Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.7201

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (6):** 592-604 (2018)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Statistical Optimization and Antimicrobial Activity of Silver Nanoparticles Synthesized by *Bacillus siralis* STRAIN UMBS1.1

Ankita Sharma* **and Poonam Shirkot**

Dr YSP University of Horticulture and Forestry, Nauni

*Corresponding Author E-mail: ankitas238@gmail.com

Received: 1.11.2018 | Revised: 6.12.2018 | Accepted: 14.12.2018

ABSTRACT

Silver nanoparticles are one of kind of nanomaterials with applications in areas like cosmetics, nanosensors, antimicrobials and bioremediation. In modern era, ecofriendly processes of silver nanoparticles synthesis using various biological systems are gaining importance, biosynthesis of silver nanoparticles using bacteria is one of such approach with benefits of easy maintenance and manipulation. Optimization of culture conditions is useful for harvesting maximum product benefit from microorganisms. Present study focuses on biosynthesis of silver nanoparticles using Bacillus siralis strain UMBS1.1 followed by optimization of its culture conditions using response surface methodology. By using a central composite design, the optimal culture conditions for silver nanoparticles synthesis were found to be as: incubation time 46.0 hrs; incubation temperature of 36.5°C; pH 8.5, 3.0 g/l tryptone and 3.0 g/l yeast extract. Under these conditions, a 16.064 fold increase in silver nanoparticles activity has been achieved as compared to unoptimized conditions. These silver nanoparticles were found to show antimicrobial activity against various fungal phytopathogens.

Key words: *Bacillus siralis strain UMBS1.1, Silver nanoparticles, Response surface methodology, Antifungal effect*

INTRODUCTION

Every year, a great loss to agricultural produce happens due to attack of various pests including pathogens, parasitic weeds and insects. Millions of dollars are invested on various pesticides to control these pests to ensure food, feed and fibre security worldwide. But, the haphazard use of these pesticides has led to pollution of environment resulting into adverse effects on human health and non-target organisms.[1] Therefore, there is a great concern regarding continuous use of pesticides forcing research towards rapid development of alternative ecofriendly approaches to control these pests.

Nanotechnology is a fast growing branch of science that deals with synthesis and development of varied nanomaterials. Silver nanoparticles (AgNPs), one of type of nanoparticles, which have become the main focus of intensive research because of their wide selection of applications in areas like catalyst, optics, antimicrobials and bioremediation[2,3,4,5,6].

Cite this article: Sharma, A. and Shirkot, P., Statistical Optimization and Antimicrobial Activity of Silver Nanoparticles Synthesized by *Bacillus siralis* Strain Umbs1.1, *Int. J. Pure App. Biosci.* **6(6):** 592-604 (2018). doi: http://dx.doi.org/10.18782/2320-7051.7201

Silver nanoparticles can be produced by physical, chemical and biological methods. However, due to the growing demand to develop eco-friendly nanoparticles using safe chemicals in the synthesis protocol, researchers are turning towards bio mimetic approaches for synthesis of silver nanoparticles (AgNPs) using different biological systems such as *Fusarium oxysporum*, *Escherichia coli*, *Aspergillus flavus* and *Bacillus licheniformis*[7,8,9,10].

The culture conditions for industrially useful microorganisms are generally optimized to obtain higher yields of their industrially important products. The classical approach employed for optimization is one-factor-at-a-time (OFAT) method which involves designing of experiments based on testing of factors, or causes, one at a time instead of multiple factors simultaneously, has severe limitation that it does not allow the investigation of interaction between variables.[11] Among multivariate optimization methods that can be used more reliably to find global maxima, the response surface methodology (RSM) has shown to be powerful and practicable for program optimization and is often used to identify the relative significance of different factors, interactions between factors and optimal level of test variables. Hence, present study focuses on statistical optimization of selected factors using response surface methodology followed by antimicrobial effect of these silver nanoparticles on various plant fungal pathogens.

MATERIAL AND METHODS

2.1 Chemicals and Apparatus- All the chemicals were purchased from HiMedia and Sigma Aldrich. All solutions were made using sterile double distilled water.

2.2 Biosynthesis of silver nanoparticles by *Bacillus siralis* strain UMBS1.1

Bacillus siralis strain UMBS1.1 previously isolated from silver mine located in Uchich village of Kullu district of Himachal Pradesh (India) was assessed for its ability to synthesize extracellular silver nanoparticles. Bacteria was grown in TY medium (Tryptone 5g/l, yeast extract 3g/l, $CuSO_4$ 40mg/l) at 37^oC for 24 hrs at 150 rpm. Ten ml of culture supernatant was mixed with 10 ml of 1.0 mM solution of silver nitrate ($AgNO_3$) and incubated at 37^oC for 1 hr followed by exposure to sunlight for 10 minutes. Formation of silver nanoparticles was indicated by colour change of the solution from light yellow to brown colour.[12]

2.3 Characterization of silver nanoparticles

Optical properties, morphology and size of the silver nanoparticles synthesized by *Bacillus siralis* strain UMBS1.1 were determined using UV- visible spectroscopy, Scanning Electron Microscopy (SEM) and Dynamic Light Scattering (DLS) respectively.

2.4 Optimization of culture conditions using Central Composite Design and Response Surface Methodology

Five independent variables i.e. incubation time (A), temperature (B), pH (C), tryptone (D) and yeast extract concentration (E) were chosen for optimization studies by employing Central Composite Design (CCD) of Response Surface Methodology (RSM). The effect of each variable was studied at three different levels, namely -l, 0, +l and a set of 50 experiments was performed in triplicate. The range and levels of this variable have been presented in Table-I.

