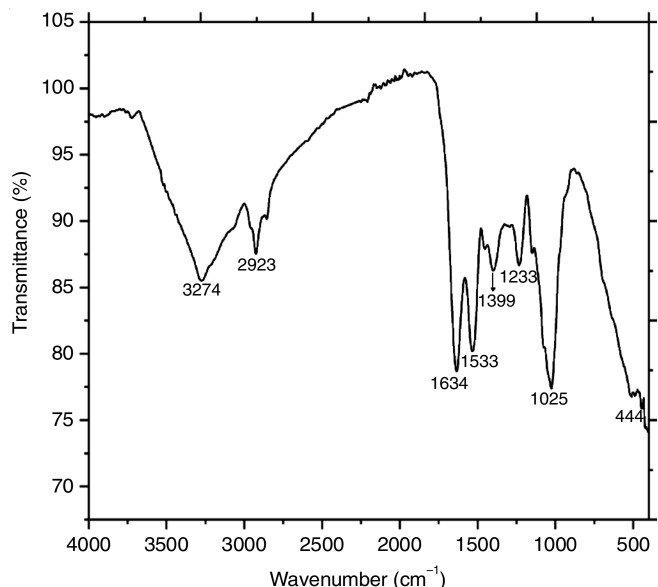

Fig. 4. FTIR spectrum indicating band stretching corresponding to the formation of silver nanoparticles

Morphology studies: The SEM images of the myco-synthesized silver nanoparticles indicated that the nanoparticles were mostly spherical in shape (Fig. 5). The SEM and EDX showed the spherical shaped silver nanoparticles of 23-26 nm size range were confirmed in the present study. The results are compared with the studies carried out by Soni & Prakash [35], where synthesized *Chrysosporium tropicum* silver nanoparticles were found to be spherical in shape with a size range of 20-50 nm.

Bioefficacy of silver nanoparticles on larval and pupal stages of *Aedes aegypti*: The larvicidal activity of myco synthesized silver nanoparticles was tested against the fourth instar larval stages of *Aedes aegyptii*. The pupicidal activity of myco-synthesized silver nanoparticles were tested against the pupae. The LC_{50} and LC_{90} values of silver nanoparticles were 352.03 µg/L and 1336.24 µg/L LC_{90}, respectively against the first instar larvae. The LC_{50} and LC_{90} values were 389.86 µg/L and 1576.86 µg/L against the second instar larvae. The LC_{50} and LC_{90} values were 397.72 µg/L and 2078.80 µg/L against the third instar larvae. The LC_{50} and LC_{90} values were 443.50 µg/L and 2317.86 µg/L against the fourth instar larvae. The LC_{50} and LC_{90} values were 540.41 µg/L and 2843.34 µg/L, respectively against the pupal stage of *Aedes aegypti* (Table-2). This studies showed larvicidal and pupicidal activity against *Aedes aegypti* species of mosquitoes at lower concentrations of silver nanoparticles such as 200 to 1000 µg/L.

