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Original Article

Evaluation of chitosan and silver nanoparticles Against isolated pathogens from Mulberry Silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) under laboratory conditions

Avaliação de nanopartículas de quitosana e prata contra patógenos isolados de bicho-da-seda, *Bombyx mori* L. (Lepidoptera: Bombycidae), em condições de laboratório

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Abstract

This study aims to isolate and identify certain bacterial and fungal pathogens from silkworm, *Bombyx mori* L. such as promising chitosan, plus silver nanoparticles as its antimicrobial activity under laboratory condition. *Silkworm*, *B. mori* (H1xKKxG2xV2-Bolgaria) eggs were attained from Sericulture Research Center from Giza Governorate, Egypt. Chitosan and silver nanoparticles materials were assembled at the laboratory of Biochemistry Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. Herein total of 7 bacterial and 5 fungal were isolated from the external and internal silkworm larvae. As a result, the mean percentage decrease in weight was elevated in diseased fifth instars (88%) compared to fourth diseased instars (62%). In addition, two bacterial species isolated from the infected larvae were identified as follows: *Staphylococcus aureus* and *Enterococcus faecalis*, whereas three fungal species were isolated as follows: *Aspergillus flavus*, *Aspergillus tamarii* and *Beauveria bassiana*. Transmission electron microscope imaging demonstrated the morphological properties and surface appearance of silver and chitosan nanoparticles which have a nearly spherical shape and smooth surface. The average particle size of 18.7 - 26.0 nm and 18.8 to 21.8 nm of silver and chitosan nanoparticles were recorded. Furthermore, the highest activity among nanoparticles tested against all pathogenic bacteria and fungi isolated and identified in our study was recorded by chitosan at 100 µg/ml in series, whilst silver nanoparticle exhibited moderate antibacterial and antifungal activity.

Keywords: chitosan, silver, silkworm, antimicrobial agent, nanoparticle.

Resumo

Este estudo visa isolar e identificar certos patógenos bacterianos e fúngicos do bicho-da-seda (*Bombyx mori* L.), como a promissora quitosana, além de nanopartículas de prata como sua atividade antimicrobiana em condições de laboratório. Ovos de *B. mori* (H1xKKxG2xV2-Bolgaria) foram obtidos do Centro de Pesquisa em Sericultura da Província de Gizé, Egito. Materiais de nanopartículas de quitosana e prata foram montados no laboratório do Departamento de Bioquímica, Faculdade de Agricultura, Universidade Al-Azhar, Cairo, Egito. Aqui, foi isolado um total de 7 bactérias e 5 fungos das larvas de bicho-da-seda externas e internas. Como resultado, a diminuição percentual média no peso foi elevada no quinto instar doente (88%) em comparação com o quarto instar doente (62%). Além disso, foram identificadas duas espécies bacterianas isoladas das larvas infectadas (*Staphylococcus aureus* e *Enterococcus faecalis*), enquanto três espécies fúngicas foram isoladas (*Aspergillus flavus*, *Aspergillus tamarii* e *Beauveria bassiana*). A imagem de microscopia eletrônica de transmissão demonstrou as propriedades morfológicas e aparência de superfície de nanopartículas de prata e quitosana que têm uma forma quase esférica e superfície lisa. Foi registrado o tamanho médio das partículas de 18,7-26,0 nm e 18,8-21,8 nm de nanopartículas de prata e quitosana, respectivamente. Além disso, a atividade mais alta entre as nanopartículas testadas contra todas as bactérias patogênicas e fungos isolados e identificados em nosso estudo foi registrada pela quitosana em 100 µg/ml em série, enquanto a nanopartícula de prata exibiu atividade antibacteriana e antifúngica moderada.

Palavras-chave: quitosana, prata, bicho-da-seda, agente antimicrobiano, nanopartícula.

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1. Introduction

Generally, silkworm a lepidopteron group of insect, is often considered as "Queen of textiles" for producing economically important silk fiber (Soumya et al., 2017). However, the agro-based silk industry is considered as a rural lifeline of India, a sit generates employment to millions of rural and sub-rural people, improving their economic status (Bukhari and Kour, 2019). *Bombyx mori* L., is considered an important economic insect, has made a great contribution to the development of national economy (Dong et al., 2014). Importantly, silk is one of the nature's gifts to mankind produced by silkworm. Among silkworms the most commercial exploited one are mulberry silkworm *B. mori* (Thirumalaisamy et al., 2009). The mulberry silkworm is of a great economic importance as a foreign exchange earner for many silk producing countries of the world (Krishnaswami et al., 1992). As numerous of other insects, mulberry silkworm, *B. mori* is also susceptible to wide range of microorganisms causing crop losses up to 20% (Jiang et al., 2013). Grasserie (Viral disease), Muscardine (Fungal disease), Pebrine (Microsporidian infection) and Flacherie, a syndrome inflected by non-occluded viruses, bacterial and both in combination are the frequently encountered diseases to silkworm (Tao et al., 2011). Further, bacterial septicemia is one of the most frequent silkworm diseases, which is usually caused by different types of pathogenic bacterium, including *Bacillus, Pseudomonas, Streptococcus* and *Staphylococcus* (Jin and Lu, 2001; Tayal and Chauhan, 2017; Javaid et al., 2021).

Moreover, Aspergillosis or *Aspergillus* disease is a mycosis or a fungal disease caused by *Aspergilus* fungi and it is one of the essential diseases of silkworm, *B. mori* (Yu et al., 2002). Among the insect pathogens, fungi constitute the largest group with more than 700 species causing mycosis in insects (Tamuli and Gurusubramanian, 2011). Furthermore, nearly a dozen species of fungi cause infections in silkworm of which most of the infections caused by the members of the genera are *Beauvaria* and *Mitarhizium*. They are found throughout the world and are most contagious (Sengupta et al., 1991).

In the regard, nanotechnology science refers broadly to a field of applied science and technology whose unifying theme is the control of matter on the atomic and molecular basis (Nanda and Saravanan, 2009). However, among different biosynthesized metallic nanoparticles, silver nanoparticles gain special attention due to their diverse sector application of biogenic silver nanoparticles was revealed in the field of biomedical science (antibacterial, antifungal, antiviral, antiinflamatory, antiangeogenic, wound healing, drug delivery and anticancer activity (Anjum et al., 2019; Aldayel et al., 2021).

Development of metal-containing preparations based on polymers (including chitosan) is a major area of nano-chemistry. It is known that Ag-chitosan composites have bacterial and bacteriostatic effects against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis,* and *Pseudomonas aeruginosa* (Tripathi et al., 2016). It is known that chitosan and its modifications exhibit biological activity against viral infections, bacteria and phytopathogenic fungi associated with silkworm (Kochkina and Chirkov, 2000; Pospieszny, 1999).

In this interim our objective of the present study to isolate of certain bacterial and fungal pathogens in silkworm which is considered promising antimicrobial activity using chitosan and silver nanoparticles under laboratory condition.

2. Materials and Methods

2.1. Chemicals and reagents

All chemicals and reagents used in this study were high purity. Silver nitrate (assay >99.9%, Merck), chitosan powder (assay 99.0%, degree of deacetylation 80.0% was produced from Suvchem, Mumbai, India) and maize starch was purchased from the Egyptian Company for Starch and Glucose manufacture, Cairo, Egypt. The other chemicals used for this study were purchased from El-Nasr Pharmaceutical Chemicals Company, Cairo, Egypt.

2.2. Collection of silkworms

Diseased larvae of silkworm *B. mori* (H1xKKxG2xV2-Bolgaria) were obtained from rearing silkworm lab. Based the fourth and fifth larvae instars were examined, and the infected larvae were separated from the healthy and their weight was determined during rearing in Plant Protection Department, Faculty of Agriculture Cairo, Al-Azhar University, Egypt. Preserved in aseptic plastic containers and transported to Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Assiut branch, Egypt, to complete the isolation and identification of associated bacteria and fungi with silkworm larvae.

2.3. Microorganisms strains

Three fungi strains (*Aspergillus flavus, Aspergillus tamari*, and *Beauveria bassiana*) and two bacterial strains (*Streptococcus faecalis and Staphylococci aureus*).

2.4. Methods

2.4.1. Preparation of nanoparticles

Chitosan and silver nanoparticles materials were prepared at laboratory of the Biochemistry Department, Faculty of Agriculture, Cairo, Al-Azhar University, Egypt.

2.4.2. Silver nanoparticles

Silver nanoparticles were prepared according to the method described by El-Rafie et al. (2014) and Shahat et al. (2020). Starch was dissolved in alkali solution (1.0 g starch in 80 ml of distilled water containing 2.0 g sodium hydroxide) by using high-speed homogenizer. After complete dissolution the temperature of the reaction medium was raised to the desired degree (60 °C). In this moment, 20 ml silver nitrate solution (10 mM) was added dropwise. The reaction medium was kept under continuous stirring for 60 min. After complete reaction, the solution was allowed to cool down slowly to 25 °C. Then the starch - silver nanoparticles were precipitated using absolute

ethyl alcohol under high-speed homogenizer. The powder precipitate was coollected by centrifugation at 4.500 rpm for 15 min, washed twice with 80/20 ethanol/water to remove the unreacted materials and impurities, and then finally washed with absolute ethanol. The collected powder was dried and identified as starch - silver nanoparticles using state of the art facilities.

2.4.3. Chitosan nanoparticles

Chitosan nanoparticles (CHNPs) were prepared as described by Vimal et al. (2013). Chitosan solution was prepared by dissolving chitosan in 100 ml acetic acid solution (1.0%) and leaving it under stirring for until the solution was transparent. The pH was adjusted to pH 5.5 with 0.01 N NaOH. Then, tripolyphosphate (TPP) solution was added to the chitosan solution dropwise under magnetic stirring (Model-MS300HS: Korea). The formation of CHNPs started spontaneously via the TPP initiated ionic gelation mechanism. Once the dropwise addition was complete, the resulting suspension was then left under stirring for 45 min.

2.5. Isolation of fungal and bacterial pathogens from silkworm

Mulberry silkworm that shows microbial infection was surface sterilized with 0.1% mercuric chloride and then washed with distilled water. The bacteria and fungi that were isolated from Mulberry silkworm larvae were streaked nutrient and potato dextrose agar media respectively (Meyling and Eilenberg, 2006, 2007). Using streak plate technique, the bacterial and fungal colonies were further purified, after attaining good growth; slants were stored in refrigerator at 4 ºC for further studies and used as stock cultures. The bacterial pathogens of silkworm larvae were identified based on biochemical, physiological and morphological characteristics such as colony morphology and staining techniques, while fungal pathogens of silkworm larvae were identified based on morphological characteristics such as colony and microscopic morphology.

2.6. Characterization studies

2.6.1. Scanning electron microscopic examination of diseased silkworm

Samples of silkworm larvae were fixed for 2 h at room temperature in 2.5% glutaraldehyde prepared in 0.2 M cacodylate buffer (pH 7.2), dehydrated in graded alcohol-acetone series, and dried in a critical point dryer (EMS-850) using CO_2 as transition fluid. A few critically dried samples were also randomly fractured to trace the routes of infection. The dried samples were mounted onto copper stubs, gold coated, and examined using a JEOL 100 CX II ASID 4D scanning electron microscope at 15 kV.

2.7. Characterization studies of the synthesized nanoparticles

2.7.1. Visual inspection

The reduction of silver ions was roughly monitored by visual inspection of the solution as the method described by Shahat et al. (2020). While, the formation of opalescent white color suspension in the reaction mixture was used as a visual indicator to confirm the synthesis of CHNPs according to the method of Morris et al. (2011).

2.7.2. Transmission electron microscopic measurements

The morphological features of silver and chitosan nanoparticles were examined by High-Resolution Transmission Electron Microscopy (TEM) which provides accurate information about the size and shape of the formed nanoparticles. TEM characterization is performed using (JEOL, JEM-1230, Japan) instrument with an acceleration voltage of 120 kV as the method described by Hebeish et al. (2016).

2.7.3. Anti-microbial assay

Screening of chitosan and silver nanoparticles for their antimicrobial activity was done by well diffusion method based on diameter inhibition zone growth by millimeter (mm) of the microorganisms.

2.7.4. Well diffusion method

Screening of antimicrobial activity with studied nanoparticles were performed by well diffusion technique. For this, the agar plates were seeded with 0.1 ml of the standardized inoculums of each test organism. The inoculums were spread evenly over plate with sterile glass spreader. A standard cork borer of 6 mm diameter was utilized to cut uniform wells on the surface of the agar using sterile aluminium borer. Then, 200 µl of each nanoparticles (dissolved in sterilized distilled water) was introduced in the well. Different concentrations (25, 50, 75, 100 µg/ml) of studied nanoparticles dissolved in sterilized distilled water were used. Distilled water was used as control. The inoculated plates were incubated at 37 °C for 24 hours and 30 °C for 5 days for bacterial and fungal tested organisms respectively, and the zone of inhibition was measured (including the diameter of the bore (7 mm) and the results were recorded.

2.8. Statistical analysis

Collected data were subjected to the Analysis of Variance (ANOVA) using Statistical Analysis System (SAS) at 5% level of significance. The mean differences were separated using Least Significant Difference (LSD) and showed as means ± SE. Shapiro-Wilk's W test was done for the assumption of normality in which the test was insignificant.

3. Results

Data in Figure 1 show the mean weight of fourth and fifth instars of healthy and infested larvae of *B. mori* (H1xKKxG2xV2-Bulgaria). The results demonstrated that, the mean weight of fourth instars of healthy larvae was 0.460 gm, while the mean weight of infested larvae was 0.172 gm. On the other side, the mean weight of the fifth instar of healthy and infested larvae were 2.143 and 0.260 gm, respectively, with significant differences between healthy and infested instars. However, data displayed

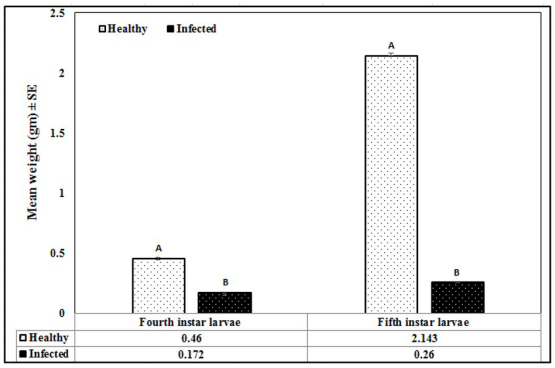

Figure 1. Mean weight of healthy and infected fourth and fifth instar larvae of *B. mori*. Isolation and identification of bacterial isolates: Total of 7 bacterial were successfully isolated from the outer surface and the inner body of silkworm larvae.

decrease in mean weight rate were higher in the infested fifth instar larvae compared to the infested fourth larvae.

These isolates were classified into two phenotypes based on the colony shape and cellular characteristics. Additionally, biochemical and physiological characterization of bacterial isolates were tested. Based on morphological, biochemical and physiological characteristics of the bacterial isolates illustrated in Table 1, two bacterial species were identified as follows: *Staphylococcus aureus* (n=3) and *Enterococcus faecalis* (n=4).

3.1. Isolation and identification of fungal isolates

Depending on microscopic and culture characteristics of all fungal isolates illustrated in Table 2, three fungal species were identified as followed: *A. flavus* (n=1), *A. tamarii* (n=2) and *B. bassiana* (n=2).

The larval integument of *B. mori* dominated various types of setae and nodules, and the conidia of different species of fungi in our study, when examined by scanning electron microscope. The aerial hyphae existed first at the intersegment regions of the mummified larvae, but later grew extensively on the larval surface forming a mycelial mat within 6-7 days (Figure 2A-C). Silkworm larvae infected with bacterial species became soft, and their skin turned dark or released a black, watery discharge as well as witnessing noticeable weight loss, compared to a normal larvae. This indicates that the larvae suffered from septicemia due to the bacterial infections, and resulted from melanization. The cocci shapes of bacteria in surface of larvae is presented in Figure 2D.

3.2. Characterization of produced nanoparticles

3.2.1. Visual inspection of silver nanoparticles

A concised time after the addition of $AgNo_3$ solution into starch solution during the synthesis of silver nanoparticles, the reaction medium acquired a clear yellow color then changed to brown and finally to dark brown color during stirring the solution along with mirror-like illumination on the walls of Erlenmeyer flask clearly indicated the

Table 1. Morphological and biochemical characterization performed for identification of bacterial isolates from *Bombyx mori* L. larvae.

General characteristics	*Enterococcus faecalis* (n=4)	*Staphylococcus aureus* (n=3)
Morphology:		
Gram staining	Positive	Positive
Shape	Cocci	Cocci
Colony color	Watery white	Golden brown
Motility	Non-motile	Non-motile
Spore formation	Non-sporing	Non-sporing
Oxygen requirements	Facultative anaerobic	Facultative anaerobic
Capsule	Non-Capsulated	Non-Capsulated
Biochemical reactions:		
Catalase	-ve	+ve
MR	-ve	+ve
VP	+ve	+ve
Oxidase test	-ve	-ve
Indole	-ve	+ve
Citrate	-ve	-ve
Urease	-ve	+ve
Nitrate reduction	+ve	+ve
Coagulase	-ve	+ve
Pigment	-ve	+ve
Fermentation of carbohydrate:		
Arabinose	-ve	-ve
Glucose	+ve	+ve
Lactose	+ve	+ve
Maltose	+ve	+ve
Mannitol	+ve	+ve
Mannose	+ve	+ve
Raffinose	-ve	-ve
Ribose	+ve	+ve
Sucrose	+ve	+ve
Enzymatic Reactions:		
Acetate utilization	-ve	+ve
Acetoin production	-ve	+ve
Acid phosphatase	-ve	+ve
Alkaline phosphatase	+ve	+ve
Arginine dehydrolase	+ve	+ve
Hyalurodinase	-ve	+ve
Lipase	-ve	-ve
No. of isolates	4	3

+ve: Positive, **-ve**: Negative, **MR**: Methyl red test, **VP**: Voges-Proskauer test, **n**: Number of isolates.

Table 2. Cultural and morphological characteristics performed for identification of fungal isolates from *Bombyx mori L. larvae.*

Colony and microscopic features	Probal fungus		
	Aspergillus flavus **(n=1)**	***Aspergillus tamarii*** **(n=2)**	***Beauveria bassiana*** **(n=2)**
Colony characteristics:	Disperse and dense	Disperse and dense	Disperse and dense
Growth pattern	Yellow/grayish geen	Olive green	White
Color	woolly with a floccose center	woolly with a floccose center	Round
Shape	Rough	Rough	Smooth and powdery
Texture	Upraised	Upraised	Raised
Elevation			
Microscopic characteristics:	800-1200 µm	600-1500 µm	600-1400 µm
Size	Pale brown roughened	Uncolored	Branched hyphae and formed conidiogene cells
	Quietly spherical	Rough walled	
Stipes color	Biseriate	Biseriate spatulate	globose ellipsoidal
			a wavy rachis
Surface	Entirely	Entirely	smooth and hyaline
Vesicle serration	Glubose ellipsoid	Spherical	
Metula covering	Smooth finely roughened	Smooth	
Shape			
Conidia surface			

n: Number of isolates.

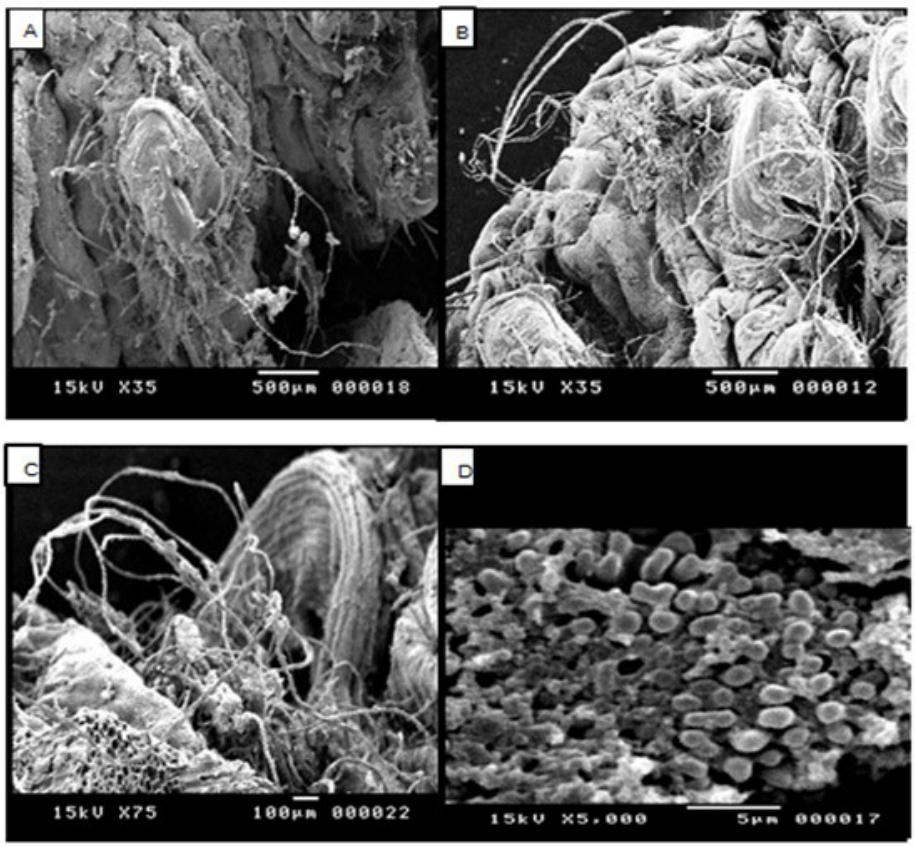

Figure 2. SEM micrographs of germination, penetration of fungi (A-C) and bacteria (D) on *Bombyx mori* larval integument.

formation of silver nanoparticles in the reaction mixture as presented in Figure 3A.

On the contrary, the formation of opalescent white color suspension was used as an indicator to confirm the synthesis of CHNPs in the reaction mixture as describe by Taha et al. (2020). Figure 3B expressed the synthesis process and color of CHNPs solution.

3.3. Particle size and morphological properties of nanoparticles

Transmission electron microscope imaging exhibited the morphological properties and surface appearance of silver nanoparticles which have a nearly spherical shape and smooth surface. As illustrated in Figure 4, it was perceive that the prepared nanoparticles were an average particle size of 18.7-26.0 nm. Furthermore, these nanoparticles are well dispersed with no sign of aggregation. Also, as illustrated in Figure 5 the imaging of TEM showed the morphological properties and surface appearance of nanoparticles, which have nearly spherical shape, smooth surface. Also, TEM analysis of chitosan nanoparticles revealed uniform size distribution in nanometer range. The average particle size of CHNPs was ranged from 18.8 to 21.8 nm.

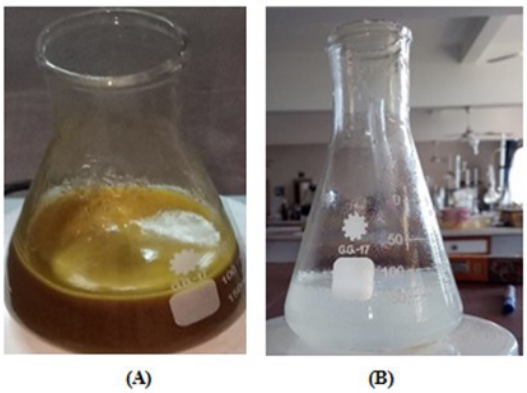

Figure 3. Final dispersion formed after reduction (A) silver and (B) chitosan.

3.4. Antimicrobial activity of silver and chitosan nanoparticles against bacterial andfungal pathogens

Results illustrated in Tables 3 and 4, showed the highest activity among nanoparticles tested against all pathogenic bacteria and fungi which have been isolated and identified in our study were recorded by chitosan at 100 µg/ml in series gram-positive bacteria *S. aureus* (47.5 mm) and *Enterococcus faecalis* (49 mm) and in series fungi against *A. flavus* and *A. tamarii* were recorded 37.5 and 36 mm respectively, and *Beauveria bassiana* (30 mm) (Table 3). Whereas, silver nanoparticle exhibited moderate antibacterial activity 38.5 and 33 mm against *S. aureus* and *E. faecalis*, and antifungal activity against *A. flavus, A. tamari and B.bassiana* (26.5, 23.5 and 27 mm) (Table 4).

4. Discussion

In general silk yield by silkworms are greatly affected by various diseases. White muscardine disease due to *B. bassiana* causes a cocoon yield loss upto 30% almost throughout the year (Isaiarasu et al., 2011). These decreases in weight may be causes malty or pulley entomopathogencis, and agreement with Seema et al. (2019) reported that, *B. bassiana* infection influenced the growth and development of silkworm larvae and ultimately the economical cocoon characters like matured larval

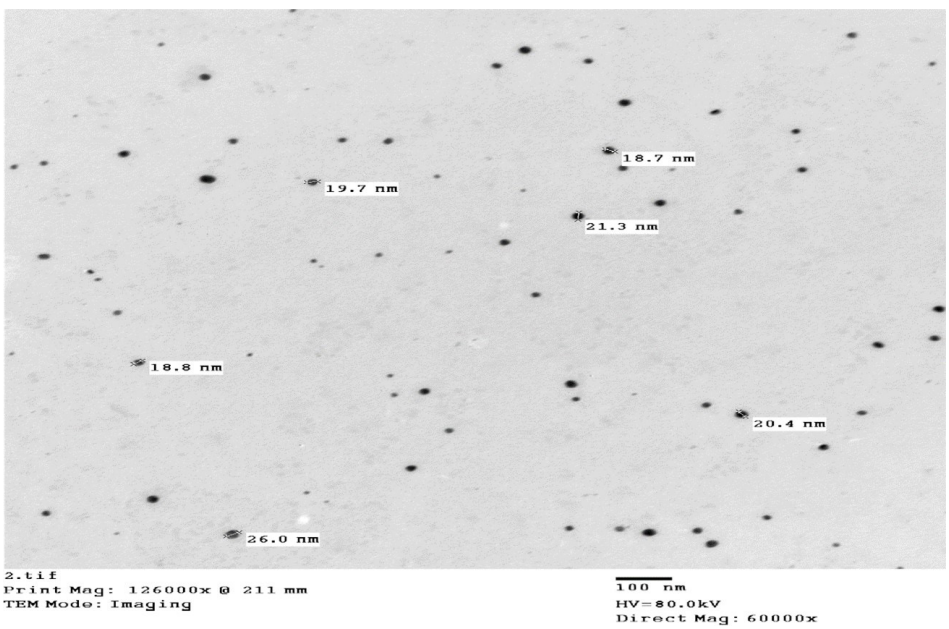

Figure 4. Transmission electron microscopy micrograph of silver nanoparticles.

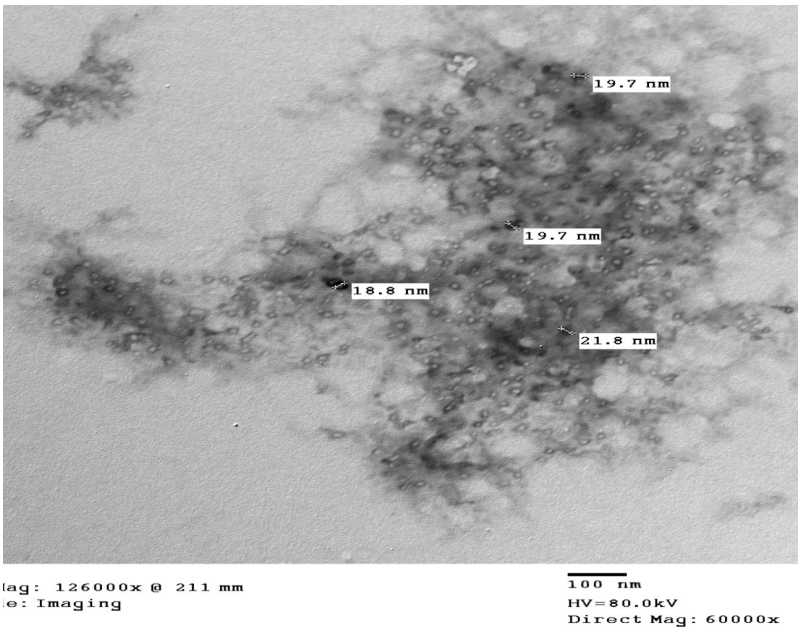

Figure 5. TEM micrograph of chitosan nanoparticles prepared by ionic gelation method.

Table 3. Antimicrobial activity of silver nanoparticles (µl) with different concentrations against fungal and bacterial isolates.

Silver nanoparticles (µl)	Pathogenic isolates				
	Fungi			Bacteria	
	Aspergillus flavus	*Aspergillus tamarii*	*Beauveria bassiana*	*Enterococcus faecalis*	*Staphylococcus aureus*
	Mean of inhibition zones (mm) ± SE				
25 µg/ml	11.5 ± 0.28^{d}	10.8 ± 0.20^{e}	13.0 ± 0.00^{c}	19.0 ± 0.00^{a}	14.6 ± 0.03^{b}
50 µg/ml	17.5 ± 0.28^{d}	15.5 ± 0.25^{e}	19.7 ± 0.00^{c}	29.0 ± 0.00^{a}	23.0 ± 0.00^{b}
75 µg/ml	25.5 ± 0.50^{c}	23.3 ± 0.13^{d}	26.0 ± 0.00^{c}	36.5 ± 0.76^{a}	32.0 ± 0.00^{b}
100 µg/ml	26.5 ± 0.50^{c}	23.5 ± 0.50^{d}	27.0 ± 0.00^{c}	38.5 ± 0.28^{a}	33.0 ± 0.00^{b}

SE: Standard error. Means, in the same row, followed by the same letter are not significantly different using the LSD test at P=0.05.

Table 4. Antimicrobial activity of chitosan nanoparticles (µl) with different concentrations against fungal and bacterial isolates.

Chitosan nanoparticles (µl)	Pathogenic isolates				
	Fungi			Bacteria	
	Aspergillus flavus	*Aspergillus tamarii*	*Beauveria bassiana*	*Enterococcus faecalis*	*Staphylococcus aureus*
	Mean of inhibition zones (mm) ± SE				
25 µg/ml	15.0 ± 0.57d	17.0 ± 0.00c	10.0 ± 0.00e	24.0 ± 0.00b	25.5 ± 0.15a
50 µg/ml	24.5 ± 0.00d	27.3 ± 0.00c	19.0 ± 0.00e	30.5 ± 0.28b	32.0 ± 0.11a
75 µg/ml	34.5 ± 0.57d	36.3 ± 0.60c	27.0 ± 0.00e	42.5 ± 0.11b	44.0 ± 0.00 a
100 µg/ml	37.5 ± 0.28c	36.0 ± 0.00d	30.0 ± 0.00e	47.5 ± 0.23b	49.0 ± 0.00 a

SE: Standard error. Means, in the same row, followed by the same letter are not significantly different using the LSD test at P=0.05.

weight, cocoon weight, shell weight, shell percentage, filament length, non-breakable filament length, number of breaks and denier .

Based on the acquired results, total of 7 bacterial isolates were successfully isolated from silkworm larvae. These isolates were classified into two species *S. aureus* and *E.*

faecalis. Plentiful other studies have also demonstrated gram positive and negative bacteria isolated from mulberry silkworm (Abou El-Ela et al., 2015; El-Adly et al., 2018). However, three fungal isolates were isolated and identified as *A. flavus*, *A. tamarii* and *B. bassiana*. Similar studies domnastred many *Aspergillus* spp. isolated from outer and inner silkworm (El-Adly et al., 2018).

Transmission electron microscope imaging demonstrated the morphological properties and surface appearance of silver nanoparticles which have a nearly spherical shape and smooth surface. It was noticed that the prepared nanoparticles were in average particle size of 18.7 - 26.0 nm. Moreover, these nanoparticles are well dispersed with no sign of aggregation. Importntly various reports have been employed for the synthesis of silver nanoparticles for its beneficial applications. Silver nanoparticle were synthesized and characterized in ambient conditions with an average size of 16 nm (Surya et al., 2016). Basically, the shape of the band was symmetrical, suggesting uniform dispersal of cubic to spherical shape nanoparticles, indicating that the silver nanoparticles are partially cubic to spherical crystalline in nature (Govindaraju et al., 2009; Rajeshkumar et al., 2014). Besides, TEM analysis of chitosan nanoparticles in our data showed uniform size distribution in nanometer range. Plus, the average particle size of CHNPs was ranged from 18.8 to 21.8 nm. SEM has been used as an efficient technique for silver nanoparticles characterization (Anandalakshmi et al., 2016).

In present study, we reported that, highest activity among tested nanoparticles against all pathogenic microorganism was counted by chitosan at 100 µg/ml in series gram-positive bacteria *S. aureus* and *E. faecalis*. In agreement with the findings of Van Toan et al. (2013) *S. aureus* was inhibited with chitosan. In disagreement with Younes et al. (2014), *E. faecalis* was resistant strain to chitosan. Antifungal activity agreed with Ing et al. (2012), the chitosan nanoparticles were detected to be natural antifungal agents.

In this interim, performance of silver nanoparticles depended on both dosage and particle size. Metal nanoparticles diosplayed a large surface to volume ratio and exhibit antimicrobial properties due to their ability to interact with cellular membranes through disruption of cell wall structure (Ullah et al., 2017; Abbaszadegan et al., 2015). Particularly, silver is known for its strong toxicity against a wide range of microbes including bacteria and fungi (Narayanan and Park 2014). In addition, silver nanoparticle in present study exhibited moderate antibacterial activity against *S. aureus* and *E. faecalis*, and antifungal activity against *A. flavus, A. tamarii* and *B. bassiana*. Similar result recorded with initial study reported that, silver nanoparticles is known for its strong toxicity against a wide range of microbes including bacteria and fungi (Narayanan and Park, 2014). Based on Li et al. (2016) silver nanoparticle affect fungus cells by attacking their membranes, thus disrupting the membrane potential. The biologically synthesised *P. hornemanni* mediated silver nanoparticles prepared by direct reduction method showed antifungal activity against silkworm muscardine pathogens of *B. bassiana* and *M. anisopliae* using agar well method. Furthermore, the anti-fungal activity of AgNPs depends on the nature and type of fungus along with size of AgNPs and also closely associated with the formation of pits in the cell wall of microorganism (Shafaghat, 2015). Interestingly, the antimicrobial activity of Solanum torvum mediated silver nanoparticles was performed against bacteria pathogens (*S. aureus, B. rhizoids, E. coli and P. aeruginosa*) of silkworm *B. mori* by Govindaraju et al. (2010).

5. Conclusion

To sum up, our results supported the hypothesis that the silver and chitosan nanoparticles may be prepared in a simple, eco-friendly and cost-effective manner and are suitable for formulation of unique technique of bacterial and fungal controls. The biosynthesized silver and chitosan nanoparticles suggested low concentration of a greater significance in the prevention of silkworm pathogens during rearing process in the future. However, further investigation on their acute and chronic toxicity are highly required.

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