Table I: Coded values of independent variables at different levels used in Central Composite Design

Factors	Independent Variables	Levels		
		-1	0	+1
A	Incubation Time (hrs)	20.0	46.0	72.0
B	Incubation Temperature (°C)	28.0	36.5	45.0
C	pH	5.0	8.5	12.0
D	Tryptone (g/l)	1.0	3.0	5.0
E	Yeast extract (g/l)	1.0	3.0	5.0

The central coded value of all variables was set at zero. The data obtained from the RSM was subjected to analysis of variance. The results of the RSM were used to fit in following second-order polynomial equation, to represent the behavior of the system:

$Y = b_0 + b_1A + b_2B + b_3C + b_4D + b_5E + b_1b_1A^2 + b_2b_2B^2 + b_3b_3C^2 + b_4b_4D^2 + b_5b_5E^2 + b_1b_2AB + b_1b_3AC + b_1b_4AD + b_1b_5AE + b_2b_3BC + b_2b_4BD + b_2b_5BE + b_3b_4CD + b_3b_5CE + b_4b_5DE$

Where, Y is the response variable representing silver nanoparticles activity, b_0 is the intercept, b_1, b_2, b_3, b_4, b_5 are the linear coefficients, b_1b_1, b_2 b_2, b_3 b_3, b_4 b_4, b_5 b_5 are squared coefficients, b_1b_2, b_1b_3, b_1b_4, b_1b_5, b_2b_3, b_2b_4, b_2b_5, b_3b_4, b_3b_5, b_4b_5 are the interaction coefficients and A, B, C, D, E, A^2, B^2, C^2, D^2, E^2, AB, AC, AD, AE, BC, BD, BE, CD, CE and DE are the levels *of* independent variables. The data analysis and generation of the response surface graphs were conducted using the statistical software Design Expert (Stat-Ease 9.0).

In vitro assay of *antifungal activity of* silver nanoparticles *preparation*, synthesized by Bacillus siralis strain UMBS1.1

The inhibitory effect of silver nanoparticles synthesized *by Bacillus siralis* strain UMBS1.1 was studied using agar dilution technique (Warnock, 1989). Malt extract agar plates containing 10% of silver nanoparticles preparations were prepared (incorporating 2 ml of silver nanoparticles preparation in 20 ml of MEA). Fungal bits of each of the actively growing six test pathogens were cut with 5 mm cork borer and placed at centre of each MEA plates. Uninoculated controls were kept for comparison of results. The plates were incubated at temperature of 28°C for one week. Colony diameter of test fungus was measured and percent growth inhibition was calculated according to Vincent (1947).[13]

$$I = \frac{C-T}{C} \times 100$$

Where, I= Percent growth inhibition, C= Growth of fungus in control, T= Growth of fungus in treatment

RESULTS AND DISCUSSION

3.1 Biosynthesis of silver nanoparticles by *Bacillus siralis* strain UMBS1.1

Biosynthesis of silver nanoparticles was carried out by using *Bacillus siralis* strain UMBS1.1 (Fig.1) (Genbank Accession No. MF372388), previously isolated from silver mine located in Uchich village of Kullu district (H.P.) with 1mM silver nitrate. Formation of silver nanoparticles was indicated by colour change of the solution from light yellow to brown colour (Fig.2). Excitation of surface plasmon vibrations in silver nanoparticles is considered as the basic mechanism of change in colour of the solution to brown depicting the formation of silver nanoparticles.[12,14]

3.2 Characterization of silver nanoparticles

3.2.1 UV-visible spectroscopy

The formation of silver nanoparticles was further confirmed by UV-Vis spectroscopy. Scanning of solution of silver nanoparticles synthesized by *Bacillus siralis* strain UMBS1.1 showed a broad spectrum of peaks in range of 400-415 nm, characteristic of silver nanoparticles (AgNPs) (Fig.3). Colloidal silver nanoparticles have been found to possess optical spectrum known as surface plasmon resonance in the range of 400-460 nm. As the diameter of nanoparticles increases peak plasmon resonance shifts to longer wavelengths and broadens. The broadening of peaks indicated that particles were spherical and polydispersed which were further confirmed by SEM results.[15] Similar studies of silver nanoparticles characterization by using UV- visible spectroscopy has been reported giving spectra in the range of 400-450 nm[16], at 420 nm [17, 18, 19] and at 450 nm[20].

3.2.2 Scanning Electron Microscopy

Scanning electron microscopy results have revealed that silver nanoparticles synthesized by *Bacillus siralis* strain UMBS1.1 were spherical in nature and were polydispersed (Fig.4). Scanning Electron Microscopy (SEM) is another technique which images the sample surface by detecting scattered or secondary electrons emitted from the surface of a sample due to excitation by the primary electron

beam. SEM has been used as an efficient technique for silver nanoparticles characterization.[16, 20, 21]

3.2.3 Dynamic light scattering (DLS)

Dynamic light scattering (DLS) technique has been used to determine the size of particles. Fig.5, depicting three peaks approximately at 50.0 nm, 80.0 nm and 400 nm. On the basis of results obtained, maximum types of silver nanoparticles synthesized were of 80.0 nm size. Anandalakshmi *et al*., 2006 reported the use of Dynamic Light Scattering to determine the size of silver nanoparticles synthesized by *Padalium murex* leaf extract and observed the size distribution of silver nanoparticles ranged from 10 to 150 nm. The calculated average particle size distribution of silver nanoparticles (AgNPs) was 73.14 nm.[22]

3.4 Statistical optimization using central composite design

The results of response surface experiments to determine the effects of incubation time (A), incubation temperature (B), pH (C), tryptone concentration (D) and yeast extract concentration (E) have been presented in Table- II., The minimum and maximum silver nanoparticles activity obtained was 0.00 and 7.92 OD respectively. The highest activity was obtained from Run number -22, which consisted of incubation time of 46.0; incubation temperature of 36.5°C; pH 8.5, 3.0 g/l tryptone and 3.0 g/l yeast extract. Whereas the lowest value of silver nanoparticles activity was obtained from Run number-33 which consisted of incubation time of 72.0; incubation temperature of 28.0°C; pH 12.0, 1.0 g/l tryptone and 1.0 g/l yeast extract.

The determination coefficiant (R^2) value of 0.9777 has indicated that the statistical model has been able to explain 97.77% of the variability in the response. For good statistical model R^2 value should be close to1.0. Also the model indicated that the predicted R^2 value 0.9139 has been in reasonable agreement with adjusted R^2 value of 0.9624. In addition, adjusted determination coefficiant (adjusted R^2=0.9624) was also found to be high, indicating high significance of the model. The significance of each term in the model has been presented in Table-III. The ANOVA for the selected quadratic model showed that the model has been significant with a model F= 63.63 and P > F value < 0.0001 (Table-III).

