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A Comparative Nano Biotechnological Study: pH Dependent Biosynthesized Silver Nanoparticles by *Aspergillus niger*, *Aspergillus flavus* and *Streptomyces fradiae*

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Abstract: ***Silver nanoparticles were biosynthesized by A. niger, A. flavus and S. fradiae with extracellular process. Maximum biosynthesized AgNPs was recorded by S. fradiae at pH9. Size and shape of biosynthesized AgNPs were examined using transmission electron microscope (TEM). Spherical nanoparticle was the most common shape at pH 9 with smallest size of 21.34 nm. The antibacterial activity of AgNPs was examined against E. coli, Pseudomonas sp. and Klebsiella sp. S. fradiae nanoparticles showed maximum antibacterial activity against E. coli and Pseudomonas sp. While AgNPs biosynthesized by A. niger exhibited greater inhibition zone diameter toward the growth of Klebseilla sp. In vitro studying the effect of AgNPs on hemolysis of human red blood cells has revealed a very low hemolytic activity, where S. fradiae considered the most little hemolytic one (5.65%). AgNPs have a potential antibacterial with negligible hemolytic activity and low cost, so it can be used safely as effective antimicrobial agent.***

Keywords: Ag-NPs, antibacterial activity, hemolytic activity, Time and pH dependent, *Aspergillus niger*, *Aspergillus flavus* and *Streptomyces fradiae*

1. Introduction

Nanoscience and nanotechnology involves the synthesis and application of nanoscale materials and structures usually in the range of 1 to 100 nm [1]. Nanotechnology is rapidly finding expanding applications in various areas of industry (e.g., semiconductors, computers and engineering), environmental sciences (e.g., water purification, pathogen detection and identification), food sciences (e.g., sterilization and prolonged shelf-life), biology (in vitro diagnostics) and medicine (drug delivery, therapeutic agent and imaging) [2]. Biological synthesis of nanoparticles is an alternative method of chemical and physical methods; various organisms are used for nanoparticles synthesis because of their effectiveness and flexible biological factors [3, 4]. In addition, the chemical routes lead to particles with a strong hydrophobic surface that need further special medication in order to overcome the resulting problems for application [5].

Biological nanoparticle synthesis (green synthesis) often yields a more consistent size distribution pattern than other methods due to direct stabilization of the nanoparticles by proteins involved in the synthesis process [6].

Silver nanoparticles are known to synthesize by bacteria [7], yeast [8], fungi [9] and actinomycetes [10]. Metal nanoparticles are having considerable interest in the fast-developing area of nanotechnology because of its applications [11]. Currently various types of metal inorganic nanoparticles viz. zinc, titanium, magnesium, copper, gold, alginate, ferrous and silver have been synthesized using various techniques [12,13].

Because of AgNPs have distinctive properties such as optoelectronic, physicochemical, good electrical conductivity, chemical stability and antibacterial activities, AgNPs are gaining more interest and most widely used [14].

Bacterial infection is a serious public safety issue associated with significant mortality and health-care costs [15]. Antimicrobial agents such as tetracycline and ampicillin are commonly used to kill or slow the growth of bacteria [16, 17]. However, because of broad and often inappropriate use, bacterial resistance to antibacterial drugs may occur. Recently, nanomaterials (NMs) have emerged as novel antimicrobial agent [18, 19].

Several classes of antimicrobial nanoparticles (NPs) have proven effective in controlling infection both in vitro and in vivo, including those which are antibiotic-resistant [20, 21]. Compared with conventional antibiotics, antimicrobial NPs have several characteristic advantages, including low acute toxicity, low cost, long-term stability, and easily modifiable surfaces. However, the benefits of nanotechnology are often tempered by concerns about the safety of these materials. Specific areas of concern include hemolytic activity for human red blood cells [22], carcinogenicity, teratogenicity, developmental toxicity, cell toxicity and interaction with components of the immune system [2].

So, our study aims to examine and compare different microbes to synthesize silver nanoparticles with biotechnologically important properties including antagonistic properties against some pathogenic bacteria. Due to the future directions of commercial nanoparticle applications in medicine are geared towards drug delivery and as antimicrobial agent, the hemolytic activity on

human blood cells was taken in consideration in our search.

2. Materials and Methods

2.1 Media Used

2.1.1 Nutrient agar medium [23]

2.1.2 Dox's medium [24]

2.1.3 Starch nitrate medium [25]

2.2 Microorganisms used

E. coli, *klebseilla* sp., *Pseudomonas* sp., *Aspergillus niger* and *Aspergillus flavus* were purchased from Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University Cairo, Egypt. *Streptomyces fradiae* was previously isolated from contaminated site in Hellwan City, Cairo, Egypt. The isolate identification was done using 16S rRNA sequencing which revealed high identity, 95 % with *Streptomyces fradiae* strain HBUM174185 (Gen Bank accession number FJ486352.1) [26].

2.3 Extracellular silver nanoparticles biosynthesis

The fungal strains were inoculated in Dox's broth medium and incubated in orbital shaker at 28 °C for 5 days, while *Streptomyces fradia*e was inoculated in starch nitrate broth medium. After that, the cultures were centrifuged at 10.000 rpm for 10 minutes, and then the obtained supernatant for each organism was collected. Only 1mM of silver nitrate was added to each culture supernatant for the synthesis of the silver nanoparticles, then the flasks were incubated at room temperature in orbital shaker for different time intervals (1, 3, 5, 7 and 9 days). The variation of culture supernatant color after the addition of silver nitrate was visually observed. Periodically, aliquots of the reaction solutions were removed and the UV absorption spectrophotometer reading from 300-700 nm was recorded.

2.4 Effect of different pH values on the extracellular synthesis of silver nanoparticles

Different pH values 5 and 9 using 1N hydrochloric acid and 1N sodium hydroxide were evaluated on the extracellular synthesis of AgNPs at the end of incubation period .The absorbance using UV- spectrophotometer was applied after 3 days for *A. niger*, *A. flavus* and 5 days for *S. fradiae*.

2.5 Antibacterial activity of biosynthesized silver nanoparticles

The antibacterial activity of biosynthesized AgNPs by *A. niger*, *A. flavus* and *S. fradiae* was carried out at optimum pH for nanoparticle biosynthesize against some pathogenic bacteria like *E.coli, Pseudomonas* sp. and *Klebsiella* sp. using the agar well diffusion method [27]. A loop full of bacterium was taken from the stock culture and dissolved in 1ml nutrient broth and kept for 12 hrs. The cultures of bacteria were sub cultured into nutrient agar. Agar medium plates were prepared for each test organism. Five wells were made on nutrient agar plates with help of gel puncture. Using a micro pipette, 0.05, 0.1, 0.15 and 0.2 ml of synthesized AgNPs were inoculated into the wells leaving one pipetted with sterile distilled water as control and then the plates were incubated at 37 °C for 24hrs. The plates were observed for the formation of inhibition zones and their diameter (mm) was determined.

2.6 Characterization of biosynthesized silver nanoparticles

2.6.1 UV-Visible spectra analysis

The silver nanoparticles were characterized by UV-spectroscopy, one of the most widely used techniques for characterization of silver nanoparticles [28]. The UV absorbance spectra (300 to 700 nm) were taken at various time intervals and different pH values by using spectrophotometer (GBC Equipment Pty Ltd. Australia).

2.6.2 Transmission electron microscope (TEM)

TEM was used to study the morphology and size of AgNPs. For such purpose, an aliquot of aqueous suspension of AgNPs was transferred onto a carbon coated copper grid and allowed to be dried [29]. The grid was then scanned using JEOL-JEM 1010, TEM operated at a voltage of 80 kV.

2.6.3 X-ray diffraction analysis (XRD)

XRD measurements of the dried powder of AgNPs were done by diffractometer (Model-D8 Advance, made in BRUKER ،Germany) at 40 kV/20m in the range of 10–70 using the continuous scanning 2Θ mode to check the formation, purity, and stability of the silver nanoparticles [30].

2.7 Human erythrocyte preparation and hemolysis assay

Hemolysis induced by AgNPs was tested according to [31]. Fresh blood sample from a healthy volunteer (25 years old) was drawn from the vein into tube containing ethylene diamine tetra acetic acid (EDTA) and immediately (within 30 min of collection) centrifuged (RCF 3000g, 10 min, 4 °C) to remove serum. Fresh RBCs were then washed thrice with sterile isotonic Phosphate-buffered saline (PBS). Following the last wash, the RBCs were diluted with sterile isotonic PBS to obtain a RBCs stock suspension (4 vol. % blood cells). The RBCs (100 µl) was added to 0.9 ml of AgNPs solution in 1.5 ml vials. After 1 h of incubation at 37 °C, each of the mixtures was centrifuged at RCF 1000g for 10 min. Hemolysis activity was determined by measuring hemoglobin absorption at 576 nm (OD 576) in the supernatant by mean of a spectrophotometer. Sterile isotonic PBS was used as a reference for 0% hemolysis. One hundred percent hemolysis was measured by adding ultrapure water to the

RBCs stock suspension. The results of the tested materials in relation to the reference materials can be used to evaluate the hemolytic activity according to the following equation.

Hemolysis (%) = [(OD $_{576AgNPs}$ - OD $_{576blank}$) / (OD $_{576\ ultra-pure\ water}$ - OD $_{576\ blank}$)] ×100

2.8 Statistical Analysis

The size of biosynthesized AgNPs was analyzed using Image Analysis docu software program.

3. Results and Discussion

There is a new trend nowadays for using metallic nanoparticles due to their antimicrobial activities. Different physical and chemical methods are used for synthesizing them, but it was found that the most simple and cheapest way of metallic nanoparticles formation is from microbial origin [7].

Silver nanoparticles have attractive physicochemical properties; so it plays an important role in the area of biology and medicine. For this reason the present study provided an ecological cost effective method for the extracellular synthesis of AgNPs using cell free extracts of *A. niger*, *A. flavus* and *S. fradiae*.

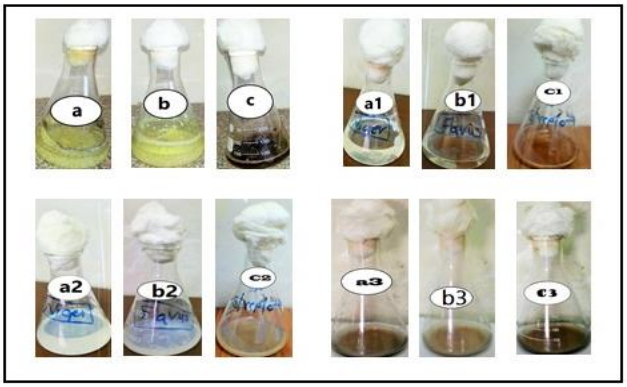
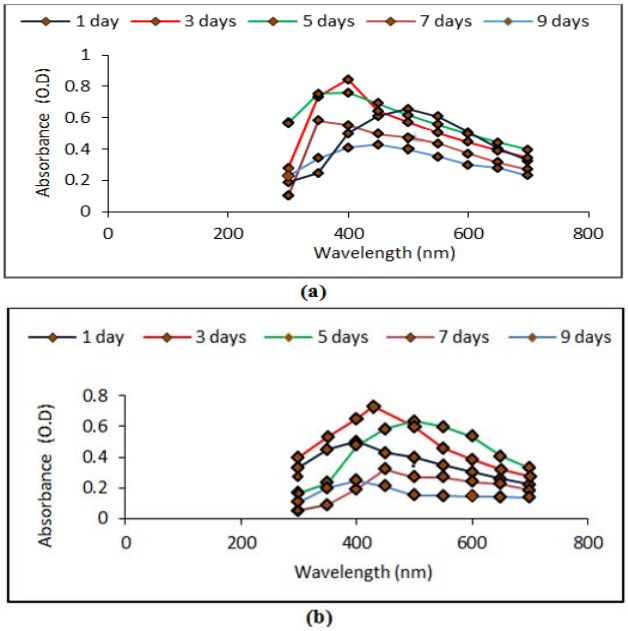


Figure1: Biosynthesis of silver nanoparticles, microbial cultures without the addition of silver nitrate (a, b and c), microbial filtrate before the addition of silver nitrate as a control (a1, b1 and c1), after immediately addition of silver nitrate to microbial filtrate (a2, b2 and c2), formation of nanoparticles (a3, b3 and c3) after 3 days incubation for *A. niger*, *A. flavus* and 5days for *S. fradiae*. Where: a= *A. niger*, b= *A. flavus* and c= *S. fradiae*

Figure 1 (a, b, and c) shows the microbial cultures under investigation. The formation of AgNPs was visually observed for the change in aqueous solution color. Before the addition of silver nitrate, there was no change in filtrate color, figure 1(a1, b1 and c1), after addition of silver nitrate, the microbial filtrate culture color was changed to white precipitate which indicates the initial formation of AgNPs, figure 1 (a2, b2 and c2) and after 3 days of reaction for *A. niger* and *A. flavus* and 5 days for *S. fradiae*, the visual maximum brown color of the solution was observed, figure 1(a3, b3 and c3). The darkening of the solution was increased with time of the reaction and is related to the reduction of silver ions by reductase enzyme present in the microbes [32]. Silver nanocrystals was synthesized using *Bacillus licheniformis*, the similar color changes during the formation of silver nanoparticles were obtained [33]. Also, Chitra and Annadurai [7] obtained a similar color changes during the formation of silver nanoparticles by using *Bacillus brevis* cell filtrate.

The UV absorption spectral studies were carried out to confirm the formation of AgNPs using *A. niger*, *A. flavus* and *S. fradiae.* Figure 2 (a, b and c) shows UV-spectrophotometer absorption of AgNPs prepared at different times intervals (1, 3, 5, 7 and 9 days), it is clear that, by increasing the incubation period of reaction there is increase in AgNPs formation**.** Ishida et al. [32] showed that, AgNPs production increased in a concentration dependent way up to 1 mM silver nitrate with increasing the reaction time until 30 days of reaction.

The maximum absorption peak is found at 400, 430 and 400 nm for *A. niger*, *A. flavus* and *S. fradiae*, respectively and occurred at 3 days for *A.niger* and *A. flavus* and 5 days for *S. fradiae* and it was found from the results that, the maximum biosynthesis of nanoparticles was recorded by *S. fradiae*. The synthesis of nanoparticles by microorganisms can produce more compatible particles than that of chemical synthesis which associated with the presence of some toxic chemical compounds adsorbed on the surface that could have adverse effects in medical applications, and by compared to bacteria and fungi, the actinomycetes can secrete much higher amounts of proteins therefore increasing the productivity of biosynthetic nanoparticles [34]. Prakash et al. [35] synthesized silver nanoparticles using *Bacillus megaterium*; they obtained the maximum absorption peak at 435 nm. In addition, Ishida et al. [32] reported the highest biosynthesis of AgNPs by *F. oxysporum* at absorbance between 340-560 nm, with maximum peak at 440 nm, for all $AgNO_3$ concentration used and reaction time tested.



(a)

(b)

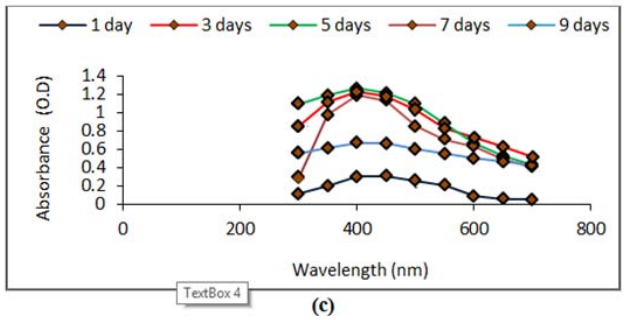
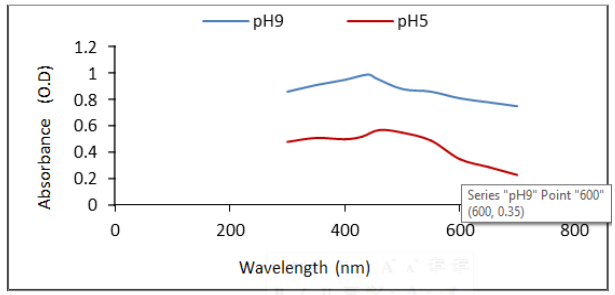
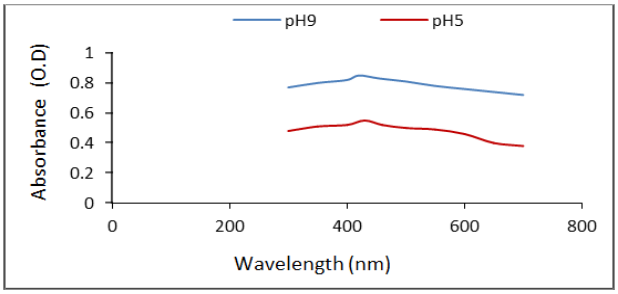


Figure 2: UV absorption spectrum of silver nanoparticles synthesized by *A. niger* (a), *A. flavus* (b) and *S. fradiae* (c)

The effect of pH on the synthesis of AgNPs by *A. niger*, *A. flavus* and *S. fradiae* indicated that, the higher amount of AgNPs formation occurred at pH9, where *S. fradiae* recorded the highest production. The absorption peak occurred at 440, 420, and 410 (nm) for *A. niger*, *A. flavus* and *S. fradiae*, respectively at pH9, while at pH 5, the absorption peak occurred at 460, 430 and 420 (nm) for *A. niger*, *A. flavus*, and *S. fradiae*, respectively as illustrated in figure 3 (a, b and c). The color variation of the medium indicated that, increased amount of silver nanoparticles formation at pH 9 for the three tested nanoparticles producers especially for *S. fradiae* exhibiting the most dark color followed by *A. niger* and *A. flavus.*

The pH of the medium plays an important role in the synthesis of controlled shape and size of nanoparticles [7]. So it is very important in the present investigation to examine the effect of medium pH used under study (5 and 9) on size and shape of biosynthesized nanoparticles by using TEM.





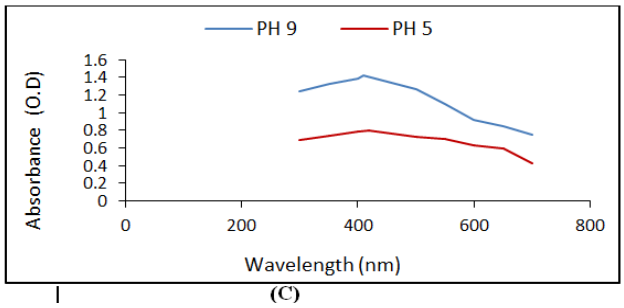
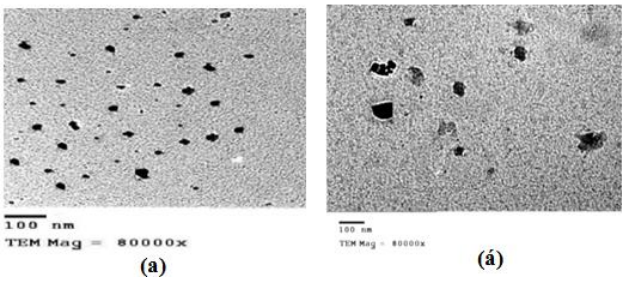


Figure 3: UV-spectrophotometer absorption showing the effect of pH on the synthesis of silver nanoparticles by *A. niger* (a), *A. flavus* (b) and *S. fradiae* (c)

Figure 4 (a, b and c) shows the TEM images of the spherical shaped biosynthesized silver nanoparticles by *A. niger* (a), *A. flavus* (b) and *S. fradiae* (c) at pH 9 which produced at 410-440 nm, so this range may play a role in the formation of spherical shape NPs as reported by Nayak et al. [36] who stated that, the absorption band at 420 nm showed the spherical shaped nanoparticles. Also Ishida et al. [32] exhibited spherical electron-dense AgNPs observed by TEM with an absorption peak at 440 nm. It is cleared from the figure the most spherical shape in a regular uniform manner was obtained by *S. fradiae* biosynthesized nanoparticles.

Figure 4 (á, ḃ and ć) shows nanoparticles shapes synthesized at pH 5 by *A. niger* (á), *A. flavus* (ḃ) and *S. fradiae* (ć).The figure shows a different shape of nanoparticles biosynthesized by *A. niger* and *A. flavus* at 460 and 430 nm, respectively. Square shaped nanoparticles can be observed for *A. flavus*. In accordance, Nayak et al. [36] stated different shapes of nanoparticles at 480 nm, whereas Chitra and Annadurai [7] reported the formation of hexagonal shapes NPs at 460 nm with nanoparticles size in the range of 60–110 nm.

Data reported in table (1) shows the statistical analysis of biosynthesized AgNPs size obtained by image analysis software program; the mean of nanoparticles size produced at pH 9 by *A. niger*, *A. flavus* and *S. fradiae* was 23.62, 24.05 and 21.34 nm, respectively. This indicated that, the smallest particle size was recorded by *S. fradiae* NPs which confirm the obtained previous result associated with the highest UV absorbance for this organism at pH 9 (i.e. the highest amount of silver nanoparticles biosynthesis was related to the smallest particle size). This is in accordance with Deepak et al. [37] who stated that, at high pH a fast nucleation process occurred because of the accessibility of -OH ions; thus high amount of small size particles formed.



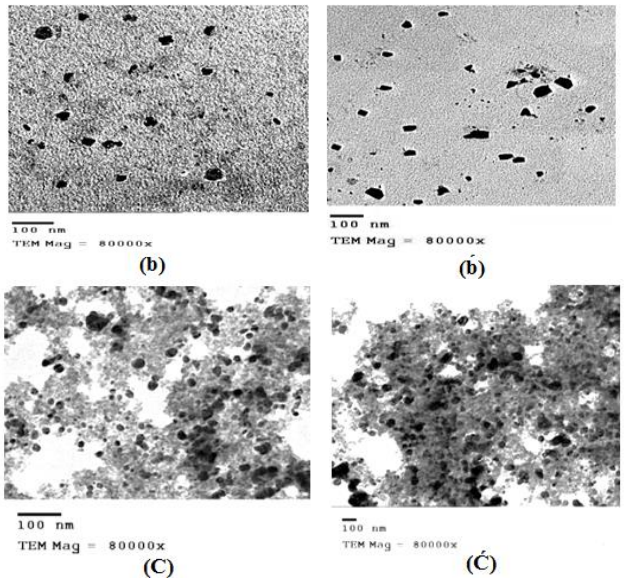


Figure 4: TEM image of the biosynthesized silver nanoparticles by *A. niger* (a, á), *A. flavus* (b, b́) and *S. fradiae* (c, ć) at pH 9 and 5 respectively

Table 1: Statistical analysis of biosynthesized AgNPs size by *A. niger*, *A. flavus* and *S. fradiae* at pH 9

Organism	*A. niger*	*A. flavus*	*S. fradiae*
Statistical Function	*NPs diameter (nm)*		
Count	10	10	10
Mean	23.62	24.05	**21.34**
Minimum	12.33	15.76	14.71
Maximum	36.23	41.11	38.23
Standard Deviation	8.20	7.36	6.39

Table (2) shows statistical analysis of biosynthesized AgNPs size at pH 5 by *A. niger*, *A. flavus* and *S. fradiae*. The mean of nanoparticles size was 63.64, 75.00, and 81.34 nm, respectively. From the result we found *A.niger* nanoparticles size represented the smallest particle at pH 5.

Table 2: Statistical analysis of biosynthesized AgNPs size by *A. niger*, *A. flavus* and *S. fradiae* at pH 5

Organism	*A. niger*	*A. flavus*	*S. fradiae*
Statistical Function	*NPs diameter (nm)*		
Count	10	10	10
Mean	**63.64**	75.00	81.34
Minimum	52.00	65.74	73.71
Maximum	76.55	91.44	108.22
Standard Deviation	6.50	5.36	5.79

Historically, silver has been well known as disinfectant for long years. The use of silver compounds is reduced due to some limitations; recently metallic silver in the form of silver nanoparticles shows well antibacterial activity against many microorganisms [38].

Small sized nanoparticles showed more antibacterial activity than large size particles because small sized particles affect a large surface area of the bacteria [39], thus we here in this research studying the antibacterial activity at pH 9 which responsible for production of small sized nanoparticles. Figure 5 (a, b and c) showing that, well diffusion method was used to determine the antibacterial activity of biosynthesized AgNPs by *A. niger* (a), *A. flavus* (b) and *S. fradiae* (c) at pH 9 against *E.coli, Pseudomonas* sp. and *Klebsiella* sp. The antibacterial activity of AgNPs was indicated by the formation of inhibition zone (mm).

From table (3) and figure (5 c) it was shown that, the antibacterial activities obtained by *S. fradiae* biosynthesized AgNPs against *E. coli* showed higher inhibition zone diameter (27 and 25mm) at two concentrations (0.2 and 0.15 ml), respectively and by increasing the concentration of the biosynthesized silver nanoparticles the inhibition zone was increased.

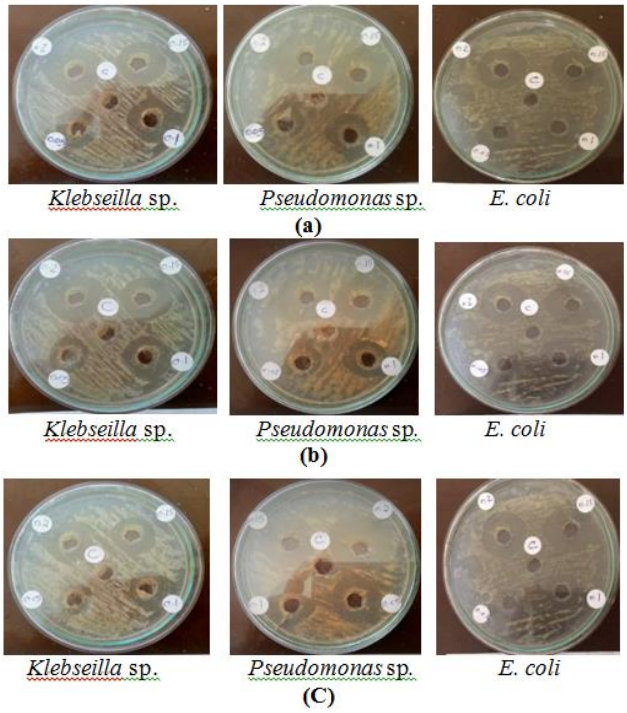


Figure 5: Antibacterial activity of AgNPs synthesized by *A.niger* (a), *A. flavus* (b) and *S. fradiea* (c) at pH 9 against *E.coli, Pseudomonas* sp. and *Klebsiella* sp.

Table 3: Average inhibition zone diameter for AgNPs biosynthesized by *S. fradiea, A. flavus* and *A. niger* against *E. coli*

Organism	*NPs concentrations (ml)*				
	Control	0.05	0.1	0.15	0.2
	Average Inhibition zone diameter (mm)				
S. fradiae	N.A.	15	17	**25**	**27**
A. flavus	N.A.	15	19	23	26
A. niger	N.A.	15	16	19	24

Abbreviation: NA, Not Appear

From table (4) and figure (5 c), the antibacterial activity obtained by *S. fradiae* AgNPs against *Pseudomonas* sp. showed higher inhibition zone diameter (39, 36 ,30 and 28 mm) at all tested concentrations (0.2, 0.15, 0.1, and 0.05 ml), respectively than that obtained by *A. flavus* and *A. niger*.

In view of the finding of other investigators stated that, the initial pH of the medium, surface area and shape plays

important role in the antibacterial efficiency of AgNPs [36]. The present study confirmed such observation by the highest antibacterial efficiency that obtained by the smallest spherical nanoparticles synthesized at pH 9 by *S. fradiea* against *E.coli* and *Pseudomonas* sp.

Table 4: Average inhibition zone diameter for AgNPs biosynthesized by *S. fradiae*, *A. flavus* and *A. niger* against *Pseudomonas* sp.

Organism	*NPs concentrations (ml)*				
	Control	0.05	0.1	0.15	0.2
	Average Inhibition zone diameter (mm)				
S. fradiae	N.A.	**28**	**30**	**36**	**39**
A. flavus	N.A.	16	19	23	34
A. niger	N.A.	16	20	25	34

Abbreviation: NA, Not Appear

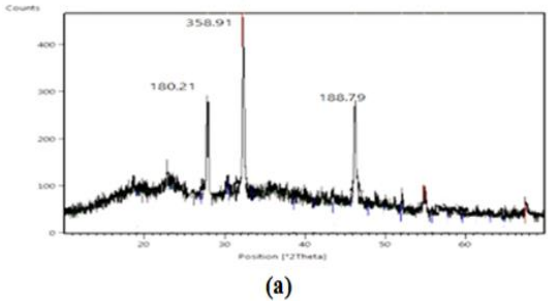
From table (5) and figure (5 a) it was shown that the antibacterial activity obtained by *A. niger* biosynthesized AgNPs against *klebseilla* sp. showed higher inhibition zone diameter (32, 30, 27 and 24 mm) at all used nanoparticles concentrations (0.2, 0.15, 0.1 and 0.05 ml), respectively than that obtained by *A. flavus and S. fradiae* .

Table 5: Average inhibition zone diameter for AgNPs biosynthesized by *S. fradiae*, *A. flavus* and *A. niger* against *Klebseilla* sp.

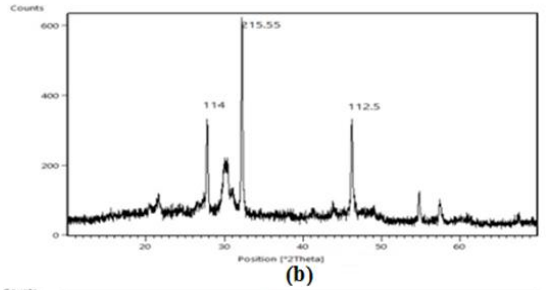
Organism	*NPs concentrations (ml)*				
	Control	0.05	0.1	0.15	0.2
	Average Inhibition zone diameter (mm)				
S. fradiae	N.A.	14	20	22	25
A. flavus	N.A.	20	25	29	31
A. niger	N.A.	**24**	**27**	**30**	**32**

Abbreviation: NA, Not Appear

Figure 6 (a, b and c) shows the XRD pattern of AgNPs synthesized by *A. niger* (a), *A. flavus* (b), and *S. fradiae* (c). The XRD pattern shows three clear peaks in the whole spectrum of 2 Θ values, ranging from 10 to 70. The peaks at 2 Θ value of 28° corresponding to 180.21, 114 and 108.76 planes of silver for *A. niger*, *A. flavus* and *S. fradiae*, respectively. The peaks at 33° value corresponding to 358.91 , 215.55 and 204.18 silver planes for *A. niger*, *A. flavus* and *S. fradiae*, respectively, and the peaks at 46° value corresponding to 188.79, 112.5 and 100.25 planes for *A. niger*, *A. flavus* and *S. fradiae*, respectively. These planes confirmed the face centered crystalline structure of nano silver [40].



(a)



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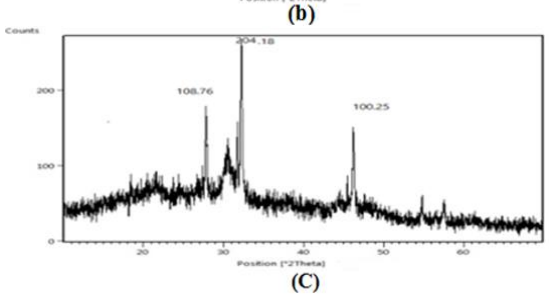

(C)

Figure 6: X-ray diffraction pattern of AgNPs synthesized by *A.niger* (a), *A.flavus* (b) *and S. fradiae* (c)

Hemolysis is a process of destruction of erythrocytes with the escape of hemoglobin in the environment. The wide use of a number of antibacterial preparations is limited as a result of their high hemolytic activity. As a consequence, when developing new pharmaceutical preparations, the test of the hemolytic activity is a necessary together with the check of their antibacterial activity [41]. Therefore, an in vitro hemolysis assay was performed to verify satisfactory biocompatibility of Ag NPs.

The hemolysis assay was performed in defibrinated human blood to test the ability of fragile red blood cells (RBCs) to withstand swelling in contact with AgNPs solution. Data presented in table (6) and figure (7) indicated that, all samples of NPs under investigation demonstrate a low hemolytic activities (17.53, 12.9 and 5.65%) for *A. flavus*, *A. niger* and *S. fradiae*, respectively. In addition *S. fradiae* AgNPs exhibited the lowest hemolytic activity (5.65%). It is very clear from the results the presence of very strong relationship between the highly NPs biosynthesis by *S. fradiae*, lowest particle size, highly antimicrobial activities against *E.coli* and *Pseudomonas* sp. in addition to lowest hemolytic activity. *A. niger* came after *S. fradiae* in consideration to its high nanoparticles biosynthesis with small size, high antimicrobial activity and low hemolytic activity (12.9 %). The NPs biosynthesized by *S. fradiae*. was promising to use as antibacterial agent considering its safety (low hemolytic activity, 5.56 %).

Table 6: Hemolytic activities of biosynthesized AgNPs by *A. niger*, *A. flavus* and *S. fradiae*

NPs producer	*hemoglobin O.D.*	*% hemolysis*
A. niger	0.023	12.9
A. flavus	0.032	17.53
S. fradiae	**0.011**	**5.65**
+ Control	0.177	100

Hemolysis activity was determined by measuring hemoglobin absorption at 576 nm (OD_{576})

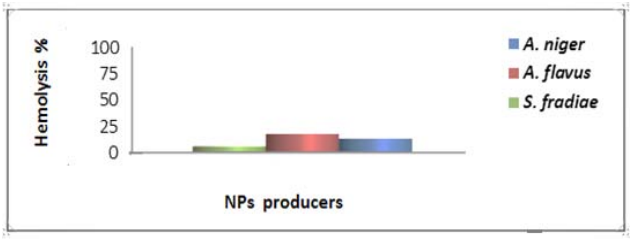

Figure 7: Comparison of AgNPs hemolytic activities biosynthesized by *S.fradiae, A. niger* and *A. flavus*

4. Conclusion

Spherical shaped silver nanoparticles were biosynthesized using *Aspergillus niger, Aspergillus flavus* and *Streptomyces fradiae* by extracellular method with maximum production by *Streptomyces fradiae* at pH 9 which showed maximum antibacterial activity toward *E. coli* and *Pseudomonas* sp., in addition to their lowest hemolytic activity to human red blood cells. In the present investigation, there is a clear correlation between highest production of AgNPs by *Streptomyces fradiae*, their smallest size induced by alkaline reaction medium and their highly antimicrobial action with lowest hemolytic activity for human red blood cells.

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