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Influence of biosynthesized silver nanoparticles on keratinase activity and mycelial growth of dermatophytes

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Objective Among different nanomaterials, silver nanoparticles (SNPs) exhibited high antifungal potency compared with other types of nanoparticles (NPs), and this property is often very helpful, particularly against fungi resistant to conventional antifungal agents. However, synthesis of SNPs can generate toxic waste during the preparation process. Accordingly, new technique using non-toxic routes have been researched for the synthesis of SNPs using cell-free filtrate of *Aspergillus niger* and evaluate their effect against some dermatophytes spp.
Methods The proposal of our study was to biosynthesize SNPs using cell-free filtrate of *Aspergillus niger* as reducing agent. The characterization of biosynthesized SNPs was carried out by UV-Visible spectroscopy, Fourier transform infrared spectroscopic analysis (FTIR) and scanning electron microscopy (SEM). The antifungal effect of the NPs against dermatophytes was also evaluated. The minimum inhibitory concentration (MIC) was determined by broth microdilution method.
Results Spherical NPs 15–50 nm in size were obtained. The biosynthesized SNPs exerted pronounced morphological alteration in the fungal mycelia. Additionally, the inhibition of keratinase activity of the tested dermatophytes was also recorded.
Conclusion The results indicate that SNPs can be synthesized in ecofriendly, inexpensive and promising technique by fungal strain of *A. niger*, and it has considerable antifungal activity in comparison with other antifungal drugs.
Keywords SNPs, biosynthesis, *Aspergillus niger*, dermatophytes, antifungal activity, keratinase, mycelium

Introduction

Dermatophytes – a group of fungi that infect the keratinized outermost layer of the skin such as nail, hair and the stratum cornium. Dermatophytes including several species belong to the *Microsporum*, *Epidermophyton* and *Trichophyton* genera. It has the ability to invade and grow in dead keratin and exhibited variable efficiency in producing extracellular enzymes particularly keratinase, which plays an important role in the virulence of this fungus. Infection is generally cutaneous and restricted to the non-living cornified layers because of the inability of the fungi to penetrate the deeper tissues or organs of hosts.[1] Several antifungal compounds, mainly azole, have been used to treat dermatophytosis, but fungal resistance to the many azole derivatives appears very commonly.[2] Moreover, the azole-containing medicines may have many adverse effects and drug interactions, it interferes with the activity of hepatic enzymes, the central nervous system, thyroid and sex hormones, and biosynthesis of testosterone.[3] Therefore, there is an urgent medical need for novel antifungal agents. The metallic NPs are most promising antimicrobial agents as they contain superior properties due to their large surface area to volume ratio.[4] SNPs have attractive the researcher interest. Compared with other metals, silver exhibits higher toxicity to microorganisms while it exhibits lower toxicity to mammalian cells.[5] The major methods used for conventional synthesis of SNPs are the physical and chemical methods. The problem with these methods is that the synthesis is energy and capital intensive and often employ toxic chemicals, as well as some chemically toxic substances being absorbed on the surface of NPs raising the toxicity issues and can hinder their usage in medical applications.[6] The biological methods for NP synthesis might offer inexpensive, nontoxic, clean, and eco-friendly alternatives. There are three major sources of biological synthesis of SNPs: plant extracts, bacteria and fungi.[7] Fungi have some distinct advantages when used as bio factories for NP production, in comparison with bacteria, fungi can secret larger amounts of proteins which directly translate to higher productivity of NPs.[8] Moreover, fungi have an additional advantage that downstream processing and handling of the fungal biomass would be much simpler.[9] Biosynthesis of SNPs by using a fungus *Aspergillus*,[10] *Trichoderma*[11] and *Fusarium*[12] has been reported. In this context, the current study focused on a cost effective and environment-friendly technique for the synthesis of SNPs using cell-free filtrate of *A. niger* and evaluate their effect against some dermatophytes spp.

Materials and Methods

Fungal Strain and Their Maintenance

The fungal strain of *A. niger* was obtained from Agriculture College/Al-Kufa University (kindly provided by Prof. Dr. Majeed M. Dewan), and it previously was isolated from soil sample and diagnosed by PCR technique. The fungus was subcultured on Potato Dextrose Agar (PDA) (Oxoid, India) at 28°C for 96 h and then refrigerated at 4°C until used for biosynthesis of SNPs.