Table II: Actual and predicted values of silver nanoparticles activity recorded in experimental set up for central composite design performed for selected parameters

Run	Factor 1 A Incubation time	Factor 2 B Incubation temperature	Factor 3 C pH	Factor 4 D Tryptone (g/l)	Factor 5 E Yeast Extract (g/l)	Response (OD at 410 nm) Actual	Response (OD at 410 nm) Predicted
1.	0	0	0	0	0	0.96	0.92
2.	0	0	+1	0	0	1.72	1.75
3.	-1	-1	+1	+1	+1	1.52	1.44
4.	0	0	0	0	0	1.02	0.92
5.	+1	-1	-1	-1	+1	4.68	4.53
6.	-1	-1	-1	-1	-1	5.53	5.68
7.	+1	-1	+1	+1	-1	0.95	1.03
8.	-1	-1	-1	+1	-1	0.63	0.82
9.	-1	-1	-1	-1	-1	0.73	0.41
10.	0	0	0	0	0	6.000E-003	0.023
11.	0	+1	0	0	0	2.56	3.07
12.	0	0	0	0	+1	1.35	1.32
13.	0	-1	0	0	0	3.73	3.37
14.	-1	-1	+1	-1	+1	3.56	3.29
15.	0	0	-1	0	0	2.00	2.01
16.	-1	-1	+1	-1	-1	0.63	0.57
17.	-1	-1	-1	+1	+1	1.45	1.56
18.	0	0	0	0	0	3.50	2.94
19.	-1	-1	+1	+1	-1	0.87	0.84
20.	-1	-1	+1	-1	+1	6.000E-003	0.87
21.	0	0	0	0	0	5.76	5.86
22.	0	0	0	0	0	**7.92**	**7.56**
23.	+1	-1	+1	+1	+1	1.25	1.12
24.	0	0	0	0	0	1.52	1.45

25.	0	0	0	0	0	1.000E-003	-0.11
26.	-1	-1	+1	+1	+1	1.000E-003	0.050
27.	+1	-1	-1	-1	-1	1.58	1.31
28.	+1	-1	-1	+1	+1	0.33	0.10
29.	0	0	0	0	0	3.92	3.54
30.	-1	-1	+1	+1	-1	3.95	4.01
31.	+1	-1	+1	+1	+1	0.72	0.93
32.	+1	-1	-1	+1	-1	0.025	0.039
33.	+1	-1	+1	-1	-1	0.000	0.22
34.	+1	-1	-1	+1	+1	0.17	0.16
35.	-1	-1	-1	+1	-1	2.87	3.58
36.	-1	-1	+1	-1	-1	1.000E-003	-0.51
37.	+1	-1	-1	+1	-1	0.37	0.31
38.	+1	-1	+1	-1	+1	4.26	4.53
39.	+1	-1	-1	-1	+1	3.76	3.62
40.	-1	-1	-1	+1	+1	0.98	1.33
41.	+1	0	0	0	0	2.56	2.59
42.	-1	-1	-1	-1	+1	2.56	2.73
43.	+1	-1	+1	-1	+1	2.85	2.89
44.	+1	-1	-1	-1	-1	2.92	2.89
45.	-1	-1	-1	-1	+1	2.92	2.89
46.	+1	-1	+1	+1	-1	2.76	2.89
47.	+1	-1	+1	-1	-1	2.92	2.89
48.	0	0	0	+1	0	2.87	2.89
49.	0	0	0	0	0	3.00	2.89
50.	0	0	0	0	0	2.72	2.89

Table III: ANOVA for Response Surface Quadratic model

Source	**Sum of squares**	**DF**	**Mean Squares**	**F values**	**p- values Prob>F**	
Model	147.3	20	7.36	63.63	< 0.0001	Significant
A- Incubation Time	8.01E-03	1	8.01E-03	0.069	0.7944	
B-Incubation Temperature	32.09	1	32.09	277.24	< 0.0001	Significant
C-pH	34.09	1	34.09	294.54	< 0.0001	Significant
D- Tryptone	9.99	1	9.99	86.35	< 0.0001	Significant
E- Yeast Extract	0.036	1	0.036	0.31	0.5834	
A2	12.66	1	12.66	109.34	< 0.0001	Significant
B2	3.17	1	3.17	27.35	< 0.0001	Significant
C2	0.38	1	0.38	3.31	0.0793	
D2	0.3	1	0.3	2.56	0.1204	
E2	0.088	1	0.088	0.76	0.3894	
AB	3.72	1	3.72	32.16	< 0.0001	Significant
AC	0.19	1	0.19	1.67	0.207	
AD	3	1	3	25.94	< 0.0001	Significant
AE	0.6	1	0.6	5.2	0.0301	Significant
BC	32.38	1	32.38	279.72	< 0.0001	Significant
BD	9.08	1	9.08	78.48	< 0.0001	Significant
BE	3.08	1	3.08	26.64	< 0.0001	Significant
CD	0.85	1	0.85	7.32	0.0113	Significant
CE	0.95	1	0.95	8.18	0.0078	Significant
DE	2.7	1	2.7	23.36	0.0078	Significant
Residual	3.36	29	0.12		< 0.0001	
Lack of Fit	3.3	22	0.15	17.48	0.0004	Significant
Pure Error	0.06	7	8.57E-03			
Cor Total	150.65	49				

The Model F-value of 63.63 implies the model is significant. There is only a 0.01% chance that large "Model F-Value" this large could occur due to noise. The "Lack of Fit F-value" of 17.48 implies the Lack of Fit is significant. There is only a 0.04% chance that a "Lack of Fit F-value" this large could occur due to noise (Software generated results).

From results, interaction effects of AE, CD, CE and DE have been found to be significant ($P<0.05$). Moreover, the linear effects of incubation temperature (B), pH (C), tryptone concentration (D), squared effects of A^2, B^2 and interaction effects of CD, CE and DE were found to be more significant than the other factors ($P<0.01$.) Thus, the response of silver nanoparticles synthesizing activity (Y) by *Bacillus siralis* strain UMBS1.1 was expressed in terms of the following regression equation:

$$Y=2.89- 0.014A- 0.86B+ 0.89C- 0.48D+ 0.29E- 0.048 A^2- 0.24 B^2-0.083C^2- 0.073 D^2-0.040 E^2-0.34AB+0.78AC- 0.31AD+0.14AE- 1.01 BC+ 0.53 BD-0.31BE- 0.16CD+0.17CE- 0.29 DE,$$

where A-incubation time; B-incubation temperature, C-pH, D-tryptone concentration and E- yeast extract concentration.

A 3D response surface was drawn based on the model equation to investigate the interaction among the variables and to determine the optimum value of each factor for silver nanoparticle synthesis activity by *Bacillus siralis* strain UMBS1.1. The final optimal medium components included 3.0 g/l tryptone and yeast extract in TY medium and incubated at final optimal cultural conditions *viz*., incubation time of 46 hrs, incubation temperature of 36.5 °C and a pH of 8.5. Also significant interactions ($P\leq 0.05$) has been observed amongst different combinations of independent variables and this synergy has been indicated by three dimensional response surface (Fig.6a,b).

An elliptical nature of the contour plots indicated that the interactions between the independent variables are significant. Further, Design Expert predicted the maximum silver nanoparticle activity to be 7.56 at 410 nm which is very close to the actual value of silver nanoparticles activity i.e. 7.92 at 410 nm. As a result of optimizing various physical and nutritional conditions,16.06 fold increase in silver nanoparticles activity (7.92 OD ar 410 nm) has been achieved as compared to 0.493 obtained previously under unoptimized conditions. Usually, it is necessary to check the fitted model to ensure that it provides an adequate approximation to the real system. By constructing a normal probability plot, of the residuals, a check was made for the normality assumption, as given in Fig. 7a. The normality assumption was satisfied as the residual plot approximated along a straight line. Fig. 7b presents a plot of residuals versus the predicted response. The general impression is that the residuals scatter randomly on the display, suggesting that the variance of the original observation is constant for all values of predicted response. Both of the plots (Fig. 7a and 7b) were satisfactory, so we concluded that the empirical model was adequate to describe the silver nanoparticles activity by response surface.The results showed a good agreement between the predicted and experimental values, thereby validating model. El-Batal *et al*., (2013) carried out the statistical optimization of silver nanoparticles synthesized by *Bacillus stearothermophilus* using Response Surface Methodology (RSM) and found that maximum silver nanoparticles activity using the optimized conditions of 0.1 glucose, 1.0% peptone, 0.4% yeast extract, 0.4% KNO_3 at pH: 7.5, incubation temperature of 25°C and incubation period of 3 days.[23]

Antimicrobial activity of silver nanoparticles against plant fungal pathogens

Antifungal activity of silver nanoparticles synthesized by *Bacillus siralis* strain UMBS1.1

Silver nanoparticles synthesized by *Bacillus siralis* strain UMBS1.1 were tested for their antifungal activity against six fungal plant pathogens *viz*., *Fusarium oxysporium*, *Alternaria zinnae*, , *Phytophthora capsici*, *Sclerotium rolfsii* , *Dematophora necatrix and Aspergillus niger* (Table-4, Fig 8a,b). Maximum percent inhibition (54.0%) was observed against *Dematophora necatrix*

Table 4: Antifungal activity of silver nanoparticles synthesized by *Bacillus siralis* strain UMBS1.1 using agar dilution technique

Sr No.	Fungal Pathogens	Percent growth inhibition (%)
1.	*Fusarium oxysporium*	20.0
2.	*Phytophthora capsici*	16.0
3.	*Alternaria zinnae*	33.3
4.	*Aspergillus niger*	4.0
5.	*Dematophora necatrix*	54.0

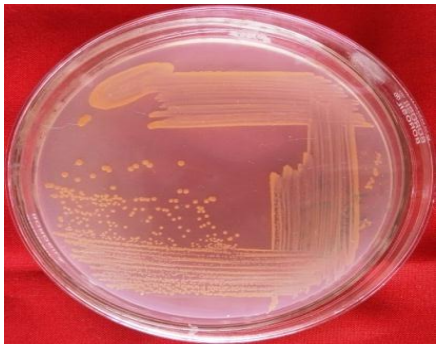

Fig. 1: *Bacillus siralis* strain UMBS1.1

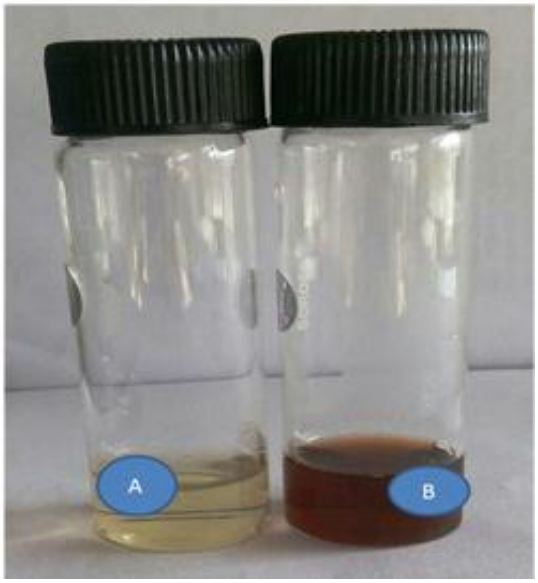

Fig. 2: Colour change from pale yellow to brown A) Control B) Silver nanoparticles solution

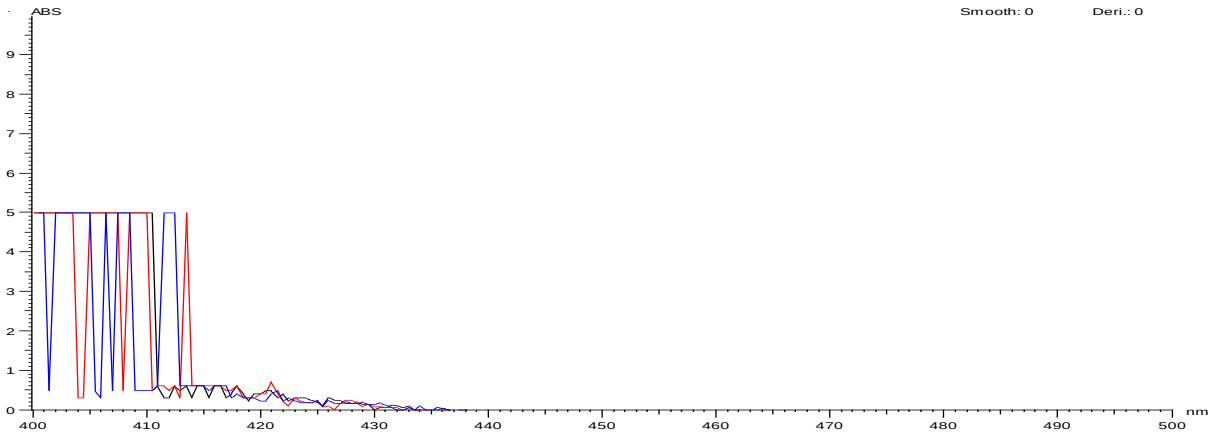

Fig. 3: UV- visible spectra of silver nanoparticles synthesized by *Bacillus siralis* strain UMBS1.1

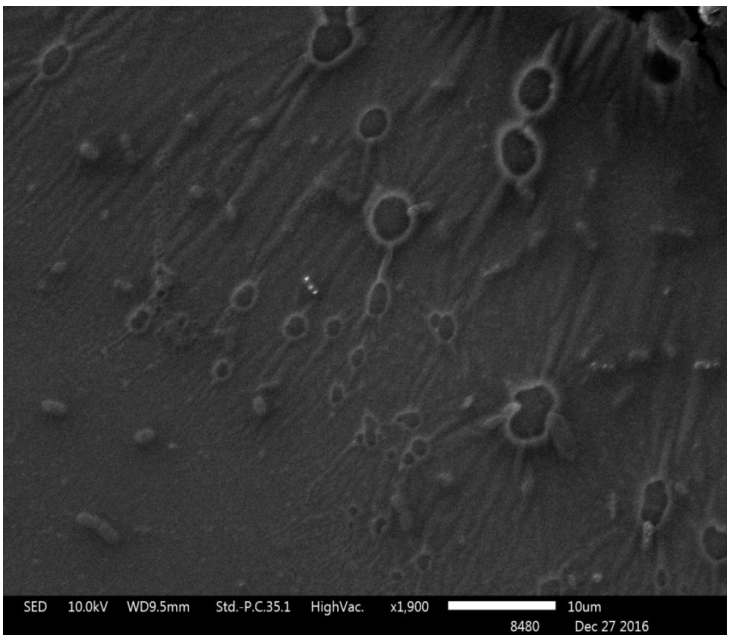

Fig. 4: SEM images of silver nanoparticles synthesized by *Bacillus siralis* strain UMBS1.1 at 1900 X magnification

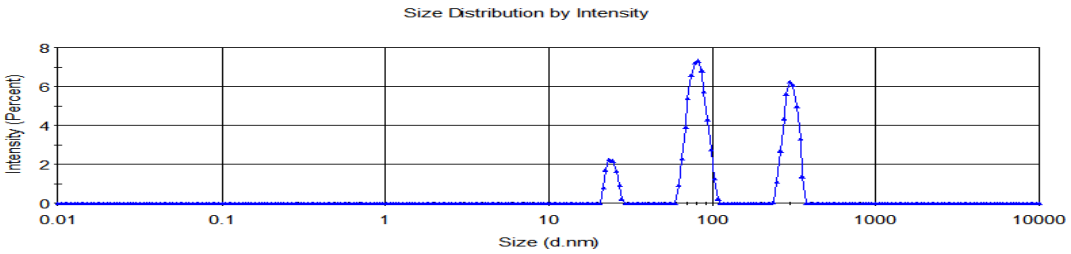

Fig. 5: DLS analysis of silver nanoparticles synthesized by *Bacillus siralis* strain UMBS1.1

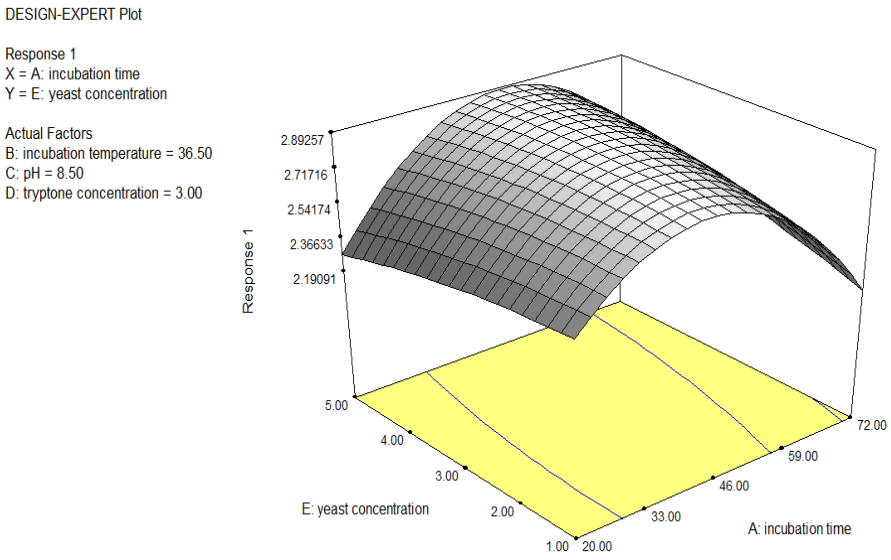

Fig. 6a: Three dimensional (3D) response surface plot of CCD experiment for silver nanoparticles activity by *Bacillus siralis* strain UMBS1.1. The interactions between incubation time and yeast extract concentrations are shown

Response 1: Silver nanoparticles activity

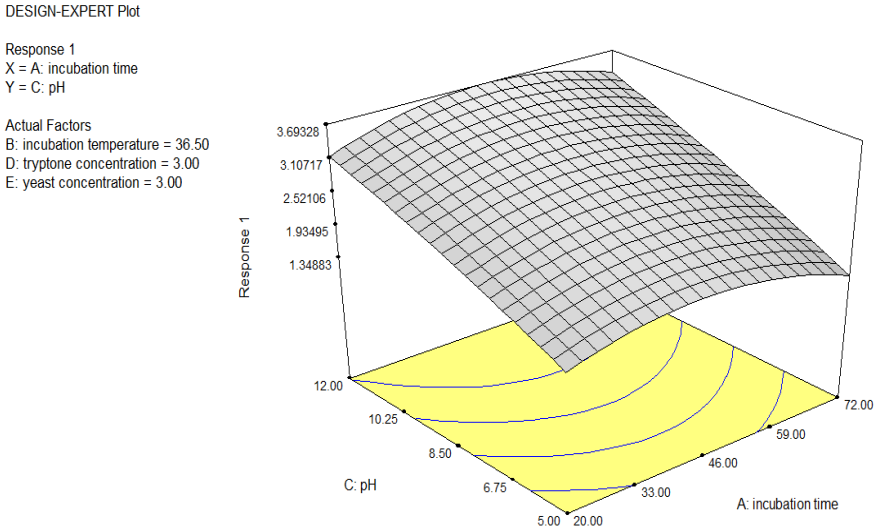

Fig. 6b: Three dimensional (3D) response surface plot of CCD experiment for silver nanoparticles activity by *Bacillus siralis* strain UMBS1.1. The interactions between incubation time and pH are shown

Response 1: Silver nanoparticles activity

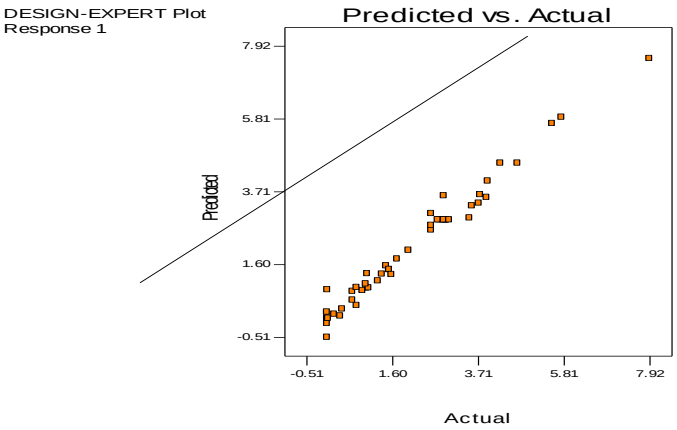

Fig. 7a: Normal probability of internally standardized residuals

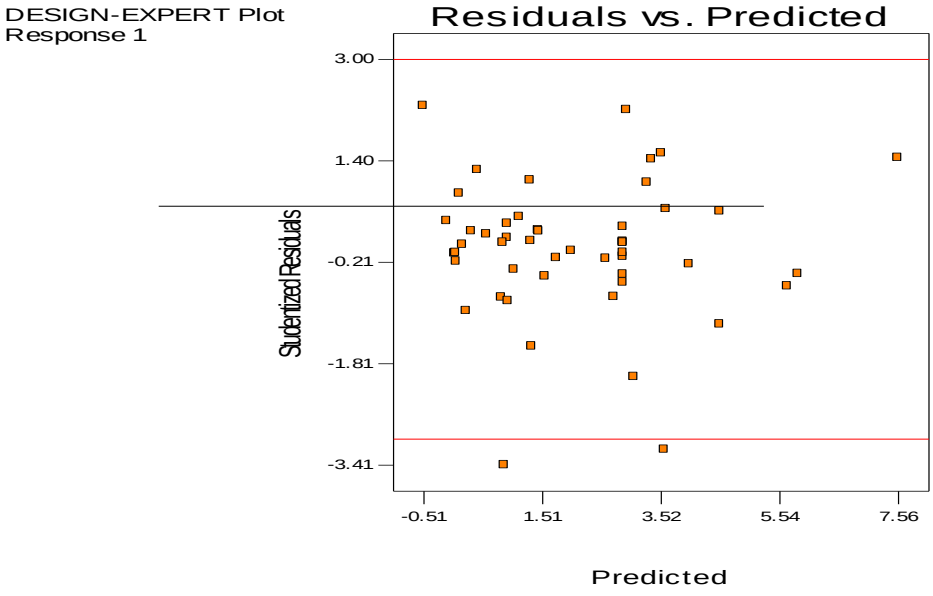

Fig. 7b: Plot of internally standardized residuals vs. Predicted response

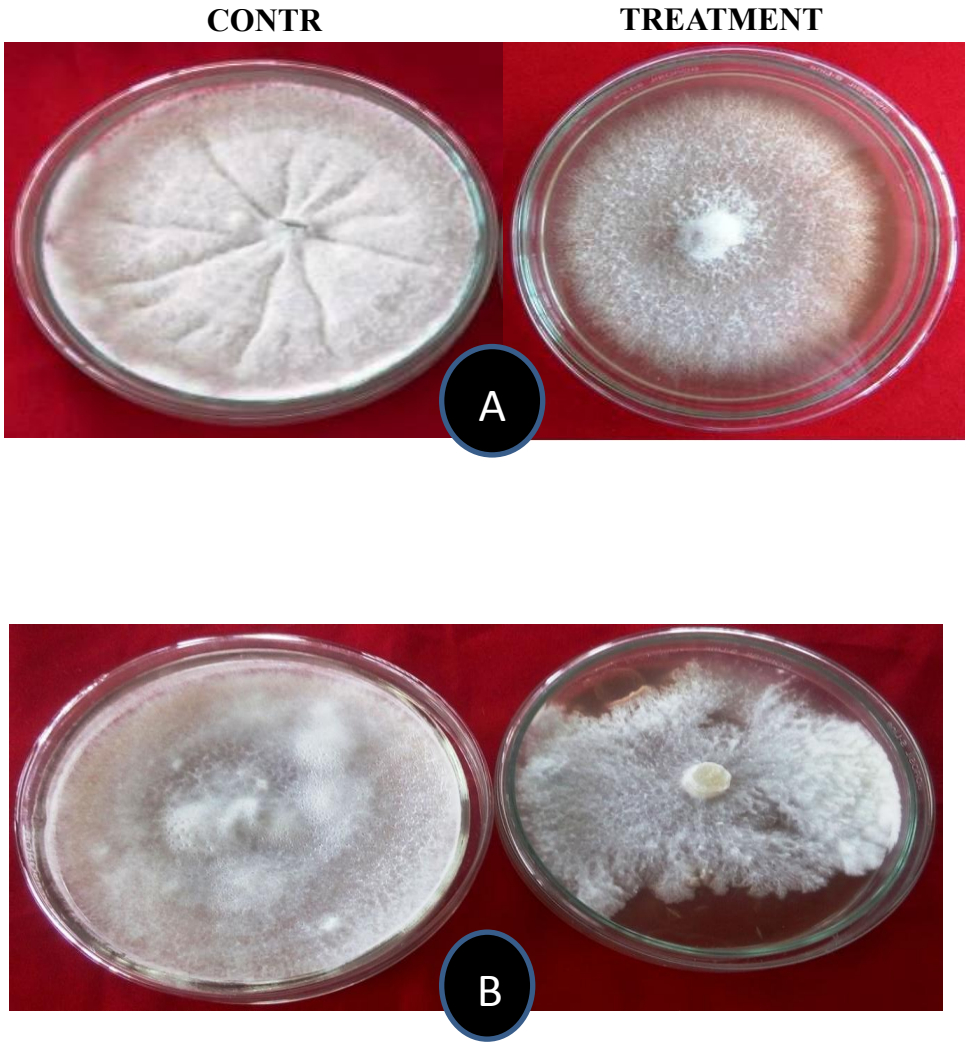

Fig. 8a: Antifungal activity of silver nanoparticles synthesized by *Bacillus siralis* strain UMBS1.1 using agar dilution technique

A) ***Fusarium oxysporium***

B) ***Phytophthora capsici***

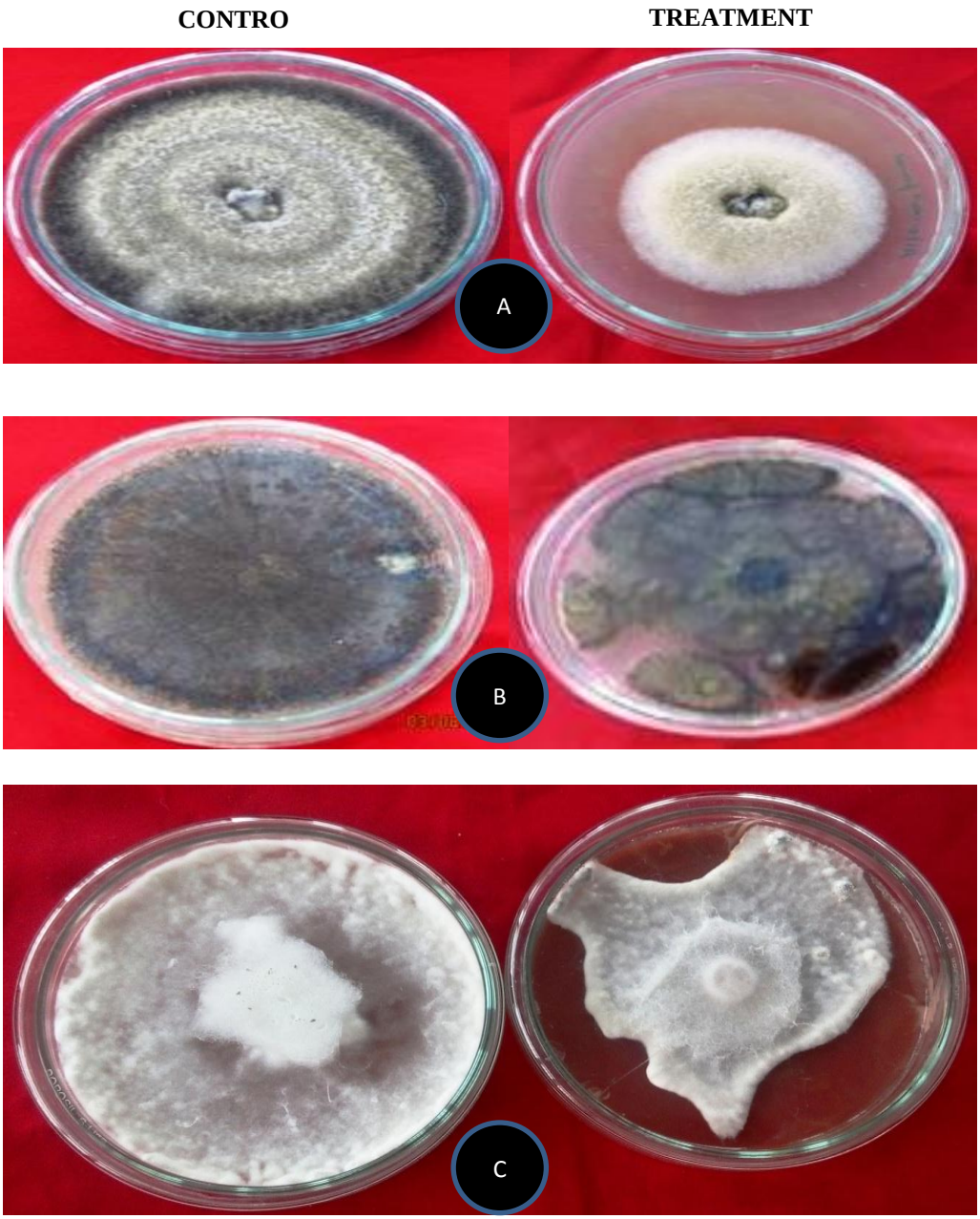

Fig. 8b: Antifungal activity of silver nanoparticles synthesized by *Bacillus siralis* strain UMBS1.1 using agar dilution technique

A) *Alternaria zinnae*
B) *Aspergillus niger*
C) *Dematophora necatrix*

It is a well-known fact that silver ions and silver-based compounds are highly toxic to microorganisms which include 16 major species of bacteria.[24,25] It has been hypothesized that silver nanoparticles can cause cell lysis or inhibit cell transduction. There are various mechanisms involved in cell lysis and growth inhibition. It has been observed that silver nanoparticles disrupt transport systems, including ion efflux[26].

CONCLUSION

Biotic synthesis method is eco-friendly, of low cost and capable of producing silver nanoparticles (AgNPs) at room temperature. In present study, 24 hrs old *Bacillus siralis* strain UMBS1.1 culture supernatant at 37°C was used for synthesis of silver nanoparticles. Cconventional one-factor-at-a-time (OFAT) method is an overlooked interaction between different variables, which is why in order to

determine the possible interactions accurately, the design of experiment (DOE) method needs to be employed. These nanoparticles were found to show antimicrobial activity against various fungal pathogens.

Data availabiliity statement

The Data (Accession No of Strain) is submitted in NCBI.

Conflict of interest

Authors declare that there are no conflicts of interest.

