ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

EVALUATION OF LARVICIDAL EFFECT OF MYCOGENIC SILVER NANOPARTICLES AGAINST WHITE GRUBS *(HOLOTRICHIA SP)*

Gyanika Shukla[1]*, Shailendra Singh Gaurav[1], Varsha Rani[2], Amardeep Singh[1], Poonam Rani[3], Pankaj Verma[4] and Bipul Kumar[5]

[1]*Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India*
[2]*Department of Biotechnology, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India*
[3]*Department of Soil Science, Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India*
[4]*Department of Biotechnology, BN College of Engineering and Technology, Lucknow, Uttar Pradesh, India*
[5]*CSIR- Institute of Genomics and Integrative Biology, New Delhi, India*
**Corresponding author: gyanikashukla@gmail.com*

ABSTRACT

In the present study pesticidal efficacy of mycogenic silver nanoparticles (AgNPs) was tested against white grubs (*Holotrichia sp*), a potent pest of sugarcane in western Uttar Pradesh (India). The AgNPs were synthesized by using *Aspergillus niger* biomass and characterized by color based method, UV (ultra voilet) - Visible Spectroscopy, Field Emission Scanning Electron Microscopy (FESEM), Energy Dispersive X-Ray (EDX), Dynamic Light Scattering (DLS), Fourier-transform infrared spectroscopy (FTIR) and Inductively coupled plasma mass spectroscopy (ICPMS). The development of paint-like mustard colored solution by color-based identification method confirmed the synthesis of AgNPs. The UV-Vis spectroscopy showed peak at 425nm, corresponding to AgNPs. The FESEM results also confirmed the synthesis of nano sized particles. EDX analysis result showed the optical absorption peak at 3 *keV* which is specific to AgNPs. The DLS result confirmed the synthesis of AgNPs with average size of 76.6 nm. FTIR analysis depicted information about all the interaction of AgNPs with other chemicals present in environment. The AgNPs were applied against first instar white grub larvae *in vitro* and lethal dosage (LD_{50}) was formulated by Probit analysis, which was further validated and found to be significant at 0.05 levels by chi-square test and analysis of variance (ANOVA) including least significant difference (LSD).

Keywords: Silver nanoparticles, myconanoparticles, nanopesticide, white grub, lethal dosage, nanoparticle toxicity, EDX, FESEM, DLS, FTIR, ICPMS.

1. INTRODUCTION

Nanotechnology is a new area of research with ocean of new possibilities. The area of nanotechnology based research is however relatively recent and needs further evaluation for its application in various fields of study. Crop protection is one of the possible approaches of nanotechnology but its use in crop protection is just in its infancy [1].

Random use of various chemical pesticides is affecting the soil fertility and enhancing pollution; hence various countries are enforcing the use of biopesticides and biological components for pest management [2]. However, there are several disadvantages associated with the use of biopesticides like high selectivity or host specificity, requirement of additional control measures, delayed effect or mortality, storage problem, difficulty of culturing in large quantities, short residual effectiveness, soil quality dependency, slow activity etc.

Nanoparticles are basically metal oxides of nano size range, typically between 1-100 nm and have been proved to be efficient agents for pest management [3]. Nanoparticles (NPs) possess insecticidal property due to their characteristics like size-shape, depth qualities, greater strength, high chemical reactivity, high electrical conductivity and optical properties [4]. Such nanoparticles can be synthesized by physical, chemical and biological methods [5], but the biogensis of nanomaterials is eco-friendly approach and their characterization is simple and reliable.

Number of studies has been made related to application of nanoparticles as insecticides and different nanoparticles have been found to be efficient insecticides against

different pests *viz s*ilver NP (AgNP) against *Aphis nerii, Anopheles subpictus, Pediculus humans* and *Lepidio ptamansueta* [6-9], zinc oxide (ZnO) NPs against *Trialeurodesva porariorum* [10], titanium oxide (TiO_2) NPs against *Spodoptera litura* and *Sitophilus oryzae* [11-12], aluminum (Al) NPs against *S. oryzae* [13] and silica NPs against *S. oryzae* [14]. The possible mechanism of action of NPs as pesticides can be discharge of larval inner contents due to recapturing of midgut [15], binding with the exoskeleton of the pest subsequently enters the pest's body and causes physiological reactions [16] or binding with the genetic material of the organisms which leads to cell proliferation [17]. Although, nanoparticles have been widely evaluated as nanopesticides against various pests, but with best of our knowledge no prior study has been done to evaluate the efficacy of nanoparticles against white grub (*Holotrichia sp*) pest. White grub is a potent plant pest of western Uttar Pradesh (India), causing great yield loss of sugarcane crop every year [18]. Its larva is found in soil and feeds on the roots of the crop, causing crop damage [19].

Various antimicrobial properties of silver are well known. Hence, it is the most commonly synthesized and applied nanoparticle among various other metals. Biologically, the silver nanoparticles can be synthesized by plants, fungi and bacteria. Biogenic silver nanoparticles (AgNPs) prepared with plant extracts of various plants *viz Pongamia pinnata* [20-21], *Azadirachta indica* [22-23], *Annona squamosa* [24], *Chrysanthemum* [25] and fungal biomass extract of different fungi *viz Aspergillus niger* [26], *A. fumigates* [27], have been utilized for many purposes in different studies.

This study deals with determination of efficacy of mycogenic silver nanoparticles (AgNPs) as nanopesticides against white grubs, with following objectives:

1. Synthesis of silver nanoparticles by fungal biomass extract of *Aspergillus niger*.
2. Characterization of synthesized nanoparticles.
3. Determination and evaluation of larvicidal properties of silver nanoparticles against white grub larvae.

2. MATERIAL AND METHODS

2.1. Culturing of *Aspergillus niger*

The pure culture of *Aspergillus niger* was obtained from Department of Biotechnology, C.C.S. University, Meerut and sub-cultured on Potato Dextose Agar (PDA) Media (to preserve) and Malt Glucose Yeast Peptone (MGYP) Media (to generate biomass for nanoparticle synthesis).

2.2. Biosynthesis of silver nanoparticles by *Aspergillus niger* biomass

Two mili molar (2mM) aqueous solution of silver nitrate ($AgNO_3$) was prepared for the synthesis of silver nanoparticles. One gram of wet fungal biomass was taken and suspended in 100 ml of the 2mM aqueous $AgNO_3$ solution in 250 ml Erlenmeyer flasks (in triplicate) for reduction of silver nitrate into AgNPs. Whole mixture was placed on a shaker at 28°C (at 150 rpm) and the reaction was carried out for a period of 120 hours. The bio-transformation was routinely monitored visually and by UV-Visible Spectrophotometer after time intervals (0 hr, 4 hrs, 12 hrs, 24 hrs, 48 hrs, 72 hrs, 96 hrs and 120 hrs).

2.3. Characterization of AgNPs

The mycogenic silver nanoparticles were characterized by chemical based color characterization method, UV (ultra voilet) - Visible Spectrophotometer, Field Emission Scanning Electron Microscopy (FESEM), Energy Dispersive X-Ray (EDX), Dynamic Light Scattering (DLS), Fourier-transform infrared spectroscopy (FTIR) and Inductively coupled plasma mass spectroscopy (ICPMS).

2.4. Larvicidal activity of AgNPs against white grub larva

The first instar white grubs *(Holotrichia sp)* were collected from agricultural fields of Meerut (Uttar Pradesh, India). After harvest of sugarcane crops, the agricultural fields were deep dug (10 inches to 30 inches) and larvae of different instar levels were collected. The collected grubs were preserved in laboratory in pots containing humus. The *in vitro* larvicidal activity of AgNPs against white grub was determined by simply taking ten first instar grubs each in a control and test plate. Further, the parts per million (ppm) concentration of AgNPs was determined by ICPMS technique availed from Indian Institute of Technology (IIT), New Delhi and on the basis of various tests performed, a series of ppm dilutions (3 ppm, 5 ppm, 10 ppm, 15 ppm and 20 ppm) were prepared to test for the mortality percentage and establishment of lethal dose (LD_{50}). Each concentration was tested in triplicate with 10 first instar white grubs per plate. Control plates were kept without nanoparticle treatment. Nanoparticle dilutions in 2 ml quantity were directly applied on larvae and mortality was observed over 72 hrs time duration. Larvae were kept at starvation during treatment. Larvae were considered dead if they were immobile and unresponsive to touch. The mortality

percentage was calculated and LD_{50} was formulated by Probit analysis. The calculated LD_{50} was further validated *in vitro* and significance of result was analyzed by chi-square test. All the statistical analysis were done using Microsoft Excel (2010) and SPSS 16.0 version.

3. RESULTS AND DISCUSSION

3.1. Biosynthesis of silver nanoparticles using *Aspergillus niger*

It is well known that the Silver nanoparticle solution has dark brown or dark reddish color [28-30]. As the fungal biomass was added with aqueous solution of the silver nitrate, it started to appear pale-yellow after 4 hrs and eventually turned brown after few hrs (fig 1) which indicated the formation of silver nanoparticles. After 120 hrs, the fungal biomass was separated by filteration (first with Whatman filter paper no. 1 and further with syringe filters of 0.45µm pore size) and AgNPs were subjected to further characterizations and application.

A number of the workers in the past have reported the synthesis of extracellular silver nanoparticles with the help of fungi. These include *Verticillium* [31], *Fusarium oxysporum* [32], *Aspergillus niger* [26], *Aspergillus fumigatus* [33-34], *Aspergillus flavus* [35] etc. Since the fungi are known to secrete much higher amount of proteins, these are expected to have significantly higher productivity of nanoparticles [36]. Fungal reductase enzymes are key role players in reduction of metal oxides to nanoparticles [37].

Fig. 1: Control (left) and three test flasks after 96 hrs incubation. Brown color in test flasks indicates the formation of AgNPs.

3.2. Color based characterization of AgNPs

The chemical based color test was performed as per the method given by Zhu *et al* [38]. In this method 5ml of AgNP solution was taken in a flask and 30 ml of 2mM solution of sodium borohydrate was added with continuous stirring. The solution was kept in ice-bath for 20 minutes. Further, a small portion of this solution was taken in a test tube and few drops of 1.5 M solution of sodium chloride were poured that lead to the development of cloudy grey color, further 4% polyvinyl alcohol solution was added and the solution turned in yellow paint like color which confirmed the synthesis of silver nanoparticles (Fig 2).

Reduction of silver by chemical method goes through a one step process to produce a colored silver solution because of the surface of a metal having freed electrons. Sodium borohydrate reduced silver ions and clusters of silver [39]. Borohydrate play a key role in stabilizing growing silver nanoparticle by providing a particle surface charge [38]. The NaCl works as electrolyte is used for aggregation of silver nanoparticles. The charge allows the particles to clump together to form aggregates. Polyvinyl alcohol works as stabilizer and needed to prevent aggregation of silver nanoparticles and capping with the particle [40].

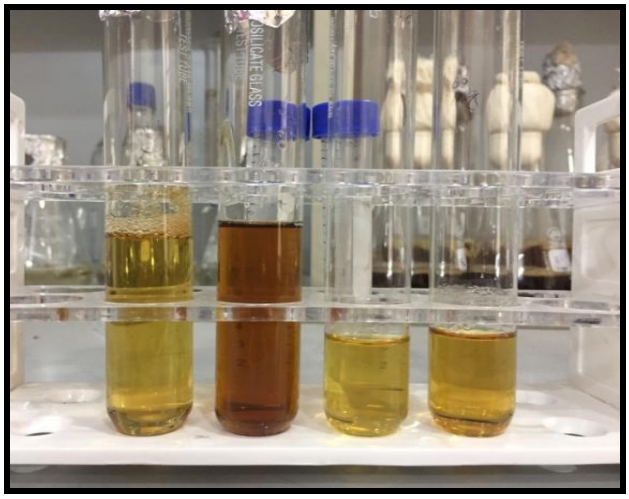

Fig 2: Chemical conformation method for the presence of AgNPs. Yellow-paint like color is developed.

3.3. UV-Vis Spectrophotometer analysis of AgNPs

The AgNPs were characterized by UV-Vis double beam spectrophotometer (Lasany LI-295). All spectra were measured at room-temperature, in a quartz cell with 1 cm optical path, to know the kinetic behavior of AgNPs. The scanning range for the samples was 200-800 nm. The spectrophotometer was equipped with "UV prov

software" to record and analyze the data. Base line correction of the spectrophotometer was carried out by using a blank reference. The samples were analyzed at 0, 4, 12, 24, 48, 72, 96 and 120 hrs. In the present study the reaction stabilized after 96 hrs and a sharp peak at 425 nm was observed (Fig 3). UV-Vis spectrophotometer based characterization of silver nanoparticles is the preliminary characterization method. According to Kamaraj *et al* (2012) the peak range of AgNPs in UV-Vis spectroscopy lies between 410 to 425 nm [41]. The peak range for silver nanoparticles may vary. In various studies vast difference in peak range of AgNPs has been observed, but generally it ranges from 400 nm to 480 nm [42-44].

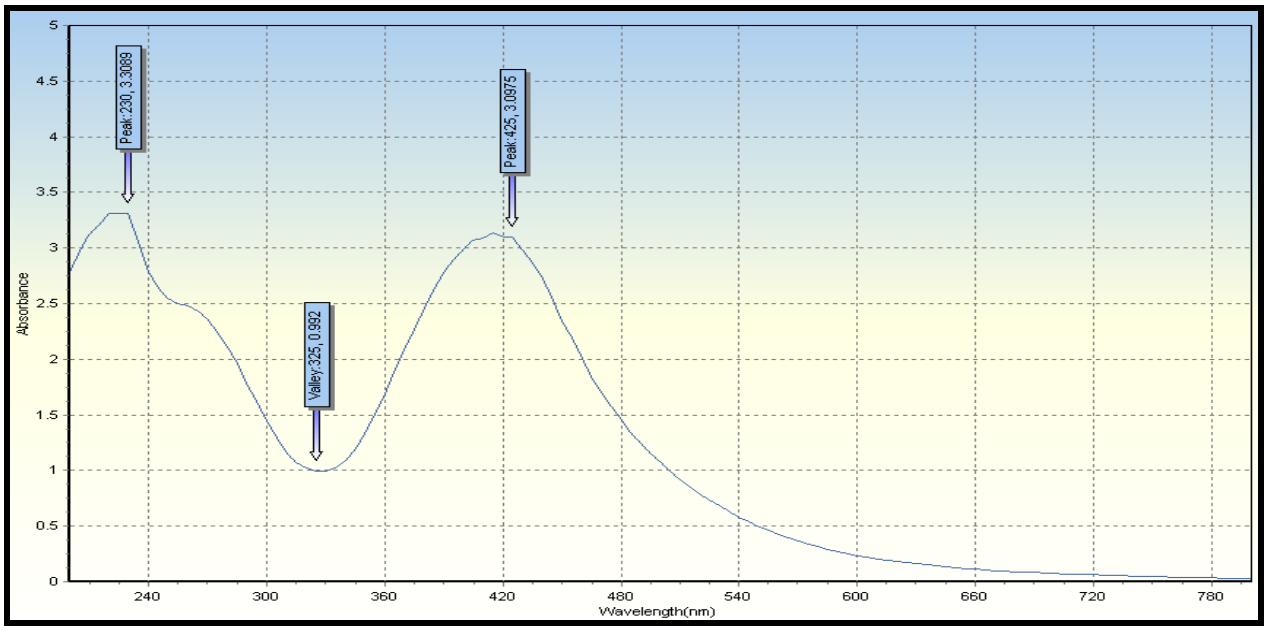

Fig 3: UV-Vis graph of 96 hrs sample

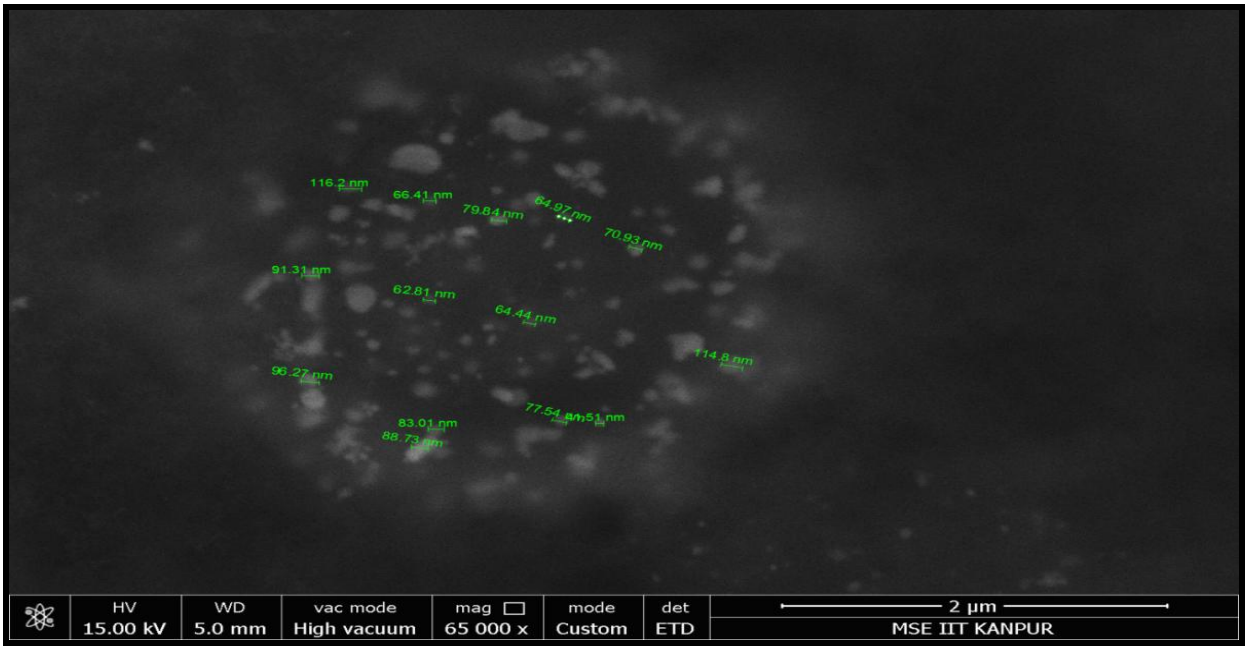

Fig 4: Image of AgNPs obtained from FESEM analysis.

3.4. Analysis of AgNPs by FESEM

The AgNPs dried samples were prepared by placing two drops of AgNP solution on aluminum foil and placing it in hot air oven at 100°C for 15 hrs. The FESEM facility was availed from Advance Imaging Centre, Indian Institute of Technology (IIT), Kanpur (UP, India). The image taken indicated that the nanoparticles are partially spherical, well distributed with the lowest agglomeration (Fig 4).

FESEM Provides topographical and elemental information at magnifications of 10X to 300,000X, with virtually unlimited depth of field. Compared with convention scanning electron microscopy (SEM), FESEM produces clearer, less electrostatistically distorted images with spatial resolution down to 1 or 2 nanometers. A study has been reported where FESEM has been used to detect the presence of nanoparticles inside cells even without disrupting cells, due to its high resolution [45]. It provides high resolution without need of coating. This may be the reason why it is most frequently used electron Microscopy for the analysis of size and structure of nanoparticles [46-48].

3.5. Analysis of AgNPs by EDX

This facility was also availed from Advance Imaging Centre, IIT, Kanpur (UP, India). EDX analysis showed the optical absorption peak at 3 *keV* (Fig 5), which is typical for metallic silver nanocrystallites, with 100% weight percentage.

EDX is a surface analytical technique where an electron beam hits the sample, exciting an electron in an inner shell, causing its ejection and formation of an electron hole in the electronic structure of element [49]. EDX is a standard method for identifying and quantifying elemental compositions in a very small sample of material [50]. This technique is generally equipped with FESEM assembly.

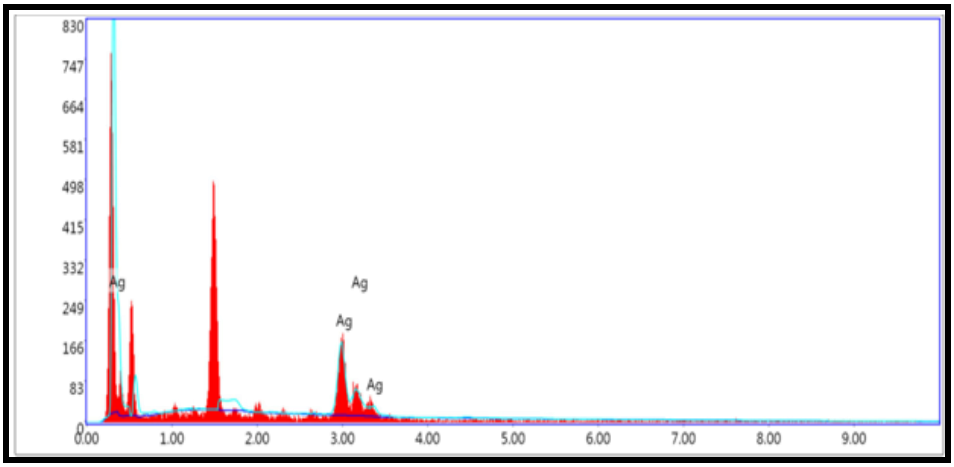

Fig 5: EDX analysis graph of AgNPs, where X-axis is showing the energy in keV and Y-axis is signifying intensity count

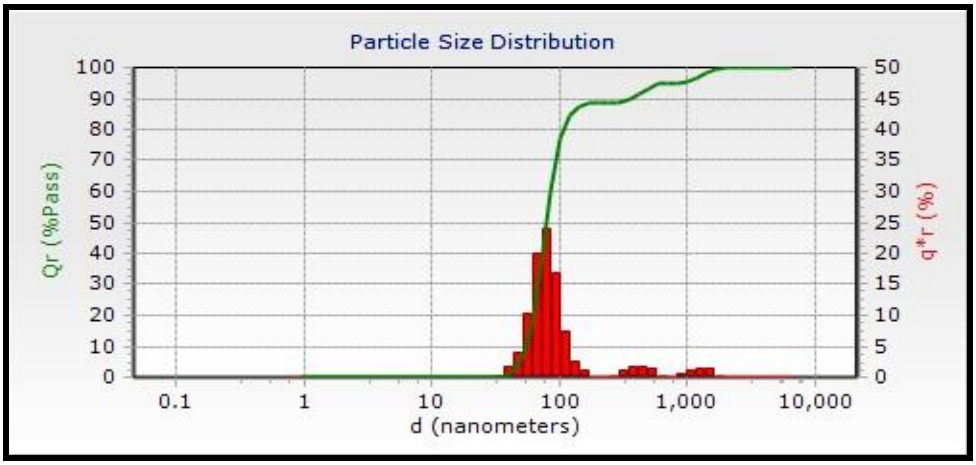

Fig 6: DLS image of AgNPs depicting size distribution of nanoparticles

3.6. Analysis of AgNPs by DLS

Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques was employed to study the average particle size of AgNPs. This analysis was facilitated by Centre for Interdisciplinary Research (CIR), Motilal Nehru National Institute of Technology (MNNIT), Allahabad (U.P.), India. The DLS size distribution image of biosynthesized silver nanoparticles is shown in Fig. 6. It was observed that the average size of majority of synthesized AgNPs (88.4% volume) was found to be 76.6 nm. DLS is used to characterize the nanoparticles in terms of their size distribution [51-53]. The theory and mathematical basics of DLS technique is already well known [54]. It measures the light scattered from the laser that passes through a colloid solution. Further, the scattered light intensity is analyzed as a function of time and the size of particles in the solution can be determined [55]. The mean diameter of NPs can also be determined by this technique.

3.7. Analysis of AgNPs by FTIR

The FTIR facility was availed from Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology (IIT), Bombay to recognize the organic, inorganic, biomolecule residues along with nanoparticle formation, which may come along via reducing agent on to the surface of AgNPs (Fig 7).

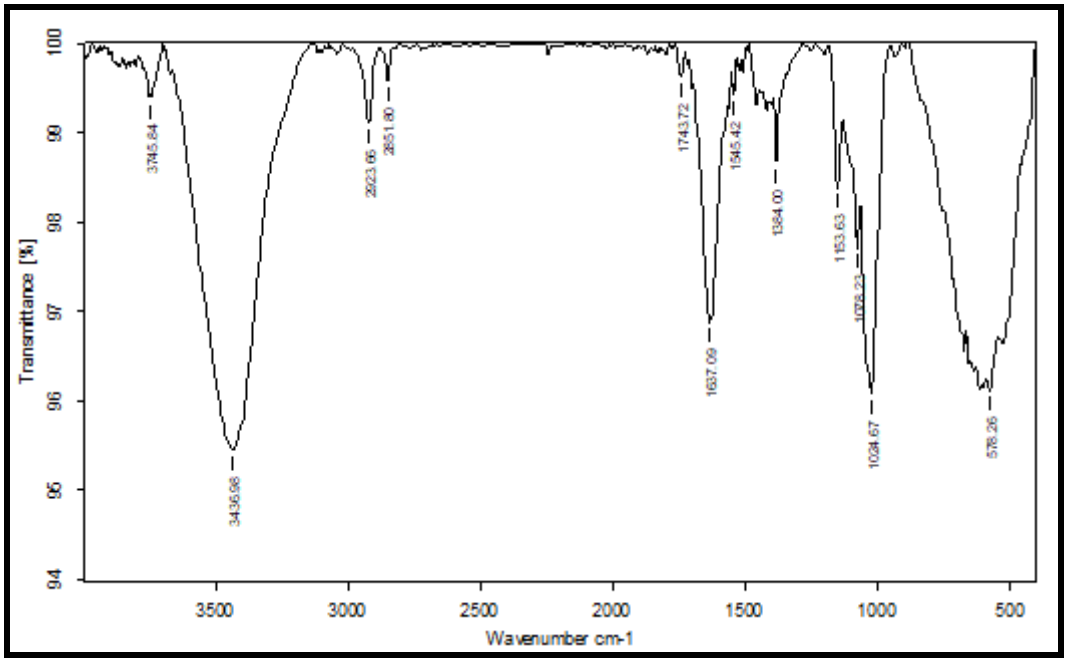

Fig 7: FTIR-spectrum of AgNPs

Absorption bands for AgNPs were found to be at 578.26 cm^{-1}, 1024.67 cm^{-1}, 1078.23 cm^{-1}, 1153.63 cm^{-1}, 1358.00 cm^{-1}, 1545.42 cm^{-1}, 1637.09 cm^{-1}, 1743.72 cm^{-1}, 2851.80 cm^{-1}, 2923.66 cm^{-1}, 3436.98 cm^{-1} and 3745.84 cm^{-1}. The intense bands at 3436.98 cm^{-1} and 3745.84 cm^{-1} correspond to O–H stretching [56]. The peaks at 2851.80 cm^{-1} indicate the presence of C-H bond. The peak at 1637.09 $cm^{-1}$is corresponding to C=C stretch in the aromatic ring. The band at 1743.72 cm^{-1} symbolizes presence of C=O stretch. Band at 1545.42 cm^{-1} indicates the asymmetric stretching of N-O bond [57]. Peak at 1358.00 cm^{-1} indicates the significance presence of C–O bending vibration. Peak at 1153.63 cm^{-1} shows presence of alkyl halides. Peak at 1024.67 cm^{-1} correspond to C-O stretch [58]. The peak at 578.26 cm^{-1} indicates the stretching of C-Br [59].

3.8. Larvicidal activity of AgNPs against white grubs (*Holotrichia sp*)

While testing for larvicidal activity of AgNPs (original concentration), it was observed that the color of larval skin started appearing reddish in the treatment plate. As compared to control plate, a more frequent egestion of undigested waste was observed by the larvae in the treatment plate. The absorption or consumption of silver nanoparticles by larval skin and mouth respectively is well known to cause the formation of reactive oxygen species (ROS) in cells [60]. The increase in egestion rate

can be correlated to the stress generated because of ROS, which interferes with proper functioning of digestive system [61]. All the larvae died within 12 hrs in the AgNPs treated plate, while all larvae were alive in control plate, which preliminarily confirmed the larvicidal properties of AgNPs.

Further, the larval mortality in control and at different ppm solutions of AgNPs was recorded (fig 8). The detail of recorded death of larvae in each replication during incubation is given in table 1.

Table 1: Record of larvae died with time in treatments.

Test sample	Replication	Exposure time and mortality counts of *Holotritia sp* larvae							Total mortality
		1 hr	2 hrs	4 hrs	12 hrs	24 hrs	48 hrs	72 hrs	
Control	1	-	-	-	-	-	-	-	0
	2	-	-	-	-	-	1	-	1
	3	-	-	-	-	-	-	-	0
3 ppm AgNPs	1	-	-	1	1	-	1	-	3
	2	-	-	-	2	-	-	-	2
	3	-	-	-	-	1	1	-	2
5 ppm AgNPs	1	-	-	-	2	1	1	-	4
	2	-	-	-	1	1	-	1	3
	3	-	-	-	-	2	1	-	3
10 ppm AgNPs	1	-	-	1	1	-	2	-	4
	2	-	-	-	1	1	1	1	4
	3	-	-	-	2	1	1	1	5
15 ppm AgNPs	1	-	-	1	1	2	1	-	5
	2	-	-	2	1	-	2	1	6
	3	-	-	1	1	1	2	-	5
20 ppm AgNPs	1	-	-	1	3	2	2	-	8
	2	-	-	-	2	3	1	1	7
	3	-	-	1	3	4	1	-	9

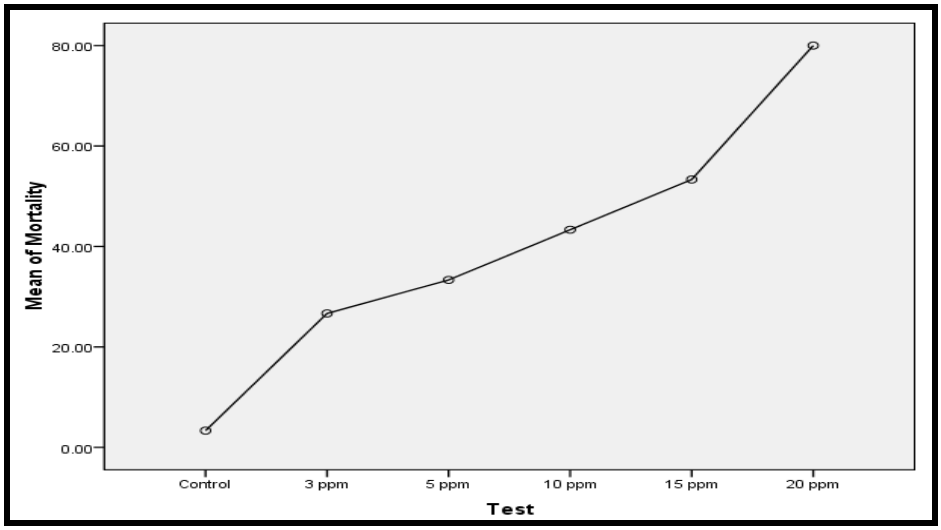

Fig 8: Mean plot of mortality in different tests, prepared by SPSS 16.0 software

The average mortality percentage in control and AgNPs ppm tests is given in table 2 and the Analysis of Variance (ANOVA) including least significant difference (LSD) was found to be significant at 0.05 levels for the difference in mortality percentage among control and AgNP treatments. The LD_{50} was calculated to be 9.03

ppm by Probit analysis. The calculated LD_{50} was validated in 5 replications (each plate containing 10 *Holotritia sp* first instar larvae) with one control plate (without treatment). The mortality data was recorded for 72 hrs. The chi square test value for LD_{50} validation was found to be significant at 0.05 significance levels. Probit Analysis is commonly used protocol to determine the relative toxicity of chemicals to living organisms [62]. Various studies have been reported for silver nanoparticles against insect larvae of animal and plant pests [63-68]. However, with best of our knowledge it is pioneer study of silver nanoparticles against *Holotrichia sp* larvae.

Table 2: Larval mortality per ppm concentration of AgNPs

Test sample	Larval mortality percentage* ± SD
Control	3.3±5.8
3 ppm AgNP	26.8±5.8
5 ppm AgNP	33.3±5.8
10 ppm AgNP	43.3±5.8
15 ppm AgNP	53.3±5.8
20 ppm AgNP	80±10

*SD: standard deviation; * values are mean ± SD of three replicates.*

4. CONCLUSION

The present study was performed to demonstrate the pesticidal effects of silver myco-nanoparticles against white grub insects. This study primarily confirms the efficacy of synthetic silver myco-nanoparticles as effective pest control agent against white grubs, which can lead to the replacement of harmful chemical pesticides in near future. It is possible that by adding silver myco-nanoparticles to formulations of pesticides, the toxicity of chemical pesticides for humans and other non-targeted organisms would be mitigated. Further study will need to focus on methods to increase stability and physiological mechanisms of nanoparticles to increase their effects in integrated pest management programs at large greenhouse and field levels.

Conflict of Interest

The authors declare no conflict of interest.

5. ACKNOWLEDGEMENT

The authors are highly thankful to Prof. Hemant Kumar Gautam (Principal Scientist, CSIR– IGIB, New Delhi) for kindly extending his help and guidance during this work.

6. REFERENCES

1. Resham S, Khalid M, Kazi AG. In *PlantOmics: The Omi Plant Scie,* 2015; 683-698.
2. Chandler D, Bailey AS, Tatchell GM, Davidson G et al. *Philos Trans Roy Socie B Biol Sci*, 2011; **366(1573)**: 1987-1998.
3. Athanassiou CG, Kavallieratos NG, Benelli G, Losic D et al. *J Pest Sci*, 2018; **91(1):**1-15.
4. Sahayaraj K, Madasamy M, Radhika SA. *J Biopest*, 2016; **9(1):**63.
5. Shukla G, Dixit R, Kumar A, Singh R et al. *Appl Nanotec an Intro*, 2017; 89.
6. Rouhani M, Samih MA, Kalantari S. *Chil J Agri Resea*, 2012; **72(4)**:590.
7. Rajakumar G, Rahuman AA. *Acta Tropica*, 2011; **118(3)**:196-203.
8. Jayaseelan C, Rahuman AA, Rajakumar G, Kirthi AV et al. *Parasit Resea*, 2011; **109(1)**: 185-194.
9. Babu YM, Devi VJ, Ramakritinan CM, Umarani R et al. *Curr Nano*, 2014; **10(3)**:374-381.
10. Khooshe-Bast Z, Sahebzadeh N, Ghaffari-Moghaddam M, Mirshekar A. *Acta Agri Slov*, 2016; **107(2)**:299-309.
11. Chakravarthy AK, Bhattacharyya A, Shashank PR, Epidi TT et al. *Afri J Biotech*, 2012; **11(38)**:9295-9301
12. Debnath N, Das S, Seth D, Chandra R et al. *J Pest Sci*, 2011; **84(1)**: 9-105.
13. Sabbour MM. *J Nov App Sci*, 2012; **1(4)**: 103-108.
14. Zhang X, Zhang J, Zhu KY. *Ins Mol Bio*, 2010; **19(5)**:683-693.
15. Ulrichs C, Krause F, Rocksch T, Goswami A et al. *Comm Agri Appl Biol Sci*, 2006; **71(2 Pt A)**:171-178.
16. Bhattacharyya A, Bhaumik A, Rani PU, Mandal S et al. *Afri J Biotech*, 2010; **9(24)**:3489-3493.
17. Huang YW, Cambre M, Lee HJ. *Inter J Mol Scie,* 2017; **18(12)**:2702.
18. Nrip MNK & Gaikwad AT. *Inter Edu Appl Scient Resea J*, 2017; **2(11)**.
19. Yadava CPS, Vijayvergia JN. *Ecol bio soil organi Udaipur Agrotech Publishing Acad*, 1994; 179.
20. Raut RW, Kolekar NS, Lakkakula JR, Mendhulkar VD et al. *Nano-Micro Lett*, 2010; **2(2)**:106-113.
21. Naik BR, Gowreeswari GS, Singh Y, Satyavathi R et al. *Adv Entom*, 2014; **2(2)**:93.
22. Khan Z, Hussain JI, Hashmi AA. *Col Surf B Biointer*, 2012; **95**:229-234.

23. Velusamy P, Das J, Pachaiappan R, Vaseeharan B et al. *Indust Crop Prod*, 2015; **66**:103-109..
24. Vivek R, Thangam R, Muthuchelian K, Gunasekaran P et al. *Proc Biochem*, 2012; **47(12)**:2405-2410.
25. He Y, Du Z, Lv H, Jia Q et al. *Inter J Nanomed*, 2013; **8**:1809.
26. Gaikwad S, Bhosale A. *Euro J Exp Biol*, 2012; **2(5)**:1654-1658.
27. Raliya R, Tarafdar JC. *Inter Nano Lett*, 2014; **4(1)**:93.
28. Moodley JS, Krishna SBN, Pillay K, Govender P. *Nanoscience Nanotech*, 2018; **9(1)**:015011.
29. Sriram T, Pandidurai V. *Int. J. Curr. Microbiol. App. Sci*, 2014; **3(3)**:146-152.
30. Behravan M, Panahi AH, Naghizadeh A, Ziaee M et al. *Inter J Biol Macromol*, 2019; **124**:148-154.
31. Mukherjee P, Ahmad A, Mandal D, Senapati S et al. *Nano Lett*, 2001; **1(10)**:515-519.
32. Ahmad A, Mukherjee P, Senapati S, Mandal D et al. *Colloids Surf B: Biointer*, 2003; **28(4)**:313-318.
33. Bhainsa KC, D'souza SF. *Colloids Surf B: Biointer*, 2006; **47(2)**:160-164.
34. Kumar P, Selvi SS, Govindaraju M. *Appl Nanosci*, 2013; **3(6)**:495-500.
35. Vigneshwaran N, Ashtaputre NM, Varadarajan PV, Nachane RP et al. *Mater Lett*, 2007; **61(6)**:1413-1418.
36. Mohanpuria P, Rana NK, Yadav SK. *J Nanoparticle Resea*, 2008; **10(3)**: 507-517.
37. Khandel P, Shahi SK. *J Nanostr Chem*, 2018; **8(4)**: 369-391.
38. Zhu H, Zhang C, Yin Y. *Nanotech*, 2005; **16(12)**: 3079.
39. Henglein A, Giersig M. *J Phys Chem B*, 1990; **103(44)**:9533-9539.
40. Van Hyning DL, Klemperer WG, Zukoski CF. *Langmuir*, 2001; **17(11)**:3128-3135.
41. Kamaraj C, Rajakumar G, Rahuman AA, Velayutham K et al. *Parasitol Resear*, 2012; **111(6)**:2439-2448.
42. Anandalakshmi K, Venugobal J, Ramasamy V. *Appl Nanosci*, 2016; **6(3)**:399-408.
43. Elamawi RM, Al-Harbi RE, Hendi AA. *Egyptian J biol pest cont*, 2018; **28(1)**:28.
44. Badi'ah HI, Seedeh F, Supriyanto G, Zaidan AH. In *IOP Conference Series: Earth Environ Scie,* 2019; **217(1)**:012005.
45. Havrdova M, Polakova K, Skopalik J, Vujtek M et al. *Micron*, 2014; **67**:149-154.
46. Alyamani AMOL, Lemine OM. 2012. In *Scanning electron microscopy*. IntechOpen. 2012.
47. Ang BC, Yaacob II, Nurdin I. *J Nanomater*, 2013; **2013:**28.
48. Mustafa F, Razwan M, Shabbir S. *Processes*, 2019; **7(5)**: 245.
49. Polini A, Yang F. In *Nanofiber Comp Biomed Appl,* 2017; 97-115.
50. Ebnesajjad S. In *Handbook Adhesives Surf Prep,* 2011; 31-48.
51. Khlebtsov N, Dykman L *Chem Society Reviews*, 2011; **40(3)**:1647-1671.
52. Jans H, Liu X, Austin L, Maes G et al. *Analyt Chem*, 2009; **81(22)**:9425-9432.
53. Zanetti-Ramos BG, Fritzen-Garcia MB, de Oliveira CS, Pasa AA et al. *Mater Scie Engi: C*, 2009; **29(2)**:638-640.
54. Evanoff DD, Chumanov G. *An Euro J Chem Phys Physical Chem*, 2005; **6(7):**1221-1231.
55. Hao E, Schatz GC. *The J Chemical Phys*, 2004; **120(1)**:357-366.
56. Oancea A, Grasset O, Le Menn E, Bollengier O et al. *Icarus*, 2012; **221(2)**:900-910.
57. Devi TR, Gayathri S. *Int J Pharm Sci Rev Res*, 2010; **2(2)**:106-110.
58. Singh AK, Talat M, Singh DP, Srivastava ON. *J Nanoparticle Resear*, 2010; **12(5)**: 1667-1675.
59. Coates J. *Encyclo analyt chem: appli theory instru*, 2006.
60. Mao BH, Chen ZY, Wang YJ, Yan SJ. *Scientific reports*, 2018; **8(1)**: 2445.
61. Krishnan N, Sehnal F. 2006. *Arch Insect Biochem Physio: Entomol Society America*, 2006; **63(1):**1-10.
62. Sakuma M. *Appl ent zoology*, **33(3)**:339-347.
63. Subramaniam J, Murugan K, Jebanesan A, Pontheckan P et al. *J Cluster Scie*, 2017; **28(1)**:413-436.
64. Adesuji ET, Oluwaniyi OO, Adegoke HI, Moodley R et al. *J Nanomater*, 2016.
65. El-Sadawy HA, El Namaky AH, Hafez EE, Baiome BA et al. *Trop Biomed*, 2018; **35(2)**:392-407.
66. Sutthanont N, Attrapadung S, Nuchprayoon S. *Insects*, 2019; **10(1)**:27.
67. Sankar MV, Abideen S. *Inter J Nanomater Biostr*, 2015; **5(3)**:32-39.
68. AS AA, Thangapandiyan S. 2019. *The J Basic Appl Zoology*, 2019; **80(1)**:55.