Fungal Biomass Production

To prepare the fungal biomass for NP biosynthesis, the *A. niger* was cultured aerobically in PDB. The broth was supplemented with chloramphenicol (50 μg/ml) as an antibacterial agent. The flasks containing above media were incubated at 28°C for 7 days in shaking incubator (Lab Tech, India) and agitated at 100 rpm. Then, fungal mycelia were separated from broth by filtration with sterile Whatmann filter paper No. 1 and the settled mycelia were washed thrice with sterile distilled water to remove any medium components from the biomass that might interact with metal ions. Twenty grams of fungal biomass was inoculated in 200 ml deionized water for 72 h and agitated as earlier described. After incubation, the cell filtrate was separated by filtration. The filtrate was further used for biosynthesis of NPs.[13]

Biosynthesis of NPs

For the biosynthesis of SNPs, 50 ml of cell-free filtrate was mixed with 50 ml of 1mM $AgNO_3$ in 250 ml Erlenmeyer flask and kept in shaking incubator at 150 rpm at 28°C for 24 h. Simultaneously, a positive control of cell filtrate without metal salts and a negative control containing only metal salts solutions were run along with the experimental flasks.[14] All reaction mixtures were kept in dark to avoid any photochemical reactions during the experiment.

Characterization of Biosynthesized SNPs

UV-Vis Spectroscopy Analysis

The detection of SNPs was primarily carried out by visual observation of color change of the fungal filtrate after treatment with silver nitrate. Appearance of dark brown colour of fungal cell filtrate indicates the formation of SNPs due to reduction of pure silver ions. Further, the formation of SNPs was confirmed with the help of dual beam UV-Visible spectrophotometer (SPEKOL1300, Germany), through sampling of 1 cm^3 of reaction solution at different time intervals and scanning the absorbance spectra in 300–700 nm range of wavelength at a resolution of 1 nm.

Fourier Transform Infrared Spectroscopy (FTIR)

The interaction between the biosynthesized SNPs and biomolecules, which responsible for reduction, capping and stabilization of the SNPs in colloidal solution; was analyzed using FTIR spectrophotometer (Bruker Tensor 27, Germany) in the range of 500–4000 cm^{-1}.

Scanning Electron Microscopy (SEM) studies

The biosynthesized SNPs were also subjected to SEM analysis (Inspect S50, The Netherlands) to evaluate their size and morphological characteristics.

Antidermatophytic Effect of Biosynthesized SNPs

Tested Microorganisms

Two types of dermatophytes were used to evaluate the antidermatophytic effect of biosynthesized SNPs, these fungi are: *Trichophyton interdigitale and Epidermophyton floccosum*. These cultures grown on Sabouraud dextrose agar (Oxoid, India) at 35°C then maintained on SDA slant at 4°C until use.

Determination of MIC Value Against Dermatophytes

The minimal inhibitory concentration (MIC) of the silver NPs and other antifungal agents for tested dermatophytes was determined by using a broth microdilutions method, when possible, according to the guidelines of the National Committee for Clinical Laboratory Standard (CLSI) as described in document M38-A2 for filamentous fungi.[15] Briefly, aliquot of 100 µl of the inoculum of spore suspensions of the tested strains (1–3 × 10^3 cells/ml) were inoculated into U-bottomed, sterile, disposable, 96-well microdilution plates filled with aliquots of 100 µl of the serially diluted SNPs (2x final concentration from 0.156 to 80 µg/ml), Inoculated medium free of SNPs was served as growth control. Miconazole (0.03 to 16 µg/ml) and fluconazole (0.125 to 64 µg/ml) were running on the same way and used as an antifungal reference standard for comparison. The micro plates were incubated at 35°C for *T. interdigitale*, while *E. floccosum* were incubated at 30°C and readings were made visually every 24 h until fungal growth in the drug-free control wells was shown. The experiments were carried out in duplicate, and the optical densities were recorded by a spectrophotometer at 450 nm in a microtiter plate reader (DNM9602, Germany). The MIC was determined as the lowest concentration resulted in inhibition of fungal growth.[16]

