INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 12, No. 6, p. 323-333, 2018

RESEARCH PAPER OPEN ACCESS

Synthesis Myconanoparticles by using *Metarhizium anisopliae* as a biological management for *Culex pipiens*

Rasha Saatam Hameed*, Sundus hameed ahmed, Rasha Saad Nuaman, Raghad J. Fayyad

Department of Biology, College of Science, University of Al-Mustansiriyah, Baghdad, Baghdad, Iraq

Key words: Green synthesis; Silver Nanoparticles; Soil Fungus; *Metarhizium anisopliae*; Mosquito Control.

http://dx.doi.org/10.12692/ijb/12.6.323-333 Article published on June 24, 2018

Abstract

By means of green synthesis and to control culex pipiens, new silver non-particles (AgNPs) were synthesized depending on soil fungus Metarhizium anisopliae and proposed a Metarhizium anisopliae-based. The silver Nano particles were synthesized by Metarhizium anisopliae, In present study, different larval stages and pupae of Cx. pipiens mosquitoes were treated with four different concentrations of Metarhizium anisopliae and silver nanoparticles. All the collected data were from instrumental analysis like UV-vis spectrophotometer, Fourier transform infrared spectroscopy (FTIR), Atomic force microscopic analysis (AFM) and X-ray diffraction (XRD) which usually confirms the structure and the identification of the biosynthesis of AgNPs. The e effectiveness tests were carried out in different time using different concentrations. The larvae of Cx. pipiens shows 100% mortality to the prepared AgNPs after// h of get in touch with, whereas, the pupae of Cx. pipiens were fewer liable to the novel AgNPs. the research concluded that the new synthesis of Metarhizium anisopliae silver nanoparticles can be used as greener method for safe environment vector control strategy throughout a biological management.

* **Corresponding Author:** Rasha Saatam Hameed ✉ rashawe.s.h8383@uomustansiriyah.edu.iq

Introduction

Culex pipienes is a dengue fever carrier virus which cause chikungunya,, dengue, in addition to dengue hemorrhagic fever (Kang *et al.,* 2009). World Health Organizarion (WHO) reported at 2009, that 40% of human being could be infected with dengue. The WHO also reported in India and at 2010, reported that 108 were died from 28,292 infected cases (Mishra *et al.,* 2011).

To minimize the mosquito masses and to bring them down, biological ways were used as alternate to currently larvicides as an alternate to larvicides, this way provide successful and environmently accepted way to reduce the mosquitoes masses to lower level. Regrettably, mosquitoes develop its resistance toward the chemical larvicides (Cadavid *et al.,* 2012; Chenniappan and Ayyadurai, 2012). An immediate necessity is needed to investigate the propagation of mosquitoes for lessening diseases by exploring a convenient method to control vectors. The control of Mosquito especially in third countries earnest heed on account of absence of awareness, resistance of insects to chemical insecticides. Currently, fungi's were used to synthsized nanoparticle, it shows friendship with the environment because of its renewal ability and could be useful as a reduction agent in synthesis of silver nanoparticles (AgNPs).

The reduction of metal biologically can be useful for nontoxic application in green environment to the production of metals nanoprticles. Some microbes like yeast (Mourato *et al.,* 2011). Fungi (Soni and Prakash, 2013) and bacteria (Najitha *et al*., 2014) were probably valuable in preparing metal nanoparticles using standard pressure and temperature. Lots of fungus were applied for nanoparticle fabrication, together with Verticillium spp. (Mukherjee *et al.,* 2001), Aspergillus fumigates (Bhainsa and D'Souza, 2006) Aspergillus niger (Soni and Prakash, 2013), and Fusarium oxysporum (Sonal *et al*., 2013). Recently, mosquitoes defend the chemical pesticides; some bacterial toxins, Bacillus thuringiensis subsp, (Bacillus sphaericus and. israelensis) (Tetreau, *et al.,* 2012) are novel environmentally safer to havoc vectors at bottom level.

The current research goal to study aims to calculate the entomopathogenic fungi, Metarhizium anisopliae-prepared silver nanoparticles to the filariass vector Culex pipiens.

Material and methods

Chemicals

PDA was made in England, $AgNO_3$ was German made, NaOH was prepared in Iraq, and PDB was made in India. Chemical used in the synthesis and experiments in this research were pure and analytic grade.

Mosquito Rearing

Larvae of *C. Pipiens* were brought from a water pool in the College of Education for Pure Sciences / Ibn al-Haytham / University of Baghdad and put in a container and reared in laboratory at Mustansiriyha University, college of sciences, as insect was identified in the natural historic museum and research center in Baghdad University. The larval instars of *Culex pipiens* were collected from stagnant fresh water bodies and reared in the laboratory. The adult mosquitoes were transferred to cages and the culture was maintained at 27±2C^{o} temperature and 75± 5% relative humidity. The mosquitoes nutrition were 10% glucose solution, while back and belly shaved rabbit render as a resource of blood food to females. The egg rafts were collected in plastic bowls containing water and kept in the same water for larval emergence. The larvae were raised in plastic trays measuring 30cmx25cmx5cm. The larvae has bee feed with yeast powder. Pupae were separated by droppers and the colony was maintained. The light periodicity was 12 h light and 12 h dark period. The life cycle of *C. pipiens* was completed within 10-12 days in laboratory (Promsiri *et al.,* 2006).