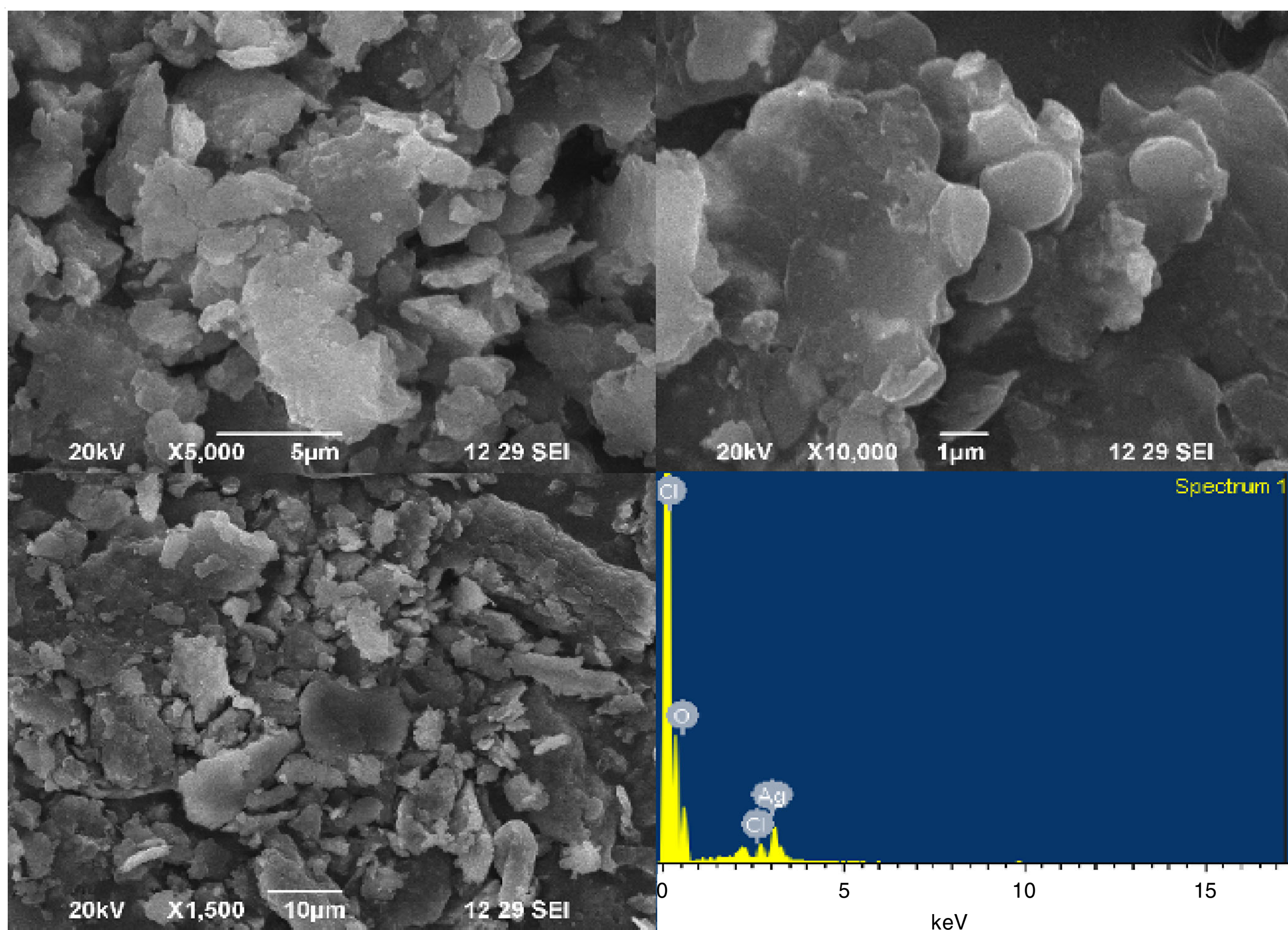

Fig. 5. SEM images of myco synthesized silver nanoparticles captured at 1500X, 5000X and 10,000X resolution and EDAX spectrum indicating the presence of silver ion

TABLE-2
LETHAL CONCENTRATION 50 (90) OF THE MYCO SYNTHESIZED SILVER NANOPARTICLES AGAINST THE DEVELOPMENTAL STAGES OF *Aedes aegypti* LARVAE

Target	LC_{50} (LC_{90})	95% confidence limit LC_{50} (LC_{90})		Regression equation	Chi-square (d.f = 3)	Signifi-cance	R^2
		Lower limit	Upper limit				
First instar larvae	352.03 (1336.24)	298.10 (1080.35)	401.56 (1830.44)	Y = -5.634+2.212x	2.760	0.430[a]	0.97
Second instar larvae	389.86 (1576.02)	332.37 (1237.86)	444.28 (2276.48)	Y = -5.473+2.113x	3.485	0.323[a]	0.95
Third instar larvae	397.72 (2078.80)	329.42 (1506.75)	462.25 (3542.65)	Y = -4.638+1.784x	1.184	0.757[a]	0.98
Fourth instar larvae	443.50 (2317.86)	373.61 (1650.15)	513.85 (4082.93)	Y = -4.723+1.784x	0.324	0.955[a]	0.99
Pupae	540.41 (2843.34)	464.28 (1947.47)	630.44 (5390.24)	Y = -4.857+1.777x	2.58	0.460[a]	0.95

No mortality was observed in the control

Similar studies conducted by Soni & Prakash [35] reported that the second instar larvae exhibited 100% mortality with an exposure to silver nanoparticles. The LC_{50} value of first, third, fourth instar larvae was reported as 3.46, 4 and 2 ppm, respectively.

Predatory efficiency of *Poecilia reticulata* on *Aedes aegypti* larvae: Biocompatibility of the myco-synthesized fungal nanoparticles was tested to check its safety in the predatory efficiency. In order to study the effect of myco-synthesized silver nanoparticles on the environment, present study was conducted to study the changes in feeding quantity before and after the exposure to silver nanoparticles in controlled environments. The experiment resulted in 77.32%, 82.66%, 82.66%, 88.64% and 90.66% of total predation on third instar larvae when exposed to 200, 400, 600, 800, 1000 µg/L of silver nanoparticles respectively for 24 h. The predatory efficiency was interestingly higher than that of the control group which was found to be 85.32%. Predation efficiency was found to be 68.64%, 79.32%, 81.98%, 87.98% and 90% at concentrations of 200, 400, 600, 800, 1000 µg/L, respectively. The predator efficiency for the control group was 81.32% for the fourth instar larvae. Table-3 indicates the mean number of predations in two time periods, such as day time feeding count (0-12 h) and night time feeding count (12-24 h). *Poecilia reticulata* are effectual predators of *Aedes aegypti* larvae at all four larval stages. Similar studies conducted by Murugan *et al.* [1] showed increased predation exhibited by the guppy fishes after the exposure to silver nanoparticles synthesized using *S. alba*. The predation was found to decrease with the increase in the size of the larvae from 82.25% to 39.06% from the first to fourth larval stages respectively. The study also shows the increase in predation from 65.06% to 82.25% when fed in the standard conditions without silver nanoparticles.