Minimum Fungicidal Concentration Assay

The minimum fungicidal concentration (MFC) of SNPs against tested dermatophytes was performed. For that aliquot of the contents of all clear wells were subcultured onto SDA plates, a positive control (from growth control well) and a negative control (from sterility control well) were included in this test. The plates were incubated until the appearance of growth in the growth control subcultures. The MFC endpoints were recorded as the lowest concentration of the tested agents which showed no fungal growth or fewer than three colonies to obtain approximately 99–99.5% killing activity.[17]

Determination of the Inhibitory Effect of Biosynthesized SNPs on the Keratinase Activity

The production of crude keratinase enzyme was performed by growing the dermatophytes on a liquid keratin induction medium described by Wawrzkiewicz et al.,[18] and incubated at 30°C for 14 days in shaking incubator (Lab Tech, India). After the incubation time, the fungal mycelia were removed from culture media by filtration. The resulted cell-free filtrate centrifuged at 4000 rpm for 5 min, and the supernatant was used as the crude enzymes.

The keratinase activity was done by using Muhsin and Aubaid[19] method with slight modification. Briefly, 0.5 ml of cell-free supernatant, 50 mg of chicken feathers were mixed in 5 ml of phosphate buffer (0.03 M). The mixture was then incubated at 37°C for 2 h with gentle shaking.

The effect of biosynthesized SNPs on the activity of keratinase enzyme was tested by incubating the enzyme with SNPs at concentration of MIC value for each isolate. The Miconazole and fluconazole were used for comparison. At the end of incubation (2 h), the reaction was stopped by keeping it in an ice for 10 min. Then, the feathers were separated from mixture by filtration using Whatmann filter papers No. 1. The keratinase activity was determined by reading the absorbance of the resulted filtrate spectrophotometrically at 280 nm using UV-vis spectrophotometer. An increase in absorbance value 0.1 was considered as equivalents to 1 unit enzyme activity (KU)/ml.

Effect of Biosynthesized SNPs on the Mycelia Development of Dermatophytes

The experiment begun by supplementation of 50 ml of SDB with SNPs at sub-MIC concentration (in order to allow some fungal growth), then a 2-mm discs of 7 days old fungal culture of dermatophytes spp. on PDA were transferred into each flask. A broth medium without SNPs was inoculated and considered as control. The inoculated flasks were incubated for 7 days at 30°C in a shaking incubator. All experiments have been performed in duplicate.

For documentation, a sample from each flask was withdrawn and observed under light microscope at 100x objective lenses and a picture of the mycelium was taken.

Statistical Analysis

Statistical analysis was performed by Social Science Statistics and the Statistical Package for Social Sciences version 19. All data were described by mean ± SD (standard deviation). ANOVA test was used to analyse the statistical significance of difference in mean between groups. The statistical tests considered that *P*-value less than the 0.05 level was statistically significant.

Results

In present study, biosynthesis, characterization and optimization of SNPs were successfully accomplished. The biological synthesis of SNPs was carried out by reduction of aqueous silver ions (Ag^+) using cell-free filtrate of *A. niger*.

Characterization of Biosynthesized SNPs

At the end of growth period of *A. niger*, the cell-free filtrate was used for biosynthesis of SNPs. The filtrate was initially pale yellow in colour. When the filtrate challenged with $AgNO_3$, the colour of the mixture was turned to yellowish brown at first and then the intensity of the colour was increased with the period of incubation, so the colour was changed to dark brown on completion of the reaction with Ag^+ ions. Color change was noticed only in the test flask and it a clear indication for the formation of SNPs in the reaction mixture. The remaining two control flasks i.e., aqueous solution of $AgNO_3$ and fungal filtrate without $AgNO_3$ showed no change in colour when incubated in the same condition (Fig. 1).

The formation and stability of the reduced SNPs in colloidal solution was detected and monitored by using UV-visible absorption spectrum (Fig. 2). The analysis was evaluated at different times after the start of the reaction. The λ max 420 nm was observed only in the test flask which confirmed the production and indicating the specific surface Plasmon resonance of SNPs. The scanning was continued and absorