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Research Article

Exploitation of *Thermomyces* fungal pigment for the synthesis of silver nanoparticles for textile application

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Abstract

Antimicrobial fabrics have gained considerable interest for use in different fields of application. However, most of these antimicrobials have many disadvantages viz., toxicity on non-targets, environment, and low durability of finished products, etc. The study aimed to develop an eco-friendly nanoparticle that can be used both as an antimicrobial and coloring agent. Fungi are known to produce a wide spectrum of colors as secondary metabolites, and they are also capable of creating nanoparticles. *Thermomyces* sp. is capable of producing a yellow pigment along with the ability to synthesize silver nanoparticles. UV-Vis spectroscopy, FT-IR, and TEM were used to analyze the synthesized silver nanoparticles. The size of the silver nanoparticles was found to be between 10 and 50 nm, and nanoparticles treated cloth displayed 90% antibacterial action against *Escherichia coli*, *Staphylococcus aureus,* and *Pseudomonas aeruginosa*.

Keywords antibacterial, fungal pigment, silk fabric, silver nanoparticle

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Introduction

Nanotechnology emerging interdisciplinary science often used to have some new revolutionary products. Nanoparticles are used as an emerging class of colorant for the textile industry. Nanoparticles have no chromophore for color when compared to synthetic or natural dyes, but their color properties are meditated by shape and size [1]. Increase in customer demand for functional apparel nanomaterials has created an opportunity to integrate nanoparticles into textile substrates. Silver nanoparticles (AgNPs) are a type of nanoparticle that has a long history of usage as a textile coloring agent. Because of their particular localized surface plasmon resonance (LSPR) capabilities, AgNPs have outstanding color stability [2].

One of the most significant inorganic nanoparticles is silver nanoparticles. It has a wide range of applications, including biolabelling and bioactive materials for antimicrobial agents. It is highly recognized for its broad-spectrum antimicrobial potential [3-4]. The property of high affinity of silver nanoparticles towards fabrics was used for imparting antimicrobial properties. It can provide high durability for fabrics when compared to conventional materials. Byproducts from the metabolism of bacteria, fungi, and plants, through the biogenic synthesis of nanoparticles, act as reducing and stabilizing agents [5]. Nanoparticles capped with biomolecules through biogenic synthesis, improved the

stability of the products [6]. Fungal nanoparticle synthesis has an advantage in large-scale manufacturing due to the generation of enormous numbers of enzymes required for silver nanoparticle synthesis. Furthermore, a fungal culture is easier to grow in the laboratory and on an industrial scale [7]. Many fungal species can synthesize silver nanoparticles; the current work presents the production of AgNPs utilizing *Thermomyces* sp and its application to fabric; it can also create color. UV-visible spectroscopy, scanning electron microscopy (SEM), and FT-IR methods were used to investigate the characteristics of AgNPs in this work. The antimicrobial activity and colorability of produced AgNPs were also tested.

Methodology

Organism and cultivation

Thermomyces sp. was isolated from a hilly environment and subcultured on potato dextrose agar slant using stock cultures. The slants were cultured for 7 days at 28 °C. 5 mL of sterile water was used to collect the spores. In 250 ml Erlenmeyer flasks, a spore suspension of 0.5 ml was employed as an inoculum for the manufacture of submerged culture Czapek yeast broth (Yeast extract-5.0, Sucrose-30, $NaNO_3$-3, KCl-0.5, K_2HPO_4 and $MgSO_4$ pH 7.0). For 5 days, the culture medium was kept at 28 °C for 72 hours in an incubator.

Extraction of yellow pigment from Thermomyces sp culture

The grown-up culture was filtered after incubation to remove the fungal biomass from the broth. One volume of 95% (v/v) methanol was added to the filtrate, and the mixture was shaken for 30 minutes at 150 rpm and 35°C on a rotary shaker. The mixture was then centrifuged for 15 minutes at 5000 rpm. The filtrate was utilized to make silver nanoparticles.

Preparation of silver nanoparticles using fungal pigment extract

One millimolar $AgNO_3$ was added to 100 mL of fungal filtrate and cultured for a day at room temperature in the dark. Conical flasks containing either fungal filtrate or $AgNO_3$ served as positive and negative controls, respectively. The formation of nanoparticles was confirmed using a Perklin Elmer FT-IR in AT mode with a resolution of 0.2 nm at 40-4000 nm.

Characterization of silver nanoparticles

A Shimadzu UV-1601 Spectrophotometer was used to quantify the silver nanoparticles generated from the fungal filtrate. TEM (The FEI Technai) and SEM were used to determine the morphology of the silver nanoparticles (FEI-Quanta 250). The presence of elemental silver was verified by EDX analysis [8].

Dyeing of cotton and silk by silver nanoparticles

Silver nanoparticles and fungal pigment were applied to cotton and silk fabric. As a control, pre-washed cotton and silk textiles were colored with fungal pigment. The antifungal activity of textiles was tested. The antifungal activity of textiles was tested by immersing textiles in 10% fungal pigment extract at 60 °C for 20 min retaining material to liquor ratio 1:25, shade dried without squeezing. Similarly, pre-washed cotton and silk were submerged for 4 hours in a silver nano solution containing fungal pigment [9].

Fastness testing

South India Textile Research Association (SITRA), Coimbatore, examined the colored samples using Indian standard procedures. The particular tests were IS-766-88 color fastness for rubbing and IS-687-79 color fastness for washing [10].

Antimicrobial activity assessment of nanomaterial dyed textile material

The colored, sterilized silk samples were inserted in a 10 ml tube of nutritional broth containing *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli*, and incubated at 37 °C for 16 hours. The bacterial reduction was estimated using the following equation:

$$RZ\ (\%) = \left(\frac{BZ - AZ}{AZ} \right) * 100$$

Where AZ is the colony-forming unit CFU / ml of the treated sample after incubation and BZ the CFU / ml of the untreated sample after incubation [11].

Results and Discussion

Due to its potential for usage in a variety of sectors, the most crucial and challenging component of nanotechnology is to develop nanostructures that are controlled. There have been various publications on adjusting the parameters of the AgNP synthesis process to boost yield, size, and stability. Furthermore, various microorganisms have been employed to synthesize AgNPs, although the majority of them have low synthesis efficiency and a long reaction time. AgNPs were developed as a new generation of antimicrobials, primarily for application as coating materials, because of their larger surface area, nanosized silver is more active than bulk silver compounds. Higher AgNP concentrations result in greater antibacterial activity. Our findings show that improved antibacterial activity may be obtained by combining AgNPs and the pigment produced by *Thermomyces* [12-13].

Biosynthesis of silver nanoparticles by Thermomyces fungal pigment extract

The first stage in the synthesis of silver nanoparticles by fungus is the trapping of Ag+ ions at the surface of the fungal cells, followed by the reduction of the silver ions by enzymes present in the fungal system [14]. The Pigment from *Thermomyces* was used to successfully generate extracellular yellow pigment and silver nanoparticles. The hue of the filtrates changed from pale yellow to reddish-brown 72 hours after the addition of $AgNO_3$. In Figure 1, the emergence of a brown hue in the solution is a clear sign of the presence of silver nanoparticles in the reaction mixture. Controls (no silver ion) showed no color change. The activation of surface plasmon vibrations (basically the oscillation of group conduction electrons) in the silver nanoparticles causes the solution's color to shift [15].

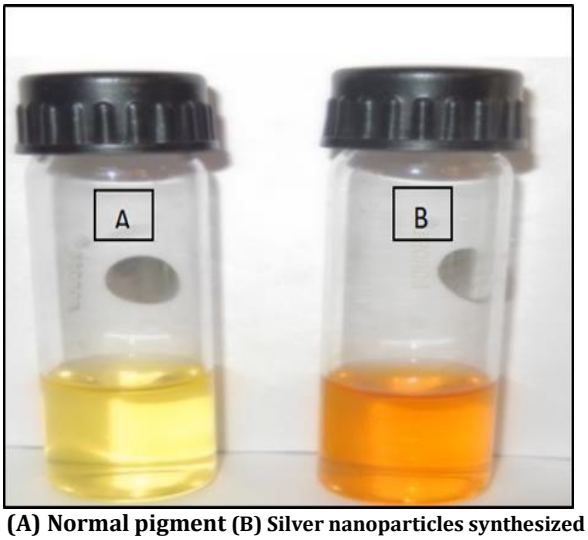

Figure 1. (A) Normal pigment (B) Silver nanoparticles synthesized pigment

UV-Vis spectroscopy

Each metal nanoparticle has a unique surface plasmon resonance (SPR) value, UV-Vis is the most significant approach for confirming the biogenesis of metal nanoparticles. UV-Visible spectroscopy was used to sample and scan the reaction mixture at 12, 24, 48, 72, and 96 h intervals. After 24 hours, a strong wide signal at 440 nm reveals the conversion of silver nitrate into silver nanoparticles in Figure 2. The absorbance maxima (max) of biosynthesized silver nanoparticles were detected in the white rot fungus at 420-430 nm [16-17]. The 480 nm peak of Aspergillus niger 2587 produced AgNPs [18].

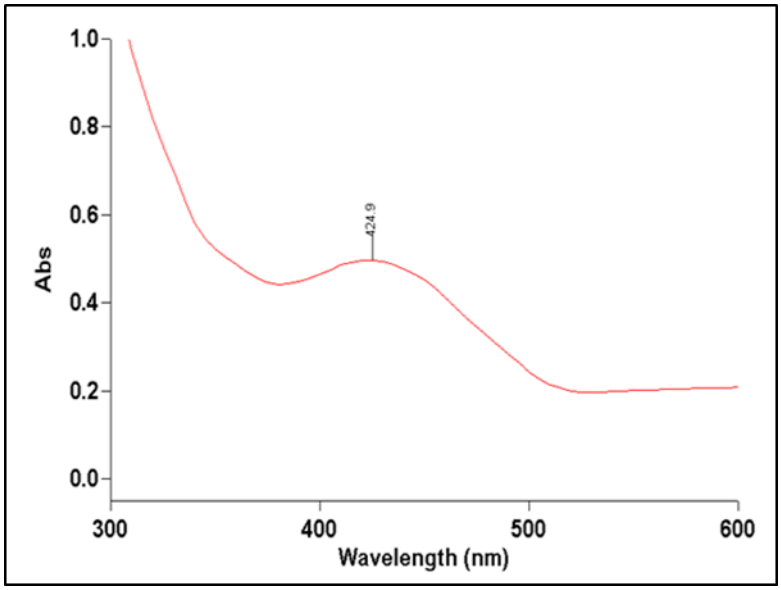

Figure 2. UV-Visible absorption spectrum of silver nanoparticles produced by *Thermomyces* sp

Transmission electron microscope (TEM)

Transmission electron microscopy was used to analyze the synthesized silver nanoparticles. The results revealed that the size of the nanoparticles ranged from 10 to 50 nm (Figure 3).

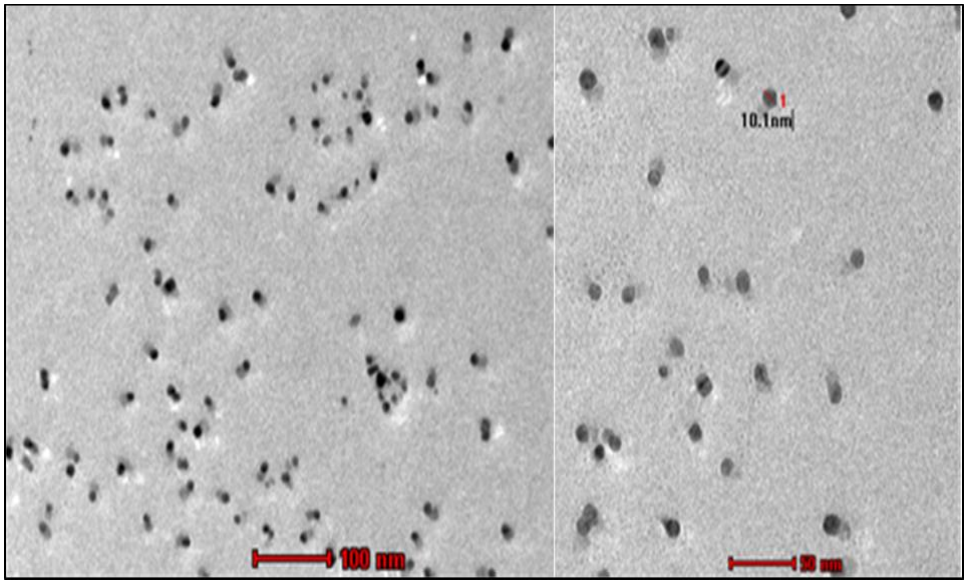

Figure 3. Silver nanoparticle synthesized by *Thermomyces* sp. visualised under TEM particle size 10.1 nm

The AgNPs formed by *A. terreus* were found to be spherical, with an average diameter of 3-27 nm. The resulting AgNPs were a polydispersed mixture with an average diameter of 35nm [19].

Scanning electron microscopy (SEM)

SEM analysis revealed that the nanoparticles are scattered and have a roughly spherical form (Figure 4). The Figure 4B depicts the elemental analysis results acquired from EDX. The presence of silver nanoparticles is confirmed by the peak of Ag at 3eV.

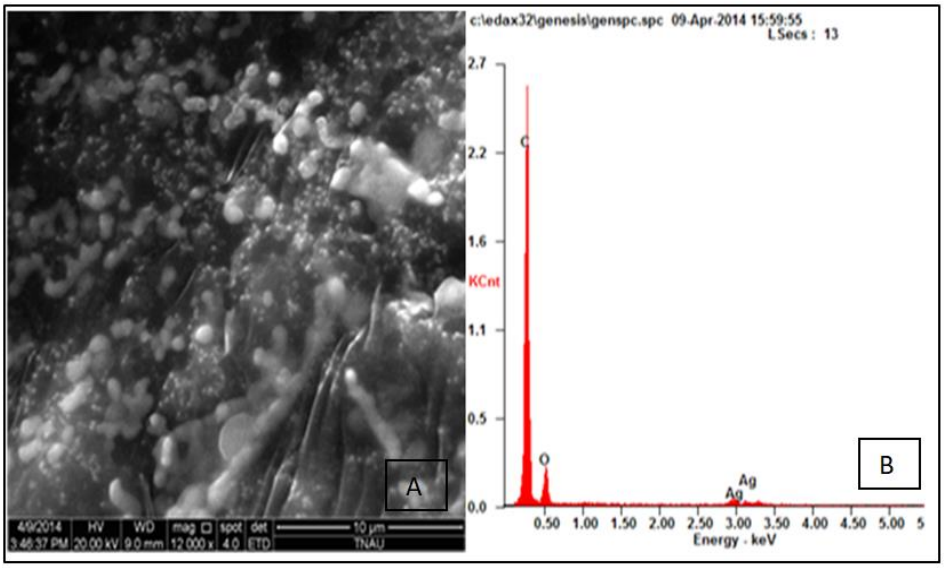

Figure 4. (A) Silver nanoparticle synthesized by *Thermomyces* sp. visualised under SEM. The particles are smooth and spear shaped (B) EDX spectrum SEM image

FT-IR analysis

The FT-IR spectra of silver nanoparticles in combination with pigment and pigment alone indicated that nanoparticles had absorption peaks at 3371, 3325, 1735, 1627, 1365, and 1219 cm1 due to N-H, C=O, C=C, C-H, and C-O functional groups (Table 1 and Figure 5).

Table 1. Comparative analysis of FT-IR spectrum of nanoparticle along with pigment and pigment alone

Pigment alone			**Pigment along with nanopartilce**		
Absorption (cm-1)	Group	Compound Class	Absorption (cm-1)	Group	Compound Class
			3371	N-H stretching	aliphatic primary amine
			3325	N-H stretching	secondary amine
1735	C=O stretching	α,β-unsaturated ester	1735	C=O stretching	α,β-unsaturated ester
			1627	C=C stretching	alkene
1365	C-H bending	alkane	1365	C-H bending	alkane
1219	C-O stretching	vinyl ether	1219	C-O stretching	vinyl ether

The peaks obtained with pigment were less strong, with a minor shift in the silver nanoparticles generated pigment. According to the FT-IR data, the pigment may be a reducing agent that aided in the creation of nanoparticles. Furthermore, the pigment is capable of producing a protective covering on the silver nanoparticles, preventing particle aggregation and stabilizing it. The pigment is water soluble due to its reducing nature [20].

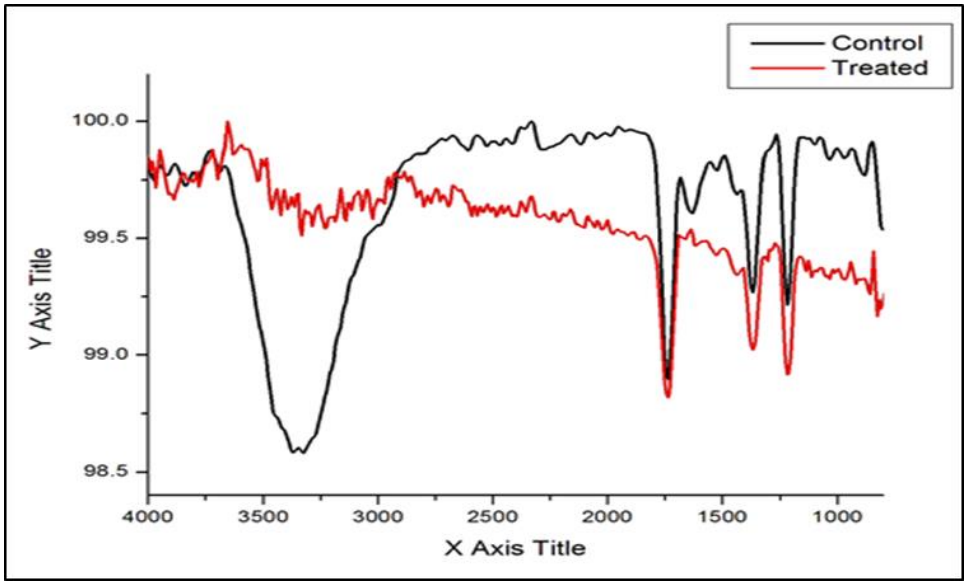

Figure 5. FTIR spectra of synthesized silver nanoparticles and pigment

Silver nanoparticle dyeing in silk and cotton

The yellow pigment made from silver nanoparticles was dyed in cotton and silk using natural mordants. The coloring behavior and fastness findings are also shown below.

Fastness properties of silver nanoparticle dyed fabric

Rub and wash fastness (dry and wet) of colored silk and cotton fabric samples are scored 4 and 5, respectively. The wash fastness grade is also tested by staining on cotton, wool, acrylic, polyester, and nylon, with 4 and 5 ratings. The findings reveal that the cloth has high fastness properties (Table 2 and Figure 6) [21]. Cotton has a limited affinity for fungal pigments, but silk has a high affinity for yellow pigments, and silk fabric is colored using natural fungal extract.

Table 2. Fastness properties of silver nanoparticle dyed fabrics

Fastness property	**Rating**	
	Silk	**Cotton**
Fastness to washing		
Change in color	**5**	**4**
Staining on (light)		
Wool	5	4
Acrylic	4-5	4-5
Polyester	5	4-5
Nylon	5	4-5
Cotton	4-5	3-4
Fastness to rubbing (Staining)		
Dry	4-5	3-4
Wet	4-5	3-4

The pigments used on silk and wool demonstrated good fastness qualities. The optimal conditions for maximal pigment synthesis should be standardized for commercialization in an environmentally responsible and cost-effective way.

Figure 6. pigment and non-pigmented silk thread along with silver nanoparticles

Antimicrobial activity of silver nanoparticle dyed fabric against human pathogens

The AgNPs showed strong antibacterial action against bacterial pathogens in this study. This pigment inhibits Gram-negative microorganisms such as *Escherichia coli* (75.13% and 72.53%), *Pseudomonas aeruginosa* (76.04% and 81.34%), and *Staphylococcus aureus* (82.32% and 85.62%) in nanoparticle colored cotton and silk fabric (Table 3).

Table 3. Percent reduction of AgNPs synthesized by *Thermomyces* sp fungal pigment against pathogenic microorganisms

Pathogenic Organisms	Reduction rate %	
	Cotton	Silk
Escherichia coli MTCC 443 -	75.13	72.53
Staphylococcus aureus MTCC 902 +	82.32	85.62
Streptococcus pyogenes +	71.25	89.28
Pseudomonas aeruginosa -	76.04	81.34
Candida albicans MTCC 227 +	82.21	85.87

Previous research has shown that the yellow pigment from *Thermomyces* sp may be used in the textile dyeing process. Pathogens were decreased in their effect by natural mordants and yellow pigment. The colored silk fabric's overall color fastness qualities were mediocre. Because of its antibacterial qualities, the fabric may be utilized in medical applications such as bandages, masks, and wound dressings [22]. Cotton textiles treated with AgNPs reduced bacterial activity by 87% to 95% against Gram-positive bacteria represented by *Staphylococcus aureus* ATCC 29213 and *Bacillus subtilis* NCTC 10400, and Gram-negative bacteria represented by *Pseudomonas aeruginosa* ATCC 9027 and *Escherichia coli* ATCC 8739 [23]. AgNPs' oxidative dissolution makes them a suitable reservoir for silver ions, which may be another major explanation for their bactericidal effect [24]

Conclusion

Silver nanoparticles and color produced by *Thermomyces* sp. are ideal for silk and cotton fabrics. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) was used to validate the size and form of AgNPs, which had a size range of 10-50 nm. The FTIR investigation revealed that silver nanoparticles had a good protein binding capacity. AgNPs treated cotton and silk textiles showed antibacterial activity by reducing 71-85% of several harmful microorganisms. The silver nanoparticle based on fungal pigments provides a revolutionary coloring and antibacterial technique for the textile sector.

References

[1] S. Riaz, M. Ashraf, T. Hussain, M. T. Hussain, A. Rehman, A. Javid and K. Iqbal et al., **(2018)**. Functional finishing and coloration of textiles with nanomaterials. Color. Technol., **134:** 327-346.

[2] P. Das, S. Barua, S. Sarkar, N. Karak, P. Bhattacharyya, N. Raza and K.-H. Kim et al., **(2018)**. Plant extract–mediated green silver nanoparticles: efficacy as soil conditioner and plant growth promoter. J. Hazard Mater., **346:** 62-72.

[3] S. Prabhu and E. K. Poulose **(2012)**. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int. Nano Lett., **2:** 32. doi: 10.1186/2228-5326-2-32.

[4] Y. Y. Loo, Y. Rukayadil, M. A. R. Nor-Khaizura, C. H. Kuan, B. W. Chieng, M. Nishibuchi and S. Radu **(2018)**. In vitro antimicrobial activity of green synthesized silver nanoparticles against selected gram-negative foodborne pathogens. Front. Microbiol., **9:** 1555. doi: 10.3389/fmicb.2018.01555.

[5] N. Durán, P. D. Marcato, M. Durán, A. Yadav, A. Gade and M. Rai **(2011)**. Mechanistic aspects in the biogenic synthesis of extracelular metal nanoparticles by peptides, bacteria, fungi and plants. Appl. Microbiol. Biotechnol., **90:** 1609-1624.

[6] D. Ballotin, S. Fulaz, M. L. Souza, P. Corio, A. G. Rodrigues, A. O .Souza and P.M. Gaspari et al., **(2016)**. Elucidating protein involvement in the stabilization of the biogenic silver nanoparticles. Nanoscale Res. Lett., **11:** 313. doi: 10.1186/s11671-016-1538-y.

[7] N. Vigneshwaran, N. M. Ashtaputre, P. V. Varadarajan, R. P. Nachane, K. M. Paralikar and R. H. Balasubramanya **(2007)**. Biolgical synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. Mater. Lett., **66:** 1413-1418.

[8] H. Borchert, E. V. Shevchenko, A. Robert, I. Mekis, A. Kornowski, G. Grübel and H. Weller. **(2005)**. Determination of nanocrystal sizes: a comparison of TEM, SAXS, and XRD studies of highly monodisperse CoPt3 particles. Langmuir, **21:** 1931-1936.

[9] M. Moresi, A. M. Gallo and M. Petruccioli **(2005)**. Assessment of the dyeing properties of pigments from *Monascus purpureus*. J. Chem. Technol. Biotechnol., **80:** 1072-1079.

[10] M. A. Seif and M. M. Hiji **(2016)**. Evaluating the effect of seams on colour fastness properties of textile fabrics. Int. J. Text. Fash. Technol., **6:** 1-14.

[11] A. I. El-Batal, A. A. Hashem and N. M. Abdelbaky **(2013)**. Gamma radiation mediated green synthesis of gold nanoparticles using fermented soybean-garlic aqueous extract and their antimicrobial activity. Springerplus, **2:** 129. doi: 10.1186/2193-1801-2-129.

[12] K. Chaloupka, Y. Malam and A. M. Seifalian **(2010)**. Nanosilver as a new generation of nanoproduct in biomedical applications. Trends Biotechnol., **28:** 580-588.

[13] A. A. Taglietti, C. R. Dagostino, A. Dacarro, L. Montanaro, L. Campoccia, D. Cucca and L. Vercellino **(2014)**. Antibiofilm activity of a monolayer of silver nanoparticles anchored to an amino-silanized glass surface. Biomaterials. **35:** 1779-1788.

[14] P. Mukherjee, A. Ahmad, D. Mandal, S. Senapati, S. R. Sainkar, M. I. Khan and R. Parischa et al., **(2001)**. Fungus mediated synthesis of silver nanoparticles and their immobilization in the mycelia matrix: a novel biological approach to nanoparticle synthesis. Nano Lett., **1:** 515-519.

[15] L. R. Jaidev and G. Narasimha **(2010)**. Fungal mediated biosynthesis of silver nanoparticles, characterization and antimicrobial activity. Colloids Surf B Biointerfaces, **81:** 430-433.
[16] R. P. Metuku, S. Pabba, S. Burra, K. Gudikandula, M. S. Charya. (2014). Biosynthesis of silver nanoparticles from Schizophyllum radiatum HE 863742.1: their characterization and antimicrobial activity. 3 Biotech, **4:** 227-234.
[17] S. Honary, H. Barabadi, E. Gharaei-Fathabad and F. Naghibi **(2013)**. Green Synthesis of Silver Nanoparticles Induced by the Fungus *Penicillium citrinum*. Trop. J. Pharmaceut. Res., **12:** 7-11.
[18] N. Soni and S. Prakash **(2013)**. Possible mosquito control by silver nanoparticles synthesized by soil fungus (*Aspergillus niger* 2587). Adv. Nanopart., **2:**125-132.
[19] A. Fouda and T. I. Shaheen **(2017)**. Silver nanoparticles: biosynthesis, characterization and application on cotton fabrics. Microbiol. Res. J. Int., **20:** 1-14.
[20] N. Jain, A. Bhargava, S.Majumdar, J.C. Tarafdar, J. Panwar **(2011)**. Extracellular biosynthesis and characterization of silver nanoparticles using *Aspergillus flavus* NJP08: a mechanism perspective. Nano, **3:** 635-641.
[21] D. S. Balaji, S. Basavaraja, R. Deshpande, D. B. Mahesh, B. K. Prabhakar and A. Venkataraman **(2009)**. Extracellular biosynthesis of functionalized silver nanoparticles by strains of *Cladosporium cladosporioides* fungus. Colloids Surf. B Biointerfaces, **68:** 88-92.
[22] D. Sharma, C. Gupta, S. Aggarwal and N. Nagpal **(2012)**. Pigment extraction from fungus for textile dyeing. Indian J. Fibre Text. Res., **37:** 68-73.
[23] R. Poorniammal, M. Parthiban, S. Gunasekaran, R. Murugesan and R. Thilagavathi **(2013)**. Natural dye production from Thermomyces sp fungi for textile application. Indian J. Fibre Text. Res., **38:** 276-279.
[24] R. Zhang, J.Yu, X.Guo, W.Li, Y.Xing and Y .Wang **(2021)**. *Monascus* pigment-mediated green synthesis of silver nanoparticles: Catalytic, antioxidant, and antibacterial activity. Appl. Organomet. Chem., **35:**.e6120. doi: 10.1002/aoc.6120.