Cytotoxicity of silver nanoparticles against C6/36 cell lines-MTT assay: To study the effects of the synthesized silver nanoparticles at the cellular level of the mosquito species, the C6/36 cell line was assessed. The absorbance obtained by conducting the experiment was used to calculate the percentage of cell viability inhibition. The percentage inhibition of cell viability at the concentration of 200, 400, 600, 800, 1000 µg/L was found to be 1.15%, 3.26%, 7.68%, 10.75% and 18.81%, respectively. The highest study concentration resulted in 18-19% of cell viability inhibition at 1000 µg/L. Therefore, the study concentration was increased to 20000 µg/L and 40000 µg/L of silver nanoparticles. The cytotoxicity of the myco synthesized silver nanoparticles were found to have an IC_{50} value at a concentration of 30,000 µg/L (Log 10 concentration = 4.48). Sujitha *et al.* [37] reported that the control cell line without exposure to silver nanoparticles showed growth, whereas cells exposed to silver nanoparticles showed cell damage with exposure to silver nanoparticles at 40 µg/mL. The study results are in agreement with the reports by Murugan *et al.* [1], which indicated a 35% decline in the Vero cell violability with the exposure to 30 µg/mL of silver nanoparticles.

Conclusion

This study highlighted the methodologies involved in the synthesis of silver nanoparticles using *Talaromyces islandicus.* Analytical tools such as UV spectrometry, FTIR, XRD and SEM with EDX results confirmed the formation of silver nanoparticles. The myco-synthesized silver nanoparticles have shown potential activity against the larval and pupal stages of *Aedes aegypti*. The study drew attention towards using the minimal concentration of the silver nanoparticles, which had shown high bioefficacy against *Aedes* stages. The study also marks the efficiency of the nanoparticles to show a positive effect on predatory behaviour of *Poecilia reticulata*, which confirmed the harmless nature of the myco-synthesized silver nanoparticles on the aquatic biosystems.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

TABLE-3
PREDATORY EFFICIENCY OF *Poecilia reticulata* AGAINST THE THIRD AND FOURTH INSTAR LARVAE OF *Aedes aegypti* IN CONTROLLED CONDITIONS AND WITH EXPOSURE TO MYCO-SYNTHESIZED SILVER NANOPARTICLES

Targeted stage of larvae	Treatment group	Treatment concentration (µg/L)	Predation during day time (1-12 h)	Predation during night time (12-24 h)	Total predation (%)
Third instar larvae	Control		24.33 ± 2.08 ^{bc}	18.33 ± 3.05 ^{a}	85.32
	Treated with TI AgNp's	200	25.00 ± 2.00 ^{bc}	13.66 ± 1.52 ^{b}	77.32
		400	22.00 ± 2.64 ^{c}	19.33 ± 1.52 ^{a}	82.66
		600	24.00 ± 3.00 ^{bc}	17.33 ± 2.08 ^{a}	82.66
		800	27.66 ± 2.08 ^{ab}	16.66 ± 1.15 ^{ab}	88.64
		1000	29.33 ± 1.53 ^{a}	16.00 ± 1.00 ^{ab}	90.66
Fourth instar larvae	Control		24.66 ± 1.52 ^{ab}	16.00 ± 1.00 ^{a}	81.32
	Treated with TI AgNp's	200	22.66 ± 2.51 ^{b}	11.66 ± 1.53 ^{b}	68.64
		400	25.33 ± 1.52 ^{ab}	14.33 ± 1.15 ^{ab}	79.32
		600	26.33 ± 1.15 ^{a}	14.66 ± 2.08 ^{a}	81.98
		800	27.66 ± 1.15 ^{a}	16.33 ± 1.53 ^{a}	87.98
		1000	28.00 ± 2.00 ^{a}	17.00 ± 2.00 ^{a}	90.00

Mean values of the predatory number of larvae ± standard deviation of triplicates (alphabets in the table indicates the significantly different values with $P\text{-value} < 0.05$) study exposure = 1 *P.reticulata* fed with 50 larvae in each replicate.

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