Identification and separation of entomopathogenic fungi

The entomopathogenic fungi were gained from the

Agricultural Research Center / Plant Protection Commission / Ministry of Agriculture and subcultured on potato dextrose agar (PDA) supported with tetracycline 2 g/L and Amoxicillin 2 g/L and were 7 days incubated at 27±2 °C (Haraprasad *et al.,* 2001)

Preparation of AgNPs (Extracellular)

The clean culture of M. metarhizium was recently inoculated using fluid medium that contains PDB in an Erlenmeyer flask which contain liquid media incubated in a rotary centrifuge at 25±2 °C using 150 rpm speed for 14 days. The biomass were harvest after 14 days of growing by sieve it using filter paper (WhatMan 1), after that distilled water was used to washing the mass to clean it from the traces remains of media. A quantity of this uncontaminated biomass (20 gm) was emptied into Erlenmeyer flask that contains 500 ml deionized water; this flask was incubated for 120 h at 25°C using dark conditions. The cells were filtered after that using filter paper (WAHATMAN 1). The filtrate cells were mixed with with 50 mM $AgNO_3$ (8.4935 g/1000 mL) in 1000 ml Erlenmeyer flask. As the final concentration, a volume of 0.8 liter was added 200 ml of cell filters and frenzied at 60 °C for time of 10 min with a continuous stirring and control the pH at 7 by addition dropwise of NaOH solution, the flasks were enclosed by aluminum foil to avoid silver nitrate reaction with light. (Elizabath *et al.,* 2017), after that incubation for 120 hours was achieved in dark at temperature 25°C. AgNPs became brownish color solution (Cölfen, 2010), at the same time an using same environment, a maintain control was achieved with no $AgNO_3$ addition in separate. The protein, enzyme and other compounds presented in the fungal liquid act as reducing agents to produce silver nanoparticles

–

Characterization of AgNPs

Nanoparticles were recorded by a using Shimadzu UV-1800 spectrophotometer from190nm to 1100 nm (Ba-Abbad *et al.,* 2012).The solution was then converted in powder for X-ray diffraction (XRD) measurements. For XRD studies, dried nanoparticles were coated on the XRD grid and the spectra were recorded by X-ray diffractometer (XRD-6000, Shimadzu). The micrographs of AgNPs were obtained by hot stage microscopy using Lecia DM 2500.

The morphology and size of the synthesized silver nanoparticles were further characterized by the atomic force microscopy (AFM) images by using Atomic Absorption Spectroscopy (AA- 3000, Angostrom Advanced Inc. USA AFM contact mode). Additional characterization of silver nanoparticles caught up using Fourier Transform Infrared (FTIR) spectra that was e recorded using Bruker Tensor-27 FTIR spectrometer with OPUS software in the range 4,000–400 cm^{-1}, at a resolution of 4 cm^{-1}.

UV–Visible Analysis

The silver nanoparticles solution, and under room temperature was incurred to the ultra-sonication, their surface plasmon resonance was noted at 400 nm the primary relation of photon transmission (T) and absorbance (A) can be expressed in the following mathematical equation:

$$A=\log_{10}\frac{1}{T} \ldots\ldots\ldots\ldots (3)$$

Using drop casting technique, a thin film was prepared on glass, its reflection (R) was counted via the following relation:

R+T+A=1..... (4)

Refraction index (n) can be measured from equation below: (Das and Pandey, 2011):

$$n=\frac{1+\sqrt{R}}{1-\sqrt{R}} \ldots\ldots\ldots\ldots (5)$$

The optimum value is 3.5. The optical absorption coefficient (α) was estimated through tauc equation:

$\alpha hv= (hv-Eg)^n$

When α=2.303 Where t is the thickness of film, hv is the photon energy,

$$Eg=\frac{1240}{\lambda\,(nm)}$$

And no= 0.5 for allow direct transition. Drawing the graph between photon energy *(hv)* versus *(αhv)2*

will give the direct band gap value, while the extrapolation of the straight line to *(αhν) 2 = 0*, which represent band gaps value (Misho and Murad, 1992) The optical band gab is 5.75 eV.

X-Ray diffraction analysis

The nanoparticles of silver were checkered by using X-ray diffractometer (XRD) to determine the formation of silver nanoparticles, the XRD instrument current was 30mM and the voltage was 40 kv in (X'Pert pro X-ray diffractometer) Cu Kα radiation in a θ-2θ configuration. The crystallite domain size was calculate from the width of the XRD peaks, supposing they aree free from nonuniform strains, using the Debye-Scherer formula (Murali *et al.,* 2008)

$$D = \frac{0.9\,\lambda}{\beta \cos(\theta)} \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where D refers to average crystallite area size vertical to the reflecting planes, λ is the X ray wavelength, the diffraction angle of full width at half maximum (FWHM) is represented by β, while (Ɛ) is microstrain value and (σ) is the dislocation density equation 2 below represent the relationship between (σ) and (Ɛ): (Kale, 2005)

$$\varepsilon = \frac{\beta \cos}{4} \ldots\ldots\ldots\ldots\ldots (2)$$

Atomic force microscopy (AFM)

Size, surface topography and granularity volume distribution of biosynthesized nanoparticles identified using Atomic Absorption Spectroscopy (AA- 680, Shimadzu- Japan). (Described by Dr. Abdul Kareem Al-Samaraii Lab. Baghdad/Iraq) (Naveen *et al.,* 2010)

Hot Stage microscopy

Using Lecia DM 2500 hot stage microscopy, the thermal analysis and microscopy of silver nanoparticles was analyzed in laboratories of College of Ibn Al-Haitham, University of Baghdad- College.

FTIR analysis Abroad and very strong band at 1600cm^{-1} is due to bounded sp2 C-X double bonds hydroxyl of Metarhizium anisopliae biomass. A shoulder peak at 3600 cm-1prove the presence of alcohols and peak at 2400 very broad. FTIR bands of silver nanoparticles, approves the presence of protein in the silver nanoparticles biosynthesized in this process, which coat covering the silver nanoparticles identified as capping proteins. Capping protein stabilizes AgNPs and prevents agglomeration in the medium. FTIR spectroscopy analysis confirmed that the *Metarhizium anisopliae* biomass extract has the ability to achieve important function of reduction of (Ag^{+}) to (Ag^{O}) and stabilization of silver nanoparticles.

Flame Atomic Absorption Spectroscopy

Atomic absorption spectrometry (AAS) is an analytical technique that calculates the concentrations of elements. Atomic absorption is so sensitive that it can measure down to parts per billion of a gram (μg dm−3) in a sample. The technique makes use of the wavelengths of light specifically absorbed by an element. They correspond to the energies needed to stimulate electrons from one energy level to another, higher, energy level, the concentration of *Metarhizium anisopliae* Silver Nanoparticles was 39.2553.

Scanning electron microscope (SEM)

AgNPs were analysis by using electron microscope AIS2300C (Oxford instruments scanning electron micrographs in College of Ibn Al-Haitham, University of Baghdad to allow visualization and shape of the AgNPs (Dakhil, 2017)

Toxicity of Metarhizium anisopliae against the Larvae and Pupae of Cx. pipiens

In order to study the toxicity *Metarhizium anisopliae* of four different concentrations i.e. 200000, 400000, 600000 and 80000 ppm concentrations were made. 10 larvae of third, fourth instars and pupae were put into plastic cups containing different concentration of the fungus in 100 ml of dechlorinated tap water. At least, six replicates were used for each tested concentration on each instar larvae. All the plastic cups were

incubated at a temperature of 27± 2 C, Relative humidity of 70± 5 % .Controls correspond to 10 larvae of third, fourth in stars and 10 pupae in 100 ml water and mortalities were daily recorded.

Toxicity of $AgNO_3$ against the Larvae and Pupae of Cx. pipiens

Pure silver nitrate solution of 4245 2122 1610 5306 ppm concentration showed2.66, 4.66, and 0.66% of mortality in 24 h of incubation respectively . At least, six replicates were used for each tested concentration on each instar larvae However, little higher percentage of mortality increases with increasing concentration and duration of exposure. The maximum mortality was recorded 4.66 with 5306 ppm of silver nitrate solution during 24 h of incubation.

Effectiveness Revision of Prepared AgNPs Using the M. Anisopliae Alongside the Larvae and Pupae of Cx. pipiens

For bioassay, 10 larvae of third, fourth in stars and 10 pupae were transferred separately into a 100ml glass containing 530, 1060 and 2120 ppm concentration. Six replications of *M. anisopliae* -AgNPs were maintained separately, every one of them was exposed to mosquito net. The system was kept at 27±2 °C and 70+ 5 % RH. The synthesized *M. anisopliae* AgNPs shows activity against the larvae and pupae of *Cx. pipiens* the larvaes of *Cx. pipiens* was highly liable to the synthesized AgNPs than the pupae at the same test concentrations. The mortality could be clearly noticed subsequent to diverse hours of exposure. Bioassay was achieved with *M. anisopliae* prepared silver nanoparticles against third, and fourth instar larvae of *C. pipienes* based on a method of the World Health Organization (WHO 2005) with minor modifications. The mortality of mosquito larvae was recorded at 24-h intervals with and without *M. anisopliae* -AgNPs. The fourth instar larvae of *cx. pipiens* concluded to be highly reliable for AgNPs synthesis and shows 10 mortality subsequent to one day (24 hours).

Statistical analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). Two way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means. $P < 0.05$ was considered statistically significant (SAS. 2010).

Results

The results in table 1 represent dimensions of *Metarhizium anisopliae* silver nanoparticles, while table 2 and table 3 shows Larvicidal and pupicidial efficacies of AgNPs synthesized by using the *Metarhizium anisopliae* against the *Cx. pipiens* . In table 4, the Larvicidal and pupicidial efficacies of *Metarhizium anisopliae* against the *Cx. Pipiens*, in table 5 Larvicidal and pupicidial efficacies of $AgNO_3$ against the *Cx. Pipiens*. Figure 1 and figure 2 shows *Metarhizium anisopliae* on PDA and *Metarhizium anisopliae* on PDB respectively. In Figure 3 it shows clearly differences between the 3 solutions, $AgNO_3$ solution, fungal cell filtrate and AgNPs solution.

Table 1. Dimensions of Metarhizium anisopliae Silver Nanoparticles.

Roughness average	0.532
Root mean square	0.615
Average diameter	95.08

Figure 4 shows the transmittance spectrum of (AgNPs) thin film, but figure 5 represent the reflectance index spectrum of (AgNPs) thin nd figure 6 shows (αhv) versus photon energy plot of (AgNPs) thin film. In figure 7, AFM topographic images nanopowder is clearly appear. Figure 8 shows Figure (8), granularity accumulation distribution chart of *Metarhizium anisopliae* Silver Nanoparticles, figure 9 hot stage images of *Metarhizium anisopliae* silver nanoparticles 500x. In figure 10 shows the data obtained from Flame Atomic Absorption Spectroscopy of *Metarhizium anisopliae* Silver Nanoparticles, while

figure 11 shows FTIR diagram for the same particles, figure 12 represent the difference in SEM images of AgNPs (440x) Figure 13 shows mosquito larva and pupa mortality with *Metarhizium anisopliae* synthesized AgNPs, figure 14 shows mosquito larva and pupa mortality with *Metarhizium anisopliae,* and finally figure 15 shows mosquito larva and pupa mortality with $AgNO_3$.

Table 2. Larvicidal and pupicidial efficacies of AgNPs synthesized by using the *Metarhizium anisopliae* against the *Cx. Pipiens*.

Group	3ed			4th			Pupa		
	24	48	72	24	48	72	24	48	72
Control	B0.00±0.00d	B0.00±0.00c	B0.00±0.00a	B0.00±0.00d	AB0.33±021bc	A0.66±021a	A0.66±0.21c	B0.00±00b	B0.00±0.00b
Com1	B9.33±0.21a	E0.66±0.21a	F0.00±0.00a	A10.00±0.00a	F0.00±0.00c	F0.00±0.00b	F5.00±0.00a	D3.33±0.21a	EF0.33±0.21ab
Con2	B8.66±0.21b	FG0.33±0.21ab	G0.00±0.00a	A9.33±0.21b	E0.66±0.21ab	F0.00±0.00b	C4.66±0.21a	D3.00±0.00a	E0.66±0.21a
Con3	B8.33±0.21b	EF0.66±0.21ab	G0.00±0.00a	A9.00±0.00b	E1.00±0.00a	G0.00±0.00b	C4.66±0.21a	D3.00±0.00a	FG0.33±0.21ab
Con4	B7.66±0.21c	E1.00±0.00a	FG0.33±0.21b	A8.66±0.21c	E1.00±0.00a	G0.00±0.00b	C3.66±0.21b	D2.66±0.21a	FG0.33±0.21ab

Means with different small letter in the same column significantly different (P< 0.05)

Means with different capital letter in the same row significantly different (P< 0.05).

Table 3. Larvicidal and pupicidial efficacies of *Metarhizium anisopliae* against the *Cx. Pipiens*.

Group	3ed			4th			Pupa		
	24	48	72	24	48	72	24	48	72
Control	B0.00±0.00a	B0.00±0.00a	B0.00±0.00b	B0.00±0.00a	AB0.33±021b	A0.66±021b	B0.00±00a	B0.00±00a	B0.00±0.00a
Com1	DB0.00±0.00a	C0.33±0.21a	BC0.66±0.21a	B0.00±0.00a	AB0.33±0.21b	A1.33±0.21a	C0.00±0.00a	CD0.33±0.21a	D0.00±0.00a
Con2	C0.00±0.00a	BC0.33±0.21a	AB0.66±0.21a	C0.00±0.00a	AB0.66±0.21a	A0.83±0.16b	C0.00±0.00a	C0.00±0.00a	C0.00±0.00a
Con3	B0.00±0.00a	B0.00±0.00a	AB0.33±0.21a	AB0.33±0.21a	AB0.33±0.21b	A0.66±0.21b	B0.00±0.00a	B0.00±0.00a	B0.00±0.00a
Con4	B0.00±0.00a	B0.00±0.00a	B0.00±0.00b	AB0.33±0.21a	0.00±0.00b	A0.66±0.21b	B0.00±0.00a	B0.00±0.00a	B0.00±0.00a

Table 4. Larvicidal and pupicidial efficacies of $AgNO_3$ against the *Cx. Pipiens*.

Group	3ed			4th			Pupa		
	24	48	72	24	48	72	24	48	72
Control	B0.00±0.00d	B0.00±0.00b	B0.00±0.00c	B0.00±0.00e	AB0.33±021c	A0.66±021a	B0.00±00b	B0.00±00d	B0.00±0.00b
Com1	B2.66±0.21a	D0166±0.21a	E0.66±0.21b	A4.66±0.21a	CD2.00±0.00a	E0.66±0.21a	E0.66±0.21a	CD2.00±0.36a	E0.66±0.21a
Con2	B2.66±0.21a	D1.33±0.21a	E0.33±0.21bc	A3.33±0.42b	C2.00±0.00a	E0.66±0.21a	E0.66±0.21a	D1.66±0.21ab	E0.33±021ab
Con3	B2.00±0.00b	CD1.00±0.36a	E0.33±0.21bc	A2.66±0.21c	C1.33±0.21b	E0.33±0.21a	DE0.66±0.00a	CD1.00±0.36bc	E0.33±0.21ab
Con4	B1.33±0.21c	CD0.33±0.00b	B1.66±0.21a	B1.33±0.21d	A2.00±0.36a	CD0.33±0.21a	CD0.33±0.21ab	C0.66±0.21bc	D0.00±0.00b

Discussion

Novel sliver nanoparicles (AgNPs) were synthesized from *M. anisopliae* mycellial as a potential biolarvicidal agent for the filariasis vector *C. pipiens*.

Fig. 1. *Metarhizium anisopliae* on PDA.

Fig. 2. Metarhizium anisopliae on PDB.

It is novel silver nanoparticles that was produced by using entomopathogenic fungus M. anisopliae for control of C. pipiens, the fungus-mediated silver nanoparticles have rapid effect on vector

mosquito population and thus determine that the fungus-synthesized as it is clearly appeared from the results that depicted in tables and figures that was listed in result section.

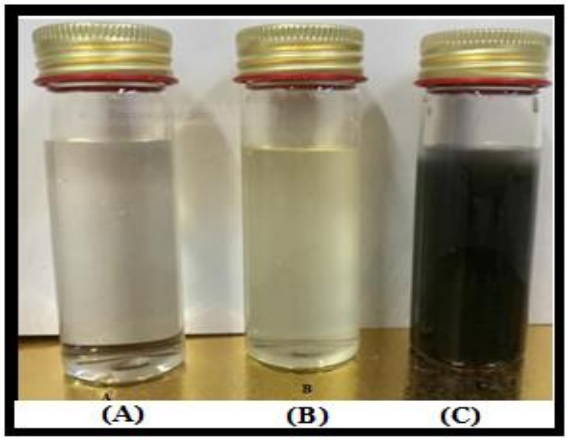

Fig. 3. Comparison between three types of solutions: A. 50mM $AgNO_3$ solution., B. Fungal cell filtrate., C. AgNPs solution.

All the silver nanoparticles (AgNPs) that was prepared from soil fungus *Metarhizium* anisopliae have higher larvicidal and pupicidal activity against Cx. Pipiens.

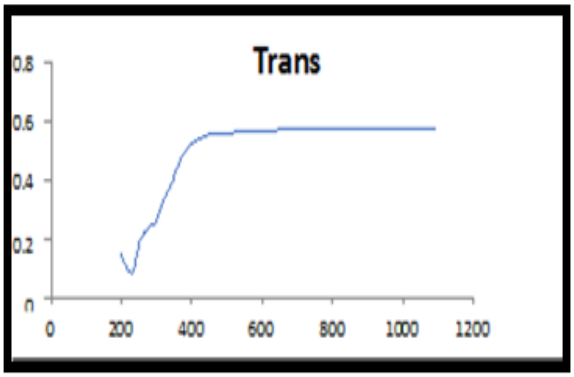

Fig. 4. Transmittance spectrum of (AgNPs) thin film.

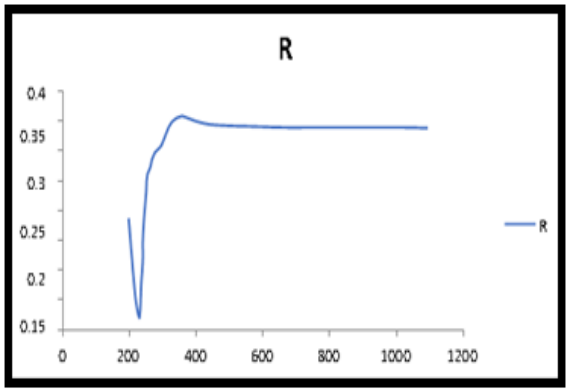

Fig. 5. Reflectance index spectrum of (AgNPs) thin.

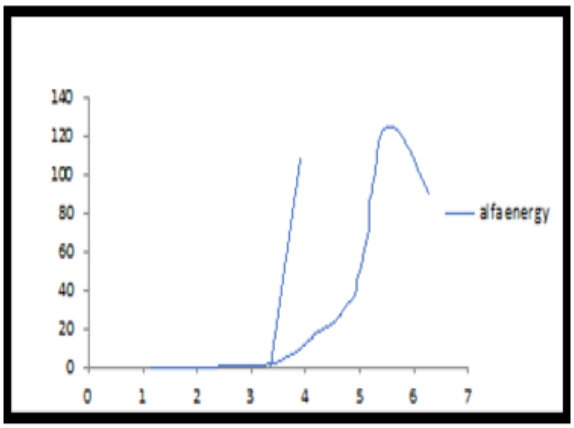

Fig. 6. (αhv) versus photon energy plot of (AgNPs) thin film.

Silver nanoparticles were characterized and identified via UV-vis spectrophotometer (wavelength length 190-1100nm).

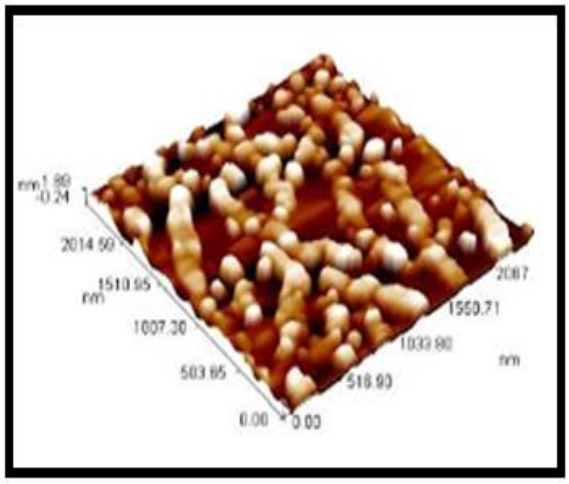

Fig. 7. AFM topographic images nanopowder.

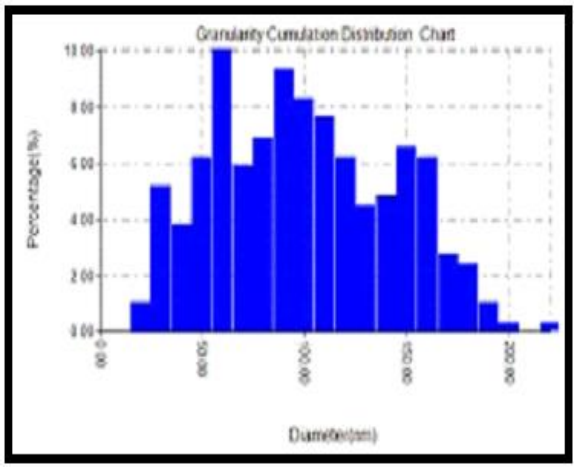

Fig. 8. Granularity Accumulation Distribution chart of *Metarhizium anisopliae* Silver Nanoparticles.

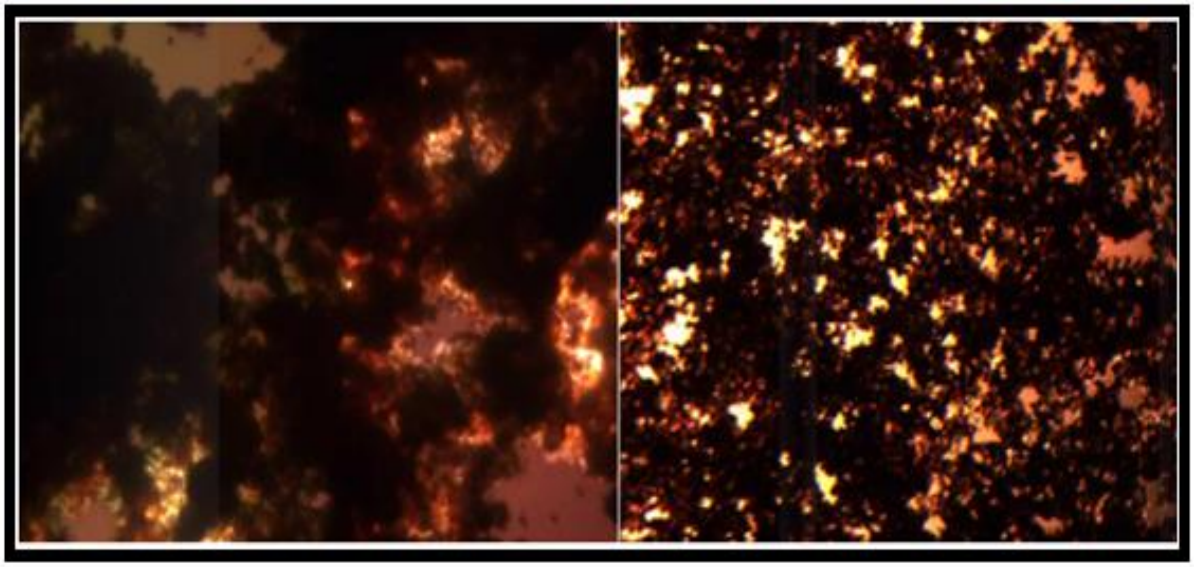

Fig. 9. Hot stage images of *Metarhizium anisopliae* silver nanoparticles 500x.

