Optimization of silver nanoparticles biosynthesis mediated by *Aspergillus niger* NRC1731 through application of statistical methods: enhancement and characterization

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Abstract

The fungal-mediated silver nanoparticles (AgNPs) biosynthesis optimization via the application of central composite design (CCD) response surface to develop an effective ecofriendly and inexpensive green process was the aim of the current study. Nanosilver biosynthesis using the *Aspergillus niger* NRC1731 cell-free filtrate (CFF) was studied through involving the most parameters affecting the AgNPs green synthesis and its interactions effects. The statistical optimization models showed that using 59.37% of CFF in reaction containing 1.82 mM silver nitrate for 34 h at pH 7.0 is the optimum value to optimize the AgNPs biosynthesis. The obtained AgNPs were characterized by means of electron microscopy, UV/visible spectrophotometry, energy dispersive X-ray analysis and infrared spectroscopy to elucidate its almost spherical shape with diameter of 3–20 nm. The produced AgNPs exhibited a considerable antimicrobial activity against *Bacillus mycoides*, *Escherichia coli* in addition to *Candida albicans*.

Keywords Nanosilver · *Aspergillus niger* · Biosynthesis · Optimization · Characterization

Introduction

The era of nanotechnology sciences is rapidly growing especially the division with the intention of nanomaterials synthesis and improvement. Nowadays, diverse types of metal nanomaterials are obtained by means of silver, zinc, copper, magnesium, titanium, alginate, and gold (AbdelRahim et al. 2017). Silver nanoparticles (AgNPs) have become a focus of interest lately; this is attributable to the fact that they have been incorporated into abundant customer products including microelectronics, cosmetics, nano-functionalized plastics, textiles, therapeutic products, household appliances, food packaging, wound dressings, biosensors, and medical imaging (McGillicuddy et al. 2017).

Numerous chemical and physical techniques have been expressed for AgNPs manufacturing throughout using reducing agents (like NaBH_4) to reduce silver ions in silver salts

solutions in the presence of stabilizing agents to prevent AgNPs aggregations. The chemical reducing and stabilizing agents used in the synthesis process influence clearly the physical characteristics of the synthesized AgNPs and lead to the adsorption of some toxic chemicals on the chemical synthesized AgNPs that cause limitations in biological applications (Gopinath et al. 2012; Mittal et al. 2014). At the same time, through mechanical bulk silver reduction it is possible to produce fine particles by laser ablation or lithography, but in lack of particles shape and size control. On the other hand, biological reduction (green synthesis) methods for AgNPs production using microorganisms like microalgae, cyanobacteria, proteobacteria and fungal species are attracting amplified attention because of moderate biosynthesis conditions as well as its less cost and toxic effects than other traditional methods (Othman et al. 2016; McGillicuddy et al. 2017).

Silver nanoparticles synthesis using plant extracts has been considered expansively as choice for a green method as defined by Banach and Pulit-Prociak (2017) to achieve nanoparticles through easy and efficient metal ions reduction by plant extracts active molecules. In this connection, *Pelargonium hortorum* leaf (Rivera-Rangel et al. 2018), green tea (Sökmen et al. 2017), *Rosa canina* (Pulit and Banach 2014)

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and raspberry fruit (Pulit et al. 2013) extracts were employed as reducing and stabilizing agents.

The classical strategy for optimization of AgNPs biosynthesis parameters through fixing all process parameters except one variable is time and labor consuming as well as not considering the interactions between different bioprocess factors (Othman et al. 2017). Application of statistical models to assist in electing the most important variables in the biosynthesis process and studying these parameters interactions is considered as the successful replacement to the conventional optimizing techniques (Lu et al. 2009).

Silver, titanium, zinc, and copper nanoparticles have demonstrated to be the mainly effective anti microbial nanomaterials due to its effectiveness in opposition to prokaryotic and eukaryotic microorganisms. Silver ion is very active, since it attaches to proteins of tissues and causes structural alteration in nuclear membrane and cell wall leading to cell death. Silver also combines with bacterial RNA and DNA to cause bacterial replication inhibition and denaturation (Pugazhendhi et al. 2018).

In the current study, AgNPs fungal-mediated biosynthesis using *A. niger* NRC1731 cell-free filtrate was conducted. The biosynthesis process was optimized using central composite design (CCD) surface response methodology (SRM) in order to study the interactions effect between the most effective biosynthesis parameters, and then the biosynthesized nanoparticles were characterized by means of different specialized techniques and tested for its efficacy as antimicrobial agent.

Materials and methods

Chemicals

Malt extract and Agar–Agar were provided by Fluka, France. Glucose was obtained from Koch-Light Lab., England. Silver nitrate was obtained from Sisco Research lab, India. Yeast extract, peptone, and sodium nitrate were purchased from S.D. Fine-Chem., India. Potassium chloride was obtained from MERCK, Germany.

Microorganisms

Aspergillus niger (*A. niger*) NRC1731 stock culture at Microbial Chemistry Department culture collection, National Research Centre (NRC) was propagated on modified Czapek-Dox's solid medium and preserved at 4 °C. The culture was reactivated periodically until use throughout the study. Gram-positive bacteria (*Bacillus mycoides*) and Gram-negative bacteria (*Escherichia coli*), as well as the non-filamentous fungus *Candida albicans*,

were preserved at 4 °C on nutrient agar medium and were employed to estimate the efficiency of produced nanosilver as antimicrobial agent.

AgNPs biosynthesis

Aspergillus niger NRC1731 was cultivated in 250 mL Erlenmeyer conical flasks contained 50 mL of a liquid medium consisting of (g/L): 3.0, yeast extract; 3.0, malt extract; 10.0, glucose; and 5.0, peptone, at 28 °C and 100 rpm for 3 days (Singh et al. 2014). The culture was filtered through Whatman filter paper No.1 (Whatman, England) and then the filtrate was used for the extracellular formation of silver nanoparticles. Aqueous solution of silver nitrate (AgNO_3) in distilled water was used as silver source for the process of AgNPs biosynthesis. Sets of reaction mixtures (50 mL) contained different silver nitrate concentrations, *A. niger* NRC1731 cell-free filtrate (CFF) %, under different pH values and reaction incubation times, according to the proposed statistical study, were incubated in dark at 100 rpm to avoid silver nitrate photo-activation. Temperature effect on AgNPs biosynthesis was studied via incubation of reaction mixtures at different temperature values (30–90 °C) at pH 7.0 for 1 h. Triplicate experiments were done under the same conditions, then the average values were presented.

Study design and optimization experiments

Central composite design (CCD) was applied for studying and optimizing the most effective variables that affect the fungal-mediated AgNPs formation. Four parameters (reaction time, silver nitrate concentration, CFF volume, and reaction pH value) were selected to study the statistical possibilities at five coded levels (− 2, − 1, 0, 1, 2). The number of offered experimental runs was calculated as: $2^k + 2k + x_0$, where k is the number of factors studied and x_0 is the number of center points (Lu et al. 2009). So, 30 experimental runs were performed according to CCD given in Table 1. Second-order polynomial equation was used to calculate the relationship between different factors and the resulted response as follows:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j$$

where Y is the response predicted, X_i , X_i^2 , X_j are factors in coded values; β_0 is the constant; β_i is the linear effect; β_{ii} is the squared effect and β_{ij} is the interaction effect. The obtained results analysis was done using Design Expert®, Version 7.0.0 graphical and statistical analysis software for

Table 1 Response surface central composite design and experiments

Run	A: time (h)	B: AgNO ₃ conc. (mM)	C: CFF (v/v, %)	D: pH value	Absorbance (430 nm)
1	44	1.5	43.75	6	1.710
2	34	1	28.125	7	0.816
3	14	1	28.125	7	0.310
4	24	1.5	43.75	6	1.324
5	24	1.5	75	6	1.593
6	24	1.5	43.75	6	1.351
7	4	1.5	43.75	6	0.059
8	34	2	28.125	7	0.549
9	34	1	59.375	7	1.273
10	14	2	28.125	5	0.000
11	24	1.5	43.75	6	1.522
12	24	1.5	43.75	4	0.000
13	24	1.5	12.5	6	0.000
14	24	1.5	43.75	6	1.376
15	14	2	59.375	5	0.196
16	14	2	28.125	7	0.136
17	14	1	59.375	7	0.233
18	14	1	28.125	5	0.000
19	34	1	28.125	5	0.000
20	34	2	59.375	5	0.353
21	24	0.5	43.75	6	0.631
22	24	1.5	43.75	6	1.357
23	34	1	59.375	5	0.210
24	24	1.5	43.75	6	0.938
25	34	2	59.375	7	1.794
26	14	2	59.375	7	0.885
27	24	2.5	43.75	6	1.284
28	14	1	59.375	5	0.146
29	34	2	28.125	5	0.000
30	24	1.5	43.75	8	2.557

data regression analysis and regression equation coefficient estimation.

Biosynthesized AgNPs characterization

Using UV/Vis spectrophotometer (Cary 100 UV–Vis; Agilent Technologies, Germany), the AgNPs spectra were measured as a function of wave lengths at data intervals of 1.0 nm. The biosynthesized AgNPs elemental analysis was studied using Scanning Electron Microscope (SEM) (Quanta FEG250, FEI company, USA) operated at 20 kV of an accelerating voltage and coupled with energy dispersive X-ray analysis (EDAX) for compositional analysis and the presence of elemental silver conformation. The shape and size of AgNPs were determined by Transmission Electron Microscope (TEM) (JEOL JEM-HR-2100, Germany) operating at 160 kV. Dry powder of the AgNPs was used

for Fourier Transform Infrared spectroscopy (FTIR) measurements using a JASCO FTIR (Japan) instrument in the diffuse reflectance mode at a resolution of 4 cm^{−1} in KBr pellets. AgNPs samples for SEM, TEM, and FTIR analysis were prepared as mentioned in Othman et al. (2016).

Effectiveness of the biosynthesized AgNPs as antimicrobial agent

Agar well diffusion assay was applied to estimate the potency of AgNPs as antimicrobial agent (Othman et al. 2016). *B. mycoides*, *E. coli*, and *C. albicans* were inoculated in nutrient agar plates, after solidification agar wells were made using cork borer (15 mm), after that 0.2 mL of the biosynthesized AgNPs solution serial concentrations (10, 20, 40, and 80 µg/mL) was added to the agar wells. Standard antibiotic (7 mm) discs from Bioanalyse[®] were also used for

comparison with the produced nanosilver. The inoculated plates were incubated for 3 h at 37 °C, after that the inhibition zone diameter was measured (Othman et al. 2017).

Results and discussion

Regression analysis interpretation

As a result of experimental data analysis using multiple regression analysis, the CCD experimental results were integrated with a second-order full polynomial equation. The practical rapport connecting the study four parameters in coded units and the resulted surface plasmon resonance (SPR) (absorbance at 430 nm) is given in this following equation:

$$\begin{aligned} \text{SPR (Abs at 430 nm)} Y = & 1.31 + 0.27A + 0.093B \\ & + 0.27C + 0.43D - 0.008AB \\ & + 0.078AC + 0.17AD + 0.11BC \\ & + 0.034BD + 0.092CD - 0.20A^2 \\ & - 0.18B^2 - 0.22C^2 - 0.10D^2. \end{aligned}$$

where Y is the resulted absorbance at 430 nm, A is the time of incubation for the reaction, B is the concentration of AgNO_3 , C is the percentage of CFF volume, and D is pH value of reaction. The analysis of variance (ANOVA) summing up for the biosynthesized AgNPs model is presented in Table 2. The F value of the applied model (4.05)

indicates the model significance. The obtained “Prob > F ” values that are less than 0.05 also indicate that model terms are significant. In the current case A , C , D , A^2 , C^2 terms are significant model terms as obtained from the ANOVA analysis. On the other hand, values superior than 0.10 be a sign of the insignificance of model terms. As obtained from Table 2, the “ F -value” of Lack of Fit is 7.41 and “Prob > F ” value is 0.0196. “Adeq Precision” concept is used to measure the signal to noise ratio, where the ratio value greater than 4 is desirable. So, the ratio of 7.072 indicates a satisfactory signal. The coefficient of variation (CV = 59.7%) value indicated the precision and reliability of the experiments.

Contour plots interpretation

The four study parameters and its interrelation effects on the *A. niger* mediated AgNPs biosynthesis were declared through the regression model and showed as contour plots using SPR values (absorbance at 430 nm) as indication for AgNPs formation. Figure 1a–c gives an idea about that the time of reaction (A) had a noteworthy outcome on the formation of AgNPs by the mediation of *A. niger* CFF. Once time of reaction (A) enlarged, the biosynthesis of AgNPs improved to extent maximum value relaying on the accompanied parameter. Othman et al. (2016) stated that metal nanoparticles biosynthesis by the microorganisms’ assist needs around from 24 to 124 h, and this time feature for reduction progression depends fundamentally on the used biomass reduction potential.

Table 2 AgNPs surface plasmon resonance model ANOVA analysis

Source	Sum of squares	DF	Mean square	F -value	Prob > F	Significance
Model	11.48	14	0.82	4.05	0.0054	Significant
A-time	1.70	1	1.70	8.41	0.0110	Significant
B-silver nitrate	0.21	1	0.21	1.03	0.3272	
C-CFF	1.74	1	1.74	8.60	0.0103	Significant
D-pH	4.34	1	4.34	21.45	0.0003	Significant
AB	0.001	1	0.0011	0.0053	0.9428	
AC	0.098	1	0.098	0.48	0.4975	
AD	0.44	1	0.44	2.16	0.1620	
BC	0.20	1	0.20	1.01	0.3311	
BD	0.018	1	0.018	0.09	0.7684	
CD	0.13	1	0.13	0.67	0.4271	
A^2	1.08	1	1.08	5.32	0.0358	Significant
B^2	0.89	1	0.89	4.38	0.0538	
C^2	1.33	1	1.33	6.57	0.0217	Significant
D^2	0.27	1	0.27	1.34	0.2646	
Residual	3.03	15	0.20			
Lack of fit	2.84	10	0.28	7.41	0.0196	
Pure error	0.19	5	0.038			
Cor total	14.52	29				

DF degree of freedom; R^2 0.7910; adjusted R^2 0.5959; CV 59.70%; adequate precision, 7.072

Fig. 1 a–f Contour plots of mediated AgNPs biosynthesis by *A. niger* CFF: two parameters were fixed at 0 levels while the effect of other two variables was considered

The importance of the AgNO_3 concentration (B) in the formation of AgNPs bioprocess in the existence of the interaction effect of reaction time (A), proportion of CFF volume (C), and value of reaction pH (D) was demonstrated by Fig. 1a, d, and e. As soon as the AgNO_3 concentration increased, the biosynthesis of AgNPs improved obviously and this effect depends on the dealings of the other factor (A), (C) or (D). The biological factor and AgNO_3 concentration have an instantly recognizable effects on silver nanoparticles synthesis, where there is a correspondence between the capacity of reductase enzymes and proteins in reaction medium as well as the concentration of Ag^+ to acquire the appropriate equilibrium intended for AgNPs biosynthesis (El-Rafie et al. 2012). In this regard, Durán et al. (2005) stated the responsibility of nitrate reductase enzyme for the Ag^+ ions reduction and the consequent silver nanoparticles formation. In addition, Golinska et al. (2014) reported that NADH-dependent nitrate reductase enzyme is known to be an important factor in biosynthesis of nanosilver. So, the probable mechanism that involves silver ions reduction possibly follows the electron shuttle metal reduction enzymatic mechanism (Golinska et al. 2014).

Figure 1b, d and f illustrates that *A. niger* CFF volume (C) had less effect on the process of AgNPs biosynthesis than the effects exerted by the other variables with the exception of interaction with pH value of reaction (Fig. 1f). This could be interpreted by the high capability of the bimolecules in *A. niger* CFF which is responsible for the biosynthesis of AgNPs by means of involvement in silver reduction process

(Ibrahim 2015). Figure 1c, e, and f demonstrates the mainly considerable effect for reaction pH values (D) on the process of AgNPs formation through mediation by *A. niger* CFF. The yield of bioprocess is enhanced by the increase in pH values from acidic toward neutral pH values, which is in agreement with our preceding results using *Penicillium. politans* NRC510 CFF (Othman et al. 2016).

On the other hand, the contour plots in Fig. 1 furthermore confirm the differences in effects among the four studied parameters on the formation of AgNPs biosynthesis, and their order on basis of its impact was $D > B > A > C$, respectively. The output of response surface central composite design reveals that the maximum production of nanosilver particles could be conducted through using 43.75% CFF in reaction mixture containing 1.5 mM silver nitrate for 24 h of incubation at pH 8.0 (Run 30), whereas the statistical optimization models indicated that using 59.37% of CFF in reaction containing 1.82 mM silver nitrate for 34 h of incubation at pH 7.0 is the optimum value to optimize the AgNPs biosynthesis. This difference could originate from the program statistical calculations considering the interactions between the tested parameters. The absorbance value at 430 nm for the optimized reaction that predicted by the optimization models was 2.16 which is close to the obtained actual response that proves the models' validity. So, throughout this study we used the values from the statistical optimization model (59.37% of CFF, 1.82 mM AgNO_3 , 34 h and pH 7.0) as optimum values for preparing nanosilver for characterization and application.

Fig. 2 UV–visible absorption spectra of the biosynthesized silver nanoparticles at different reaction temperature, (solid line) 30 °C, (dotted line) 40 °C, (dashed line), 50 °C, (dashed double dotted line) 60 °C, (spaced dashed line) 70 °C, (space ndash dotted line), 80 °C, (spaced dashed line) 90 °C. The experimentation average data were cited from triplicate results

Effect of reaction temperature

Reaction incubating temperature is obviously affecting the process of mediating silver ion reduction by *A. niger* CFF (Fig. 2). At lower temperature degrees, the formation of AgNPs process necessitates longer time than metal reduction at elevated degrees of temperature. The highest strength for SPR peak was obtained at 90 °C (Fig. 2), representing the development of Ag nanoparticles at high-speed rate, causing the configuration of nanoparticles in little dimensions (Fayaz et al. 2010). In agreement with our results, Sintubin et al. (2012) declared that silver ion reduction was approved with higher velocity at high temperatures. Resulted absorbance peaks at 70, 80, and 90 °C exhibited strong absorbance at around 430 nm. On

the other hand, incubating the reaction mixture between 30 and 60 °C resulting in SPR spectra with maximum absorbance close to 480 nm, which is slightly shifted than our previous work using *T. viride* ATCC36838 extract (Othman et al. 2017).

Analysis of SEM and EDAX micrographs

The micrographs obtained by SEM (Fig. 3a, b) indicate the presence of the prepared AgNPs sample in a mixture from other elements. The evidence of silver element present in the obtained AgNPs was established by means of EDAX and mapping spectrum. Quantitative assessment for the elements present in the biosynthesized nanoparticles sample was made in the course of EDAX analysis. Typically and in

Fig. 3 Profiles of green synthesized silver nanoparticles by *A. niger* mediation: **a** SEM micrograph (500X), **b** SEM micrograph (5000X), **c** EDAX profile of nanosilver and other combined metals, the input is EDAX mapping micrograph showing nanosilver percentage as

brown dots in the total sample black background. Samples used were produced from reaction mixture containing 59.37% of *A. niger* CFF, 1.82 mM AgNO₃ and incubated for 34 h at pH 7.0

agreement with our findings, superior counts at 3 keV were revealed by silver nano-crystals as a reason of its SPR (Ibrahim 2015). The occurrence of silver element was established by the outcome obtained from EDAX investigation (Fig. 3c) through well-built signals for energy peaks of silver particles in the region of 3.0 keV. In this connection, a significant peak at 2.9 keV was obtained from the EDAX spectrum of silver nanoparticles mediated by *Thymra spicata* extract viewing that the elemental silver is a principal component (Veisi et al. 2018). These findings inveterate the effective presence of silver element in the biosynthesized AgNPs by *A. niger* CFF. The EDAX mapping micrograph indicates the well arrangement and homogenous distribution of the biosynthesized AgNPs (the input in Fig. 3c) (Helaly et al. 2017).

TEM analysis

Using the means of drop-coated films, the biosynthesized AgNPs by approach of *A. niger* CFF mediation were inspected using TEM. The achieved TEM micrographs illustrate silver nanoparticles in diverse nano-sizes as individual units or in some collections (Fig. 4). The capping agent's (protein) function in the stabilization of nanoparticles is confirmed by the less direct connection between the produced nanoparticles (Devi and Joshi 2015). The acquired micrographs exposed the collection of the biosynthesized AgNPs to be approximately spherical in shape inside diameter assortment of 3–20 nm (Fig. 4). In this regard, the biosynthesized AgNPs mediated by *T. viride* ATCC36838 were within diameter range of 4–16 nm in spherical and oval shapes (Othman et al. 2017).

FTIR spectroscopy

FTIR spectroscopic examination was carried out to identify the key functional groups found in the biosynthesized AgNPs by mediation of *A. niger* CFF. The importance of the presence of these functional groups is due to its proposed responsibility toward the AgNPs capping, and stabilization. The FTIR spectrum of the produced AgNPs by aid of *A. niger* CFF mediation was illustrated in Fig. 5 and gives an idea about diverse absorption peaks at dissimilar locations. As of the spectrum achieved, the peak displayed around the range of 600 cm^{-1} refers to the band of alkyl halides (C–Cl) (Bagherzade et al. 2017). In addition, the peak identified at 1183 cm^{-1} can be consigning to C–N stretching vibration for aromatic and aliphatic amines. The protein amide bond N–C=O peak could be detected at 1588 cm^{-1} due to proteins' carbonyl stretching (AbdelRahim et al. 2017). In contrast, C–H bond peak could be distinguished at 2923, and 2856 cm^{-1} as a result of methylene, methoxy, and methyl groups stretching vibrations, while O–H group stretching due to the proposed presence of alcohols, flavonoids, and phenols was viewed at 3426 , and 3760 cm^{-1} (Mittal et al. 2014), at 3415 cm^{-1} for alcohol and phenol O–H stretch (Veisi et al. 2018), or at 3650 for alcohol O–H stretch, whereas C=O bond stretching peak was detected at 2362 cm^{-1} (Misra et al. 2006). The detected sharp peak at 3432 cm^{-1} could refer to the typical phenomenon of proteins due to –NH overlapping stretching vibration (Manjari Mishra et al. 2015). Based on the detected peaks from FTIR spectroscopy, we can wrap up the involvement of the protein agent which could be reductase enzymes in Ag^+ reduction in order to mediate the AgNPs biosynthesis.

Fig. 4 TEM micrograph for *A. niger* mediated AgNPs biosynthesis. Samples used were produced from reaction mixture containing 59.37% of *A. niger* CFF, 1.82 mM AgNO_3 and incubated for 34 h at pH 7.0

Fig. 5 Fourier transform infrared spectrum for the biosynthesized AgNPs by *A. niger* CFF mediation. Samples used were produced from reaction mixture containing 59.37% of *A. niger* CFF, 1.82 mM AgNO₃ and incubated for 34 h at pH 7.0

Table 3 Effectiveness of the biosynthesized AgNPs by mediation of *A. niger* as antimicrobial agent in comparison to some standard antibiotics

Sample	Inhibition zone (mm)		
	<i>Bacillus mycoides</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
Colistin 10 µg	0	0	0
Tobramycin 10 µg	10	13	12
Gentamicin 10 µg	11	12	12
Ampicillin 10 µg	12	20	14
Streptomycin 10 µg	17	15	17
Erythromycin 15 µg	17	29	25
Cell free filtrate (blank)	0	0	0
AgNPs (80 µg/mL)	26	23	26
AgNPs (40 µg/mL)	25	24	25
AgNPs (20 µg/mL)	25	25	25
AgNPs (10 µg/mL)	24	24	23

Antimicrobial activity

The inhibition zone obtained against *B. mycoides*, *E. coli* and *C. albicans* noticeably illustrates the effective nature of the biosynthesized AgNPs. A considerable diameter of inhibition zone was detected with the various concentrations of AgNPs as presented in Table 3. The results obtained also indicated a good antimicrobial activity against the three tested species of microorganisms. AgNPs exhibited its effect

even at the lowest applied concentration (10 µg/mL) which reflects its good attitude as antimicrobial agent which resemble to the AgNPs biosynthesized by *T. viride* ATCC36838 (Othman et al. 2017). The resulted inhibition zones by action of AgNPs are almost greater than the used standard antibiotics. Numerous mechanisms of AgNPs antimicrobial action have been reported to state that the particle size of 10 nm of AgNPs or below exhibits activity by itself due to proper cell interaction and its smaller particle size. While the effect caused by the range of 20–80 nm AgNPs is endorsed to the liberation of silver ions inside the cell. In addition, AgNPs interact with the protein thiol groups and obstruct the DNA replication which leads to bacterial cell inactivation (Kumari et al. 2016).

Conclusions

Optimization of AgNPs biosynthesis affecting variables and its interactions was studied via application of central composite design statistical method in order to build up an inexpensive and ecofriendly advance in this fast grown field. The results obtained from the statistical models revealed the clear effect of the parameters under study in the order of: reaction pH value > AgNO₃ concentration > reaction incubation time > *A. niger* CFF percentage, respectively.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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