Acknowledgement

The authors thank the Department of Biotechnology, Dr YS Parmar University of Horticulture and Forestry, Nauni for providing research facilities for use in this study.

REFERENCES

1. Basavaraj, U., Praveenkumar, N., Sabiha, T., S., Rupali, S., Samprita, B., Synthesis and characterization of silver nanoparticles, *Int. J. Pharm. Bio. Sc*i. **3: 10**–14 (2012).
2. Rao, C., Kulkarni, G., Thomas, P., Edwards, P., Metal nanoparticles and their assemblies, *Chem. Soc. Rev*. **29:** 27–35 (2000).
3. Zhong-jie, J., Chun-yan, L., Lu-wi, S., Catalytic properties of silver nanoparticles supported on silica spheres, *J. Phys. Chem. B.* **109:** 1730–1735 (2005).
4. Rai, M., Yadav, A., Gade, A., Silver nanoparticles as a new generation of antimicrobials, *Biotechnol. Adv*. **27:** 76–83 (2009).
5. Qin, X., Lu, W., Luo, Y., Chang, G., Sun, X., Preparation of Ag nanoparticles-decorated polypyrrole colloids and their application for H_2O_2 detection, *Electrochem. Commun.* **13:** 785–787 (2011).
6. Zhang, Y., Wang, L., Tian, J., Li, H., Luo, Y., Sun, X., Ag poly (m-phenylenediamine) core–shell nanoparticles for highly selective, multiplex nucleic acid detection, *Langmuir*. **27:** 2170–2175 (2011).
7. Kim, T., Feng, L., Kim, J., Wang, H., Chen, G., Antimicrobial effects of metal ions (Ag, Cu^{2+}, Zn^{2+}) in hydroxyapatite, *J. Mater. Sci. Mater. Med.* **9:** 129–134 (1998).
8. Ahmad, A., Mukherjee, P., Senapati, S., Mandal, D., Khan, M., Kumar, R., Sastry, M., Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*, *Colloids. Surf. B*. **28:** 313–318 (2003).
9. Cho, K., Park, E., Osaka, T., Park, S., The study of antimicrobial activity and preservative effects of nanosilver ingredient, *Electrochem. Acta*. **51:** 956–960 (2005).
10. Shahverdi, A., Fakhimi, A., Shahverdi, H., Minaian, S., Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*, *Nanomed. Nanotechnol. Biol. Med.* **3:** 168–171 (2007).
11. Frey, D.D., Engelhardt, F., Greitzer, E.M., A role for 'one-factor-at-a-time' experimentation in parameter design, *Res. Eng. Des*. **14:** 65-74 (2003).
12. Das, V.L., Thomas, R., Varghese, R.T., Soniya, E.L., Mathew, J., Radhakrishnan, E.K., Extracellular synthesis of silver nanoparticles by *Bacillus* strain CS 11 isolated from industrialized area, *3 Biotech*. **4:** 121-126 (2014).
13. Vincent, J. M., Distortion of some fungal hyphae in presence of certain inhibitors. *Nature.* **150:** 850-85(1947)
14. Saifuddin, N., Wong, C.W., Yasumira, A.A.N., Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation, *J. Chem.* **6:** 61–70 (2009).
15. Bonnie, N.N., Kamaruddin, M.S., Nawawi, M.H., Ratim, S., Azlina, H.N., Ali E.S., Green biosynthesis of silver nanoparticles using 'Polygonum *hydropiper'* and study its catalytic degradation of methylene blue, *Procedia Chem*. **19:** 594 – 602(2016).

16. Priyaragini, S., Sathishkumar, S.R., Bhaskararao, K.V., Biosynthesis of silver nanoparticles using *Actinobacteria* and evaluating its antimicrobial and cytotoxicity activity, *Int. J. Pharm. Pharm. Sci* **5:** 709-712 (2013).
17. Bhainsa, C.K., D'Souza, F.S., Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus funigatus*, *Colloids. Surf. B. Biointerfaces*. **47:** 160–164 (2006).
18. Malarkodi, C., Rajeshkumar, S., Paulkumar, K., Gnanajobitha, G., Vanaja, M., Annadurai, G., Bacterial synthesis of silver nanoparticles by using optimized biomass growth of *Bacillus* sp, *J. Nanosci. Nanotechnol*. **3:** 26-32 (2013).
19. Gopinath, V., Priyadarshini, S., Loke, M.F., Arunkumar, J., Marsili, E., Mubarakali, P.V., Vadivelu, J., Biogenic synthesis, characterization of antibacterial silver nanoparticles and its cell cytotoxicity, *Arab. J. Chem*. **30:** 311–314 (2015).
20. Thomas, R., Janardhanan, A., Rintu, T., Varghese. E.V., Soniya, J.M., Radhakrishnan, E.K., Antibacterial properties of silver nanoparticles synthesized by marine *Ochrobactrum* sp *Braz. J. Microbiol*. **45:** 1221-1227(2014).
21. Vithiya, K., Kumar, R., Sen, S., *Bacillu*s sp. mediated extracellular synthesis of silver nanoparticles, *Int. J. Pharm. Sci.* **6:** 525-527 (2014).
22. Anandalakshmi, K., Venugobal, J., Ramasamy, V., Characterization of silver nanoparticles by green synthesis method using *Pedalium murex* leaf extract and their antibacterial activity, *Appl. Nanosci.* **6:** 399–408(2016).
23. El-Batal, A.I., Amin, M.A., Mona, M.K.S., Merehan, M.A.H., Synthesis of silver nanoparticles by *Bacillus stearothermophilus* using gamma radiation and their antimicrobial activity, *World Appl. Sci. J.* **22:** 1-16 (2013).
24. Slawson, R. M., Van-Dyke, M. I., Lee, H., Trevor, J. T., Germanium and silver resistance, accumulation and toxicity in microorganisms, *Plasmid.* **27:** 73–79(1992).
25. Zhao, G., Stevens, S., Multiple parameters for the comprehensive evaluation of the susceptibility of *Escherichia coli* to the silver ion, *Biometals.* **11:** 221-226(1998).
26. Morones, J. R., Elechiguerra, L. J., Camacho, A., Holt, K., Kouri, B. J., Ramirez, T. J. Yocaman, J. M., The bactericidal effect of silver nanoparticles, *Nanotechnol*. **16:** 2346-2353 (2005).