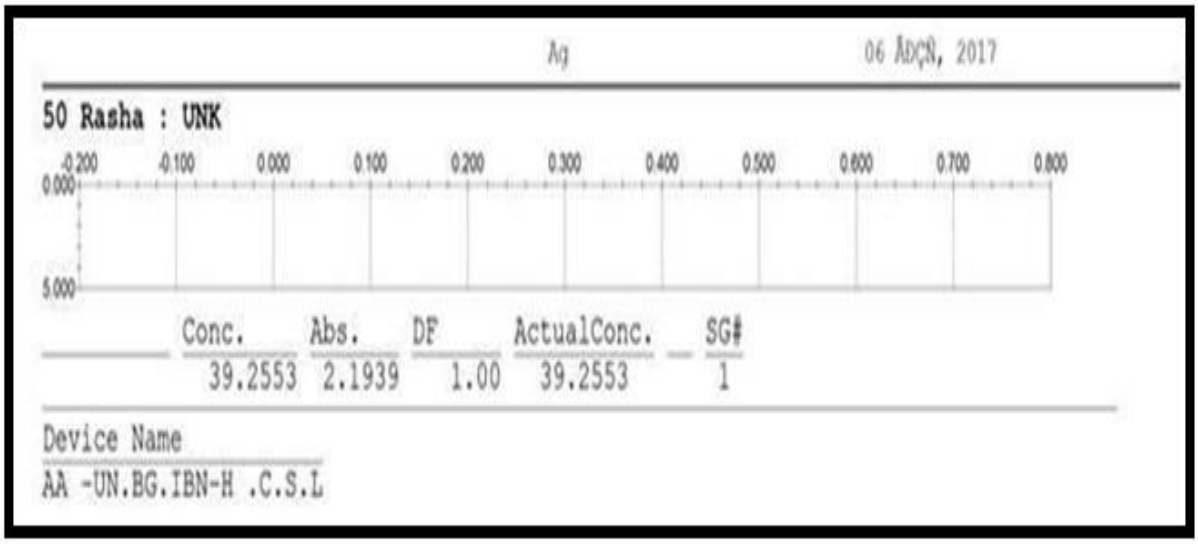

Fig. 10. Flame Atomic Absorption Spectroscopy of *Metarhizium anisopliae* Silver Nanoparticles.

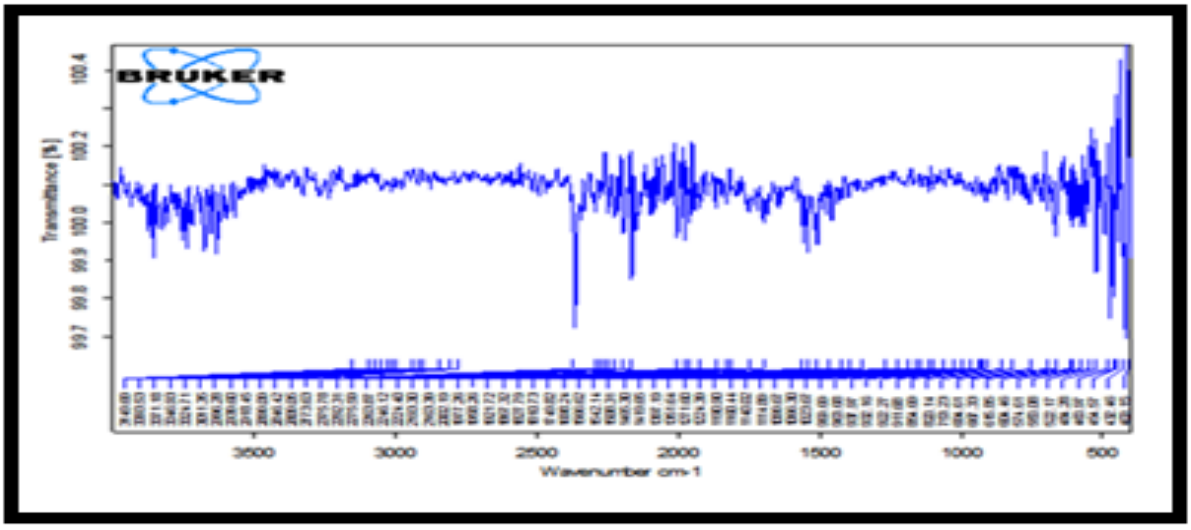

Fig. 11. FTIR Spectra of *Metarhizium anisopliae* Silver Nanoparticles.

This discovered peak at 400 nm in the fungal liquid component of soil fungus Metarhizium anisopliae representing the formation of AgNPs. also results were not similar to previous study which observed mortality rates 60.00%, 70.00%, 80.00%, 90.00% and 100% in third instar larvae, 0.00% mortality rate in fourth instar larvae, after 1 hour of exposure to 2, 4, 6, 8, 10 and 12ppm of *Aspergillus niger* silver nanoparticles while the mortality rates of pupa were 40.00%, 45.00%, 50.00%, 65.00%, 70.00% and 80.00% after 20 h of exposure to the same concentration of A. niger silver nanoparticles (Soni and Prakash 2013).

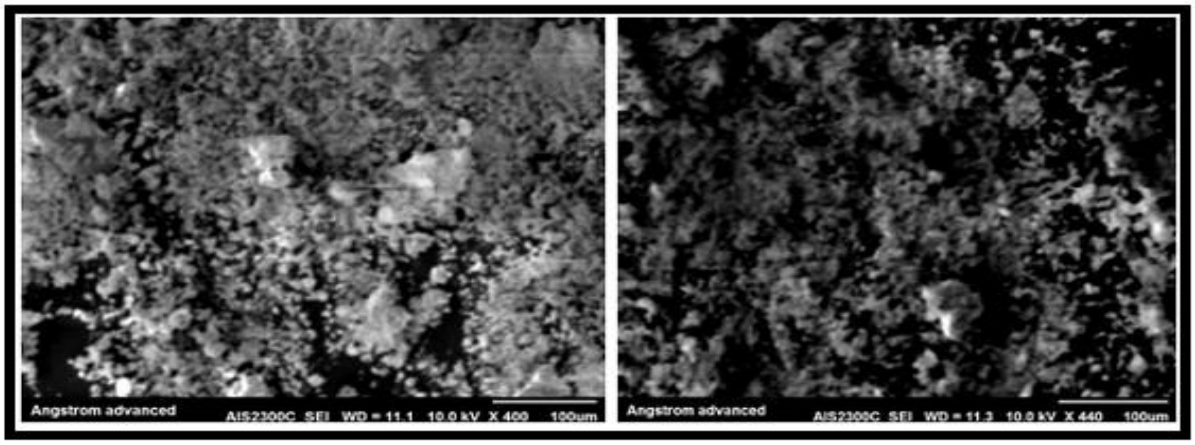

Fig. 12. SEM images of AgNPs (440x).

