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Antifungal Effects of Silver Nanoparticles Against Various Plant Pathogenic Fungi and its Safety Evaluation on *Drosophila melanogaster*

Pankaj K. Tyagi [1*], Rashmi Mishra [1], Fahad Khan [1], Dhriti Gupta [1], Deepak Gola [1]

[1] Noida Institute of Engineering and Technology, Greater Noida, Uttar Pradesh, India
* Correspondence authors e-mail address: pktgenetics@gmail.com;

Scopus Author ID: 34881106500

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Abstract: In the present study, silver nanoparticles (Ag-NPs) were synthesized by a chemical and biological method. Further, nanoparticles were characterized for their morphological feature using techniques like UV-Visible, TEM, XRD, and zeta potential. Sharp UV-visible absorption maximum at 410 was observed for biological synthesized silver nanoparticles (Bio-AgNPs), whereas for chemical synthesized silver nanoparticles (CH-AgNPs) peak was observed at 414 nm. TEM micrograph confirmed the formation of spherical nanoparticles dominantly via both protocols with an average size of nanoparticles was 50 nm and 25 nm for CH-AgNPs and Bio-NPs, respectively. Further, the antimicrobial potential of AgNPs was evaluated at different concentrations (25-100 ppm), against three pathogenic plant fungus plant (*Alternaria solani*, *Corynespora cassiicola*, and *Fusarium* spp.), in two different fungal media in term of inhibition of radial growth. Up to 100% inhibition for *Alternaria solani* and *Fusarium spp*. and 85% inhibition for *Corynespora cassiicola* was observed at 100 ppm AgNPs concentration on potato dextrose agar (PDA). Further, exposure of AgNPs on *Drosophila melanogaster* confirmed that Bio-AgNPs are nontoxic as compared to CH-AgNPS. Hence it can be concluded that Bio-AgNPs are safe to use due to their nontoxic nature.

Keywords: Silver nanoparticles; antifungal; Drosophila melanogaster

1. Introduction

Nanotechnology research has wide applications in the field of medical sciences, agriculture, electronic, cosmetics, biosensor, etc. [1–6]. Multiple studies utilize the different methods for the synthesis of nanoparticles, which come under either of these three categories physical, chemical, and biological [7–11]. The methods used for the synthesis of nanoparticles significantly affect the shape and size of the metallic nanoparticles [12–14]. Biological methods utilize plants, fungi, bacteria, and algae for the formation of nanoparticles and are nontoxic [15–17]. On the other hand, the main drawback of the physical and chemical protocol for the formation of nanoparticles is the cost and involvement of toxic chemicals [18].

Silver is well known for its antimicrobial potential [19,20]. However, in the form of nanoparticles, the antimicrobial activity of silver can be much superior [18]. The synthesis and antimicrobial potential of silver nanoparticles synthesize via multiple chemicals as well as biological entities (plant leaf, fungus, bacteria, etc.) are studied widely [15,19–21]. The way in which the utility of silver nanoparticles is increasing day by day, different methods of nanoparticles are evolving in terms of less toxic chemical involvement, cost-effectiveness, and

stability of nanoparticles. In the present study, two different methods (chemical and biological) have been employed, and comparative analysis in terms of morphology (size and shape) and antimicrobial potential against plant pathogenic fungus (*Alternaria solani, Corynespora cassiicola,* and *Fusarium* spp) was performed. Further, the toxicity of nanoparticles was examined on *Drosophila melanogaster* in terms of fecundity, hatchability, and viability. In addition to this, the morphology of the nanoparticles was examined via TEM (Transmission electron microscopy), XRD (X-ray powder diffraction), and zeta potential. The novelty of this work is the comparative analysis of CH-AgNPs and Bio—AgNPs, that help us to generate information about their antimicrobial as well as toxic nature.

2. Materials and Methods

2.1. Synthesis of nanoparticles.

For the biological synthesis of nanoparticles (Bio-AgNPs), fresh leaves were plugged from the plant, washed with distilled water, and dried at room temperature. After drying, 100 gm of leaves were crushed into the motor pestle and to this 100 ml distilled water was added, and the mixture was boiled at 80◦C. After boiling, 95ml of plant leaf extract (supernatant) is obtained via filtration in a flask and to this 5ml of 0.75mM AgNO3 aqueous solution was added. The mixture was incubated at 30◦C and 150rpm for 72 h. For the chemical synthesis of silver nanoparticles (CH-AgNPs), 50ml AgNO3 (0.75mM) mixed thoroughly with 10ml trisodium citrate (38mM) dropwise at 90°C. It kept for 24 h in the dark at room temperature. After getting the silver nanoparticles in solution (biological and chemical), solutions were lyophilized, and a stock solution of 1000 ppm was prepared for both. Different working concentrations (10 ppm, 25 ppm, 50 ppm, and 100 ppm) were prepared by diluting the original silver nanoparticles stock solution with distilled water. All AgNPs solutions were stored at 4°C for further experiments.

2.2. Morphological characterization of silver nanoparticles.

The CH-AgNPs and Bio-AgNPs in the form of colloidal solutions were primarily characterized by UV-visible spectrophotometry, Transmission Electron Microscopy (TEM), and zeta potential and for the same sample were prepared according to the previously defined protocol by [21].

2.3. Pathogenic fungi and growth media.

Three fungal species (*Alternaria solani, Corynespora cassiicola,* and *Fusarium* spp.) were obtained from the microbiology laboratory of NEIT (Noida Institute of Engineering and Technology, Gr, Noida). All three fungal pathogens are known to cause various diseases on vegetables and crop plants.

2.4. Antifungal activity of nanoparticles.

In vitro antifungal assay at different concentrations (10, 25, 50, and 100 ppm) of AgNPs (biological and chemical) was performed on two types of fungi growth medium (PDA and CMA). 5 mL of AgNPs having different concentrations was poured into growth medium before plating in a petri dish and incubated at room temperature for 30 min. In addition to this, control plates containing only growth media were also prepared in parallel. After incubation, plates

were inoculated by agar plugs of uniform size containing fungi at the center of each petri dish, followed by incubation at 28 ± 2°C for 14 days. The radial growth of fungal mycelium was measured and recorded after the incubation period of fungi on different culture medium containing different concentrations of AgNPs. Radial inhibition was calculated when the growth of mycelia in the control plate reached the edge of the petri dish. The following equation was used for calculation of the inhibition rate (%).

Inhibition rate (%) = R – r/R

where "R" is the radial growth of fungal mycelia on the control plate sample, and "r" is the radial growth of fungal mycelia on the plate containing AgNPs.

2.5. In vivo toxicity analysis on Drosophila melanogaster.

In vivo toxicity effect of CH-AgNPs and Bio-AgNPs were investigated using the Drosophila melanogaster. Life-history traits, i.e., fecundity, hatchability, and viability parameters, were used to assess the toxic effect of nanoparticles at different concentrations (10, 25, 50, and 100 ppm). Nanoparticles were supplemented with the food medium of Drosophila melanogaster. Further, control food media was not supplemented with any type of nanoparticles

3. Results and Discussion

3.1. UV-visible spectrum.

The change in solution color is the primary indication of nanoparticle synthesis. Further, the synthesis of nanoparticles can be confirmed by the maximum absorption peak between 400 to 500 nm, which corresponds to the sharp surface Plasmon resonance. As illustrated in Figure 1 (A and B), the maximum absorption peak was observed at 414 nm and 410 nm for CH-AgNPs and Bio-AgNPs, respectively.

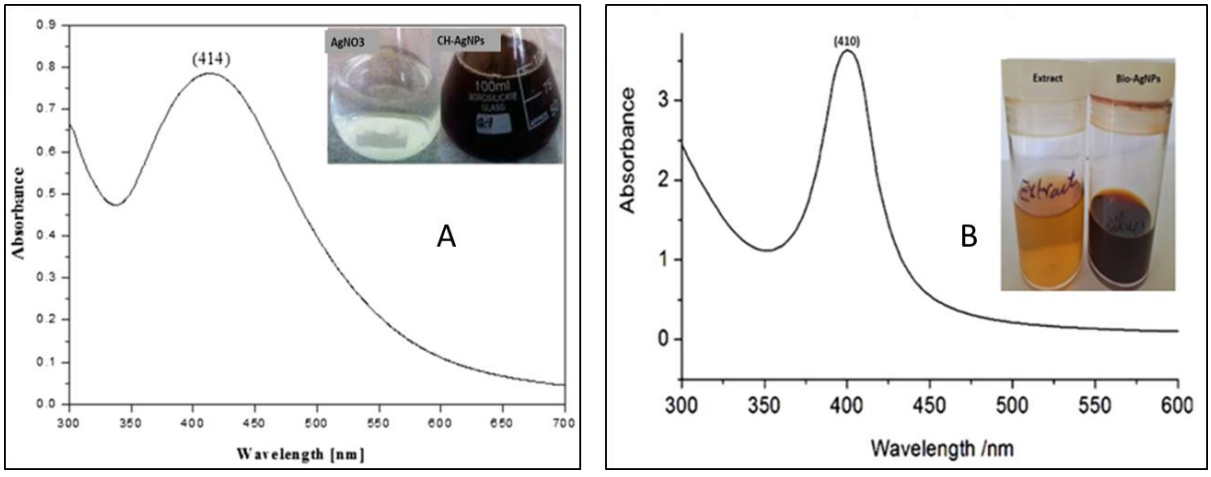

Figure 1. UV-visible spectrum of CH-AgNPs (A) and Bio-AgNPs (B).

The sharp surface Plasmon resonance peak symbolizes the formation of monodispersed nanoparticles, and the UV-visible spectrum of Bio-AgNPs showed much sharper peak as compared to the spectrum of CH-AgNPs [18]. The possible reason behind the sharp spectrum of Bio-is the plant extract that contains a number of secondary metabolites, proteins and enzymes. All these components act as the reducing agent as well as capping during the formation of metallic nanoparticles [19].

3.2. TEM.

The TEM micrograph provides insight into the morphology feature of nanoparticles, such as shape and size. It was observed that chemically synthesized nanoparticle was cuboidal, hexagons, and spherical shapes. However, spherical shape nanoparticles were leading with an average size of 50.56 nm (Figure 2A). Further, no significant agglomeration was observed between the nanoparticles. On the other hand, the TEM micrograph of Bio- AgNPs indicates the synthesis of spherical and smaller nanoparticles with size ranging from 14-39 nm (Figure. 2B).

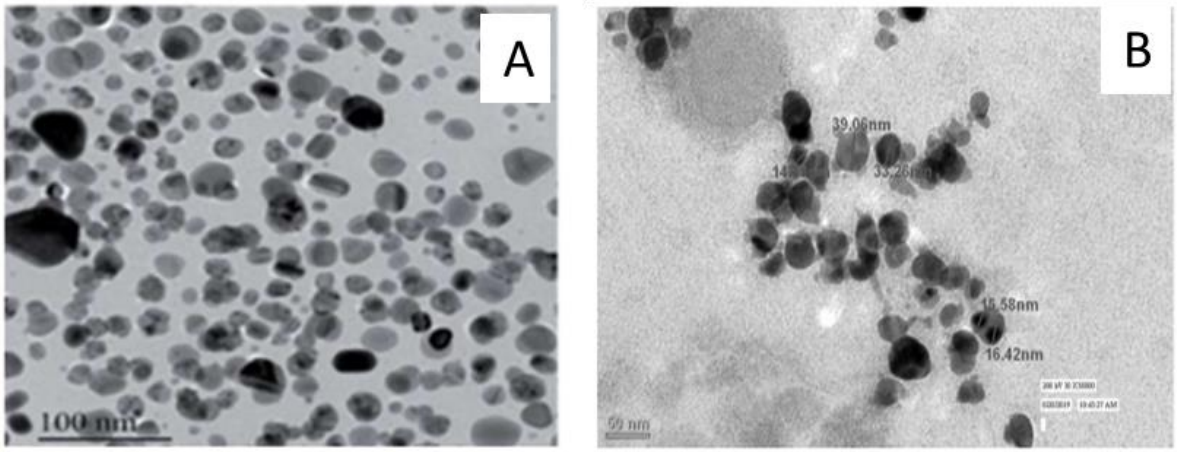

Figure 2. TEM micrograph of CH-AgNPs (A) and Bio-AgNPs (B)

The morphology (size and shape) of the nanoparticles can be further enhanced by varying the concentration of chemical and plant extract in the reaction mixture, incubation time, pH of the reaction mixture, and the temperature of the reaction mixture [22]. The shape of the nanoparticles can directly affect its antibacterial characteristics. For example, it was investigated that triangular-shaped metallic nanoparticles were more antibacterial as compared to spherical or rod shape metallic nanoparticles [18]. This may be attributed to the sharp ends formed by the triangular shape nanoparticles as sharp ends can penetrate the cell membrane easily.

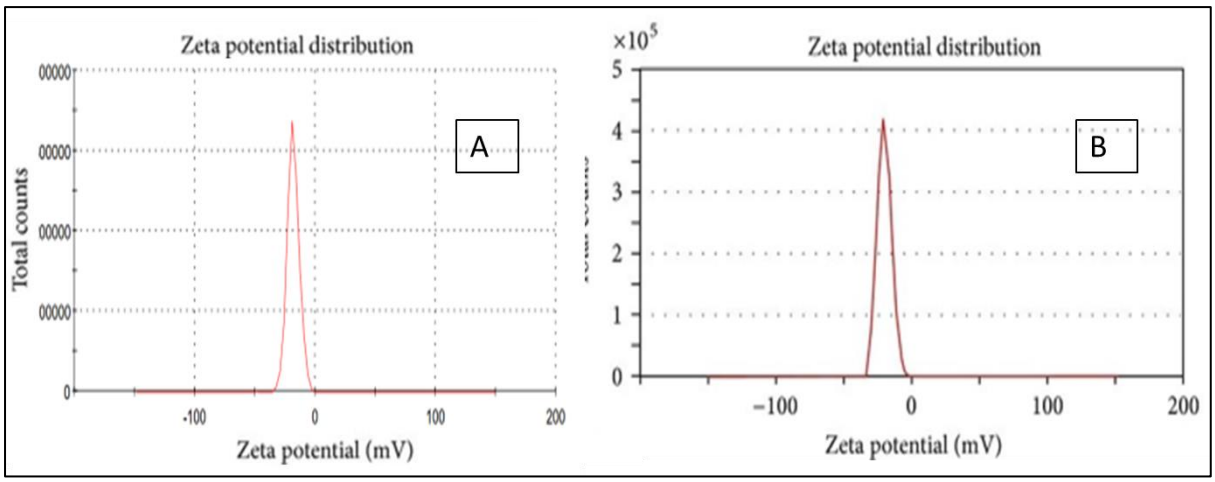

Figure 3. Zeta potential of CH-AgNPs (A) and Bio-AgNPs (B).

3.3. Zeta potential.

The zeta potential value for CH-AgNPs and Bio-AgNPs was −17.24 mV and 19.1 mV, respectively (Figure 3). It was observed that negative charge exists between the biologically as

well as chemically synthesized AgNPs, indicating the stable synthesis. This negative charge value will cause repulsion between AgNPs and hence will reduce their aggregation tendency (Tyagi et al., 2019). A similar negative zeta potential value of −7.66 mV was detected for Bio-AgNPs produced from *Pedalium murex* leaf extract [23]. Table 1 represents the morphological characteristics of CH-AgNPs and Bio-AgNPs**.**

Table 1. Characteristics of silver nanoparticles used in this study.

Type of nanoparticles	Physical Characteristics	Range of Particles Size (nm)	UV-vis peak range (nm)	Zeta potential size (mV)
CH-AgNPs	Reddish brown colloidal suspension	15-60	414	-17.24
Bio-AgNPs	Dark brown colloidal suspension	14-39	410	-19.10

3.4. In vitro antifungal analysis.

Table 2 illustrate the rate of inhibition (%) of *Alternaria solani*, *Corynespora cassiicola*, and *Fusarium spp* by CH-AgNPs and Bio-AgNPs at different concentration in PDA and CMA media.

Table 2. Inhibitory rate (%) caused by CH-AgNPs and AgNPs against three plant pathogenic fungi on PDA and CMA media.

Types of AgNPs	Pathogenic fungus	Type of media	Inhibition rate (%)				
			Control	10 ppm	25 ppm	50 ppm	100 ppm
CH-AgNPs	*Alternaria solani*	PDA	0	70.90	75.12	85.67	100
		CMA	0	63.55	72.76	84.66	90.00
	Fusarium spp.	PDA	0	40.00	51.87	64.87	85.00
		CMA	0	36.90	45.87	58.98	75.00
	Corynespora cassiicola	PDA	0	50.76	68.65	82.57	100
		CMA	0	48.99	64.77	78.86	85.00
BIO-AgNPs	*Alternaria solani*	PDA	0	65.88	70.76	83.76	95.05
		CMA	0	60.47	64.65	80.56	94.78
	Fusarium spp.	PDA	0	32.78	49.90	62.86	90.50
		CMA	0	31.87	45.90	56.59	90.05
	Corynespora cassiicola	PDA	0	52.09	66.77	78.89	90.00
		CMA	0	45.65	60.88	74.76	88.89

*Inhibition rates were determined based on 5 replicates of both experiment

Absolute inhibition (100%) was observed against *Alternaria solani*, and *Corynespora cassiicola* in PDA media via CH-AgNPs, whereas Bio-AgNPs showed up to 95% and 90% inhibition at the same concentration, respectively. The lowest level of inhibition (85%) at 100 ppm on the PDA was observed with *Fusarium spp.* by CH-AgNPs followed by *Corynespora cassiicola* (90%) against Bio-AgNPs. On the other hand, 90% inhibition growth was observed for *Alternaria solani*, followed by *Fusarium spp.* (85%) and *Corynespora cassiicola* (75%) at 100 ppm concentration in CMS medium. It was observed that as the concentration of nanoparticles in the growth media increases, the growth inhibition rate percentage of fungus increase in all the cases (Figure 4).

The maximum increase in inhibition rate, i.e., is 57%, and 58% was observed for *Fusarium spp.* against Bio-AgNPs as the concentration increased from 10 ppm to 100 ppm in the PDA and CMA media, respectively. On the other hand, the minimum increase in inhibition rate i.e, is 29 % and 26 % was observed for *Alternaria solani* against CH-AgNPs as the concentration increased from 10 ppm to 100 ppm in the PDA and CMA media, respectively. Up to 90% inhibition was observed in the growth of *Fusarium solani* at 20 µg/ml of AgNPs

[24]. In another study, inhibition up to 71% -77% was observed with different strains of *R. solani*on CDA medium at 0.0019 mol/L of AgNPs [25].

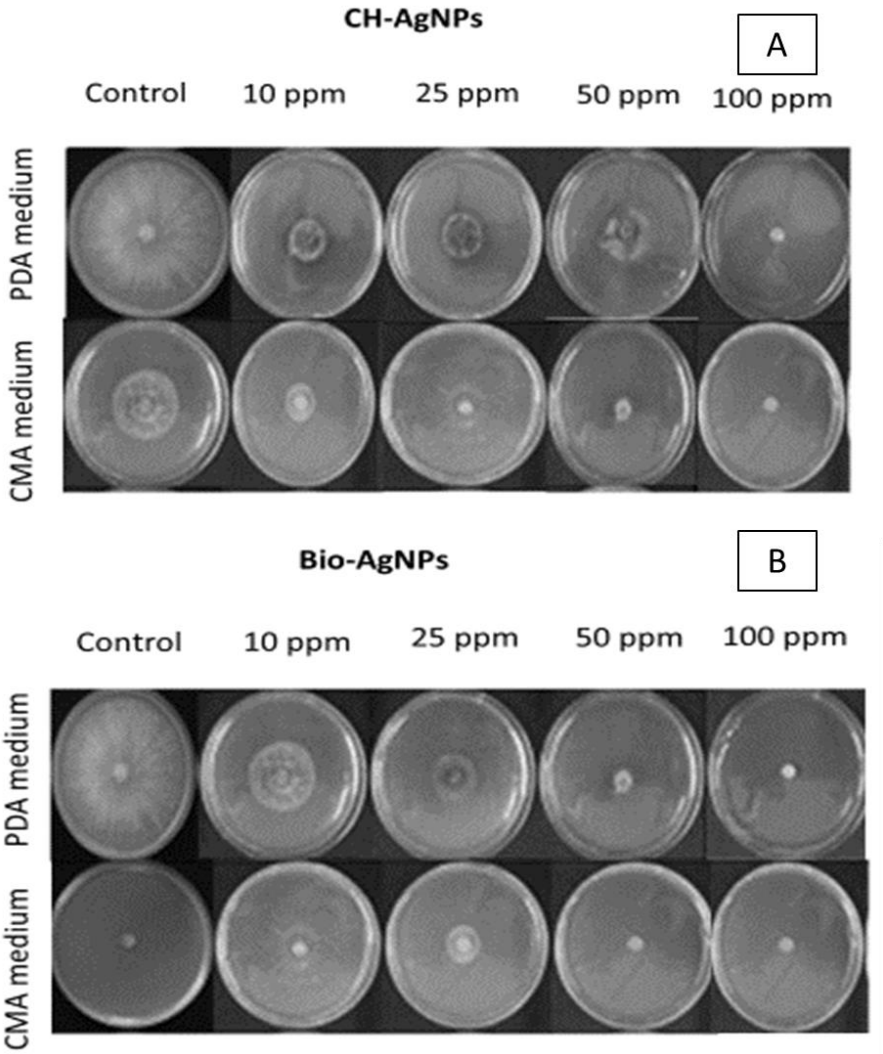

Figure 4. *In vitro* inhibition effects of CH-AgNPs and Bio-AgNPs against fungus on PDA and CMA medium.

The results indicate that both CH/Bio-AgNPs are capable of inhibiting the growth of fungal pathogens; however, results vary according to the concentration dose and type of AgNPs (chemical/biological) exposed against fungal pathogens. Most fungi showed a high inhibition effect at a 100 ppm concentration of silver nanoparticles. In addition to this, the results indicate that higher inhibition growth rate was observed on PDA media as compared to CMS medium. As the concentration of AgNPs increases inhibition rate increases, this might be due to the high density at which the solution was able to saturate and cohere to fungal hyphe as well as to deactivate plant pathogenic fungi.

The probable mechanism of inhibition may include loss in the ability to replicate DNA and inactivation of certain other cellular proteins as well as enzymes essential to the ATP production [21,26–28].

3.5. In vivo toxicity analysis on Drosophila melanogaster.

After identifying the rate of inhibition in the percentage of the CH-AgNPs and Bio-AgNPs, the next steps are to evaluate the toxicity of these nanoparticles. To examine the *in vivo* toxicity, we selected *Drosophila melanogaster* as the model organism. The *in vivo* toxic

effects of nanoparticles on the developmental stages of *Drosophila melanogaster* were investigated at 4 different concentrations. The control fly laid on average 60 eggs/fly/day with 98% hatchability and 96% viability in all the replicates. It was observed that as compared to control Bio-AgNPs did not cause any significant difference in fecundity, hatchability, and viability (Figure 5, 6, and 7). The above results clearly indicate that Bio-AgNPs were nontoxic to *Drosophila melanogaster*. Whereas with the CH-AgNPs, reduction in fecundity, hatchability, and viability was observed as the concentration increases in the food media. Therefore, the results suggested that the Bio-AgNPs did not induce any toxicity on the developmental stages of *Drosophila melanogaster*.

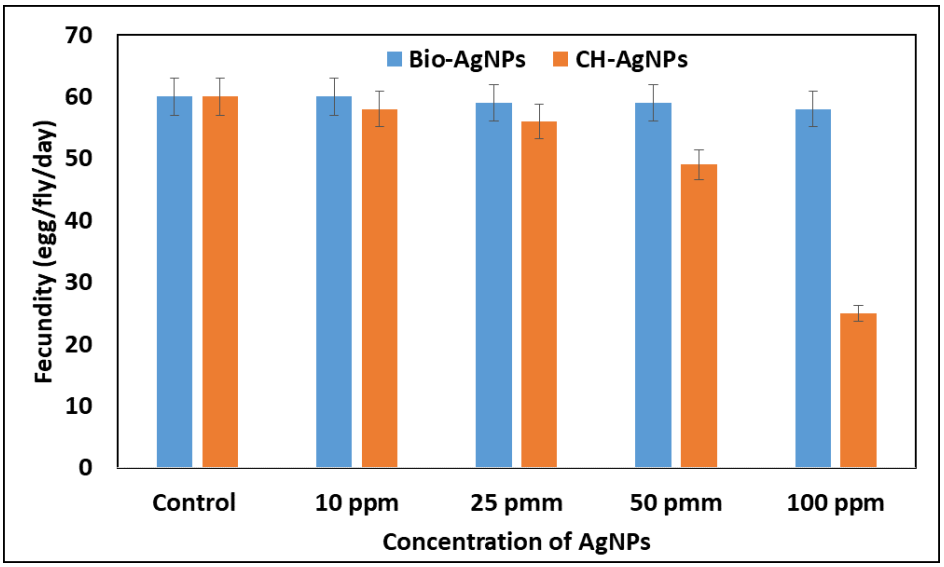

Figure 5. Fecundity of *Drosophila melanogaster* against Bio-AgNPs and CH-AgNPs.

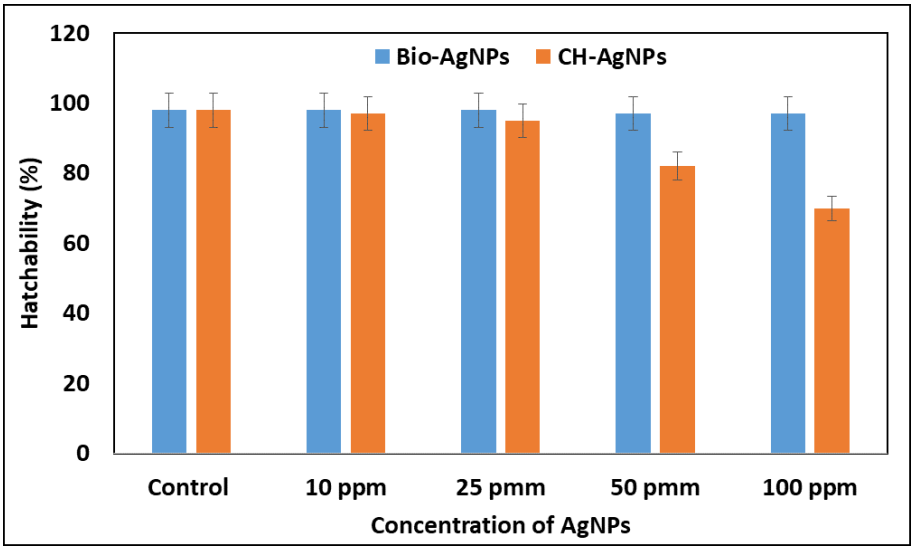

Figure 6. Hatchability of *Drosophila melanogaster* against Bio-AgNPs and CH-AgNPs.

On the other hand, we can say, CH-AgNPs were toxic for *Drosophila melanogaster* as compare to Bio-AgNPs. It was observed that no significant change was occurred up to 25 ppm (CH-AgNPs), but above 25 ppm fecundity, hatchability, and viability decrease significantly. Fecundity was reduced to 25 egg/fly/day from 60 egg/fly/day (control) at 100 ppm. Similarly, up to 27% and 50% reduction in hatchability and viability was observed as compared to Bio-AgNPs (Figures 6 and 7). The above results clearly indicate that no toxic effect was observed for Bio-AgNPs. In contrast, chemically synthesized AgNPs were toxic to *Drosophila melanogaster*. Hence it can be concluded that biologically synthesized nanoparticles are safe

to use as compared to chemically synthesized nanoparticles. In summary, the Bio-AgNPs have considerable antifungal activity as well as found to be nontoxic to *Drosophila melanogaster*. Researchers examined the in vivo toxicity of AgNPs (purchased from Sun Innovations Corp., USA) on *Drosophila* and observed that ingestion of AgNPs with food medium at higher doses significantly affects the egg-laying ability as well as impaired growth of ovary [29]. Moreover, AgNPs (with Ag concentration: 20 mg/L) were observed to cause acute toxicity in *Drosophila* as 50% of the tested population were incompetent to leave the pupae by hindering their development cycle [30]. De Lima et al. (2012) reviewed the comparative toxicity of chemically and biogenic generated AgNPs and concluded that biogenic AgNPS were less toxic as compared to chemically generated AgNPs [31].

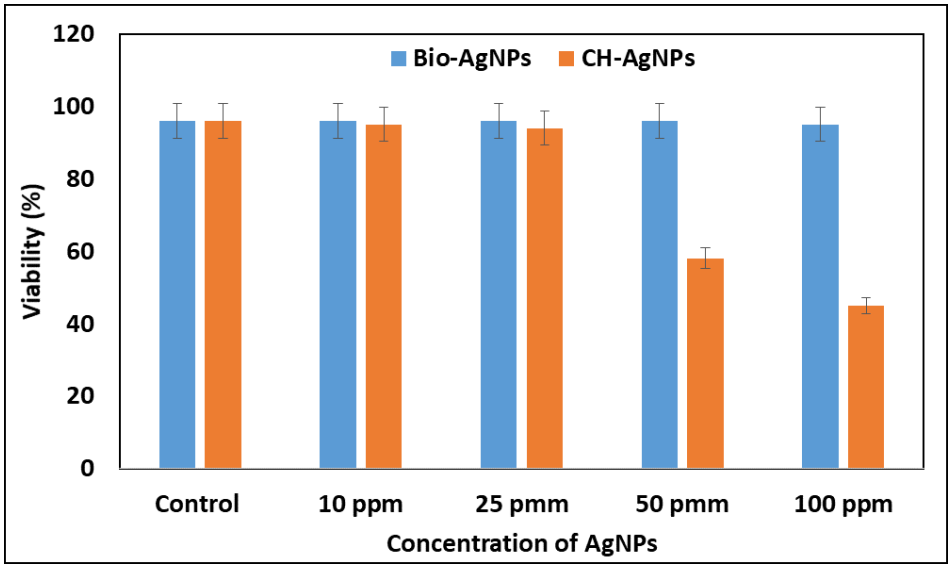

Figure 7. Viability of *Drosophila melanogaster* against Bio-AgNPs and CH-AgNPs.

4. Conclusions

The present study is a comparative analysis of AgNPs synthesizes by biological and chemical methods. It was observed that Bio-AgNPs were more stable, uniformed in shape, and smaller in size as compared to CH-AgNPs. Further, it can be concluded that in term of antifungal activity, both types of nanoparticles were showing inhibitory effect, and this inhibitory effect was dependent on AgNPs dose, type of growth media, and type of fungal species. In addition to this, Bio-AgNPs were found to be nontoxic against Drosophila melanogaster. In contrast, CH-AgNPs were toxic, and its toxicity is dependent on the concentration of nanoparticles. So it can be concluded that Bio-AgNPs are safe to use as compared to CH-AgNPs.

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Conflicts of Interest

The authors declare no conflict of interest.

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