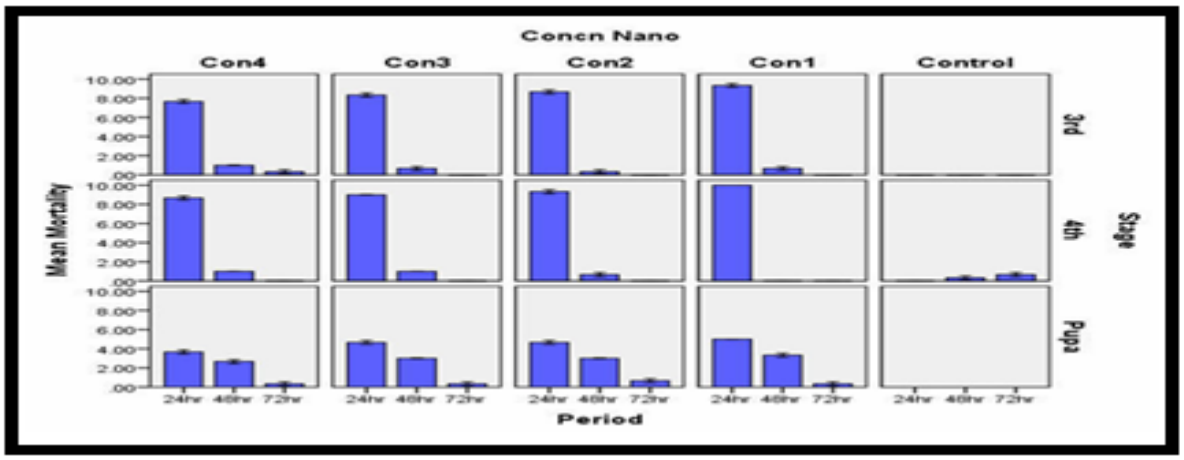

Fig. 13. Mosquito larva and pupa mortality with *Metarhizium anisopliae* synthesized AgNPs

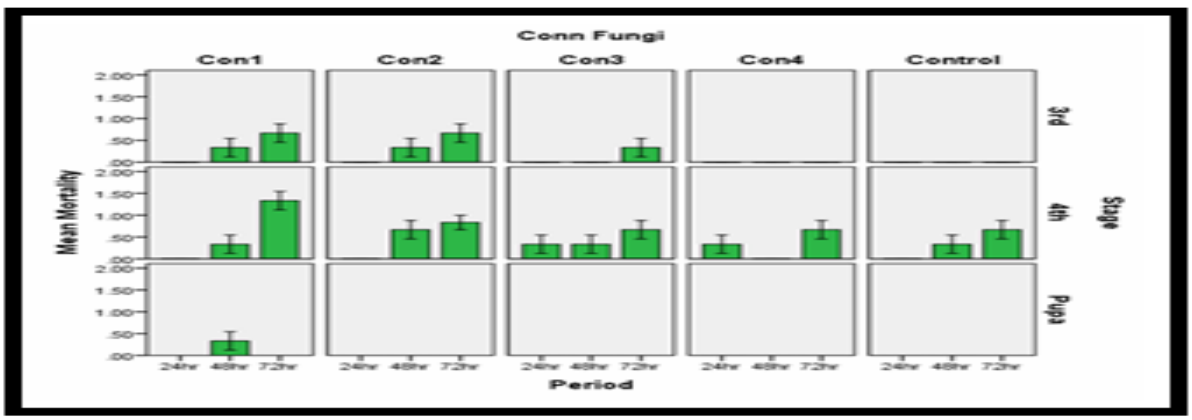

Fig. 14. Mosquito larva and pupa mortality with *Metarhizium anisopliae.*

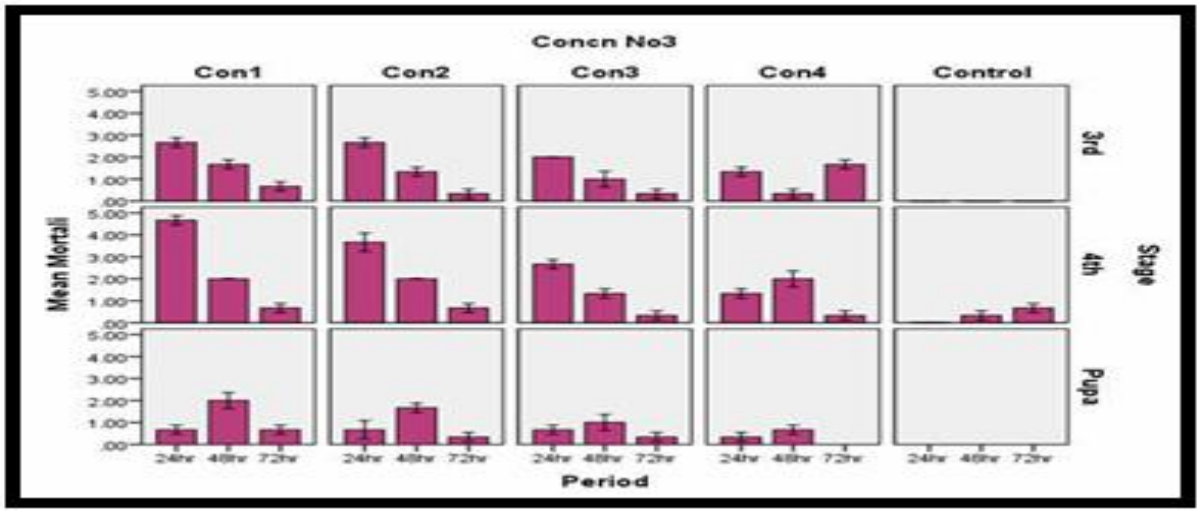

Fig. 15. Mosquito larva and pupa mortality with $AgNO_3$.

Conclusion

The synthesized fungus-mediated silver nanoparticles (AgNPs) have rapid effect on vector mosquito population and thus determine that the fungus-synthesized. Silver nanoparticles can be be considered as a green method for vector control strategy in addition to its safe to the environment.

Acknowledgements

My sincere thanks to Dr Ahmed Naji Abd Physics Department, Science, Faculty, University of Al-Mustansiriyah, and Dr. Maan Abdul Azeez Shafeeq Biology Department Science, Faculty, University of Al- Mustansiriyah, for being a constant source of inspiration and valuable suggestions.

Conflict of interest

We have no conflict of interest.

References

Kang SH, Kim MK, Seo DK, Noh DJ, Yang JO, Yoon Cl. 2009. Comparative repellency of essential oils against Culex pipiens pallens (Diptera: Culicidae). Journal of the Korean Society for Applied Biological Chemistry **52(4)**, 353-359.

Mishra B, Sharma M, Pujhari SK, Ratho RK, Gopal DS, Kumar CN. 2011. Utility of multiplex reverse transcriptase-polymerase chain reaction for diagnosis and serotypic characterization of dengue and chikungunya viruses in clinical samples. Diagnostic Microbiology and Infection Disease **71(2)**, 118-125.

Cadavid-Restrepo G, Sahaza J, Orduz S. 2012. Treatment of an Aedes aegypti colony with the Cry11Aa toxin for 54 generations results in the development of resistance. Memórias do Instituto Oswaldo Cruz **107(1)**, 74-79.

Chenniappan K, Ayyadurai N. 2012. Synergistic activity of Cyt1A from Bacillus thuringiensis subsp. israelensis with Bacillus sphaericus B101 H5a5b against Bacillus sphaericus B101 H5a5b- resistant strains of Anopheles stephensi Liston (Diptera: Culicidae). Parasitology research **110(1)**, 381-388.

Mourato A, Gadanho M, Lino AR, Tenreiro R. 2011. Biosynthesis of crystalline silver and gold nanoparticles by extremophilic yeasts. Bioinorg Chem Appl. http://dx.doi.org/10.1155/2011/546074.

Soni N, Prakash S. 2013. Possible mosquito control by silver nanoparticles synthesized by soil fungus (Aspergillus niger 2587). Advances in Nanoparticles **2,** 125–132.

Najitha-Banu A, Balasu-bramanian C, Vinayaga-Moorthi P. 2014. Biosynthesis of silver nanoparticles using Bacillus thuringiensis against dengue vector, Aedes aegypti (Diptera: Culicidae). Jjournal Parasitology Research **113,** 311–316.

Mukherjee P, Ahmad A, Mandal D. 2001. Bioreduction of $AuCl_4$– ions by the fungus, Verticillium sp. and surface trapping of the gold nano- particles formed. Angewandte Chemie International Edition in English **40(19),** 3585–3588.

Bhainsa CK D'Souza FS. 2006. Extracellular biosynthesis of silver nanoparticles using the fungus Aspergillus fumigatus. Colloids and Surfaces. B, Biointerfaces Journal **47,** 160–164.

Sonal BS, Swapnil C, Gaikwad K, Gade AK, Mahendra R. 2013. Rapid synthesis of silver nanoparticles from Fusarium oxysporum by optimizing physicocultural conditions. Science World Journal, **2013**, 1–12.

Tetreau G, AlessiM-Veyrenc S, Périgon S, David JP, Reynaud S, Després L. 2012. Fate of Bacillus thuringiensis subsp. israelensis in the Field:Evidence for Spore Recycling and Differential Persistence of Toxins in Leaf Litter. Applied and Environmental Microbiology, **78(23),** 8362–8367.

Promsiri S, Naksathit A, Kruatrachue M, Thavara U. 2006. Evaluations of larvicidal activity of medicinal plant extracts to Aedes aegypti (Diptera: Culicidae) and other effects on a non-target fish. Journal of Insect Science **13 (3),** 179-188.

Haraprasad N, Niranjans SR, Prakash HS, Shetty HS, Seema W. 2001. Beauveria bassiana—a potential mycopesticide for the efficient control of coffee Berry Borer, Hypothenemus hampei (Ferrari). Indian Biocontrol Science and Technology **11,** 251–260.

Elizabath A, Mythili S, Sathiavelu A. 2017. Synthesis of silver nanoparticles from the medicinal plant bauhinia acuminata and biophytum sensitivum–a comparative study of its biological activities with plant extract. International Journal of Applied Pharmaceutics, **9,** 22-29.

Cölfen H. 2010. Biomineralization: a crystal-clear view. Nat mat, 9, 960-961.

Ba-Abbad MM, Kadhum AH, Mohamad AB, Takriff MS, Sopian K. 2012. Synthesis and catalytic activity of TiO_2 nanoparticles for photochemical oxidation of concentrated chlorophenols under direct solar radiation. International Journal of Electrochemical Science **7,** 4871-4888.

Das R, Pandey S. 2011. Comparison of Optical Properties of Bulk and Nano Crystalline Thin Films of CdS Using Different Precursors. International Journal of Material Science **96,** 101-1052.

Misho RH, Murad WA. 1992. B and gap Measurements in Thin Films of Hematite Fe203, Pyrite FeS2 and Troilite FeS Prepared by Chemical Spray Pyrolysis. Solar Energy Materials and Solar Cells **27,** 335-345.

Murali KR, Elango P, Andavan P, Venkatachalam K. 2008. Preparation of CdSxSe1-x films by brush plating technique and their characteristics.Journal of Materials Science: Materials in Electronics, **19,** 289–293.

Kale RB. 2005. Genetic diversity among summer and winter Beauveria bassiana populations as revealed by AFLP analysis. Journal of Asia-Pacific Entomology **20,** 1-9.

Naveen HKS, Gaurav K, Karthik L, Bhaskara-Rao KV. 2010. Extracellular biosynthesis of silver nanoparticles using the filamentous fungus Penicillium sp. Archives of Applied Science Research **2(6),** 161-167.

Dakhil AS. 2017. Biosynthesis of silver nanoparticle (AgNPs) using Lactobacillus and their effects on oxidative stress biomarkers in rats. Journal of King Saud University-Science **29,** 263-388.

SAS. 2010. SAS/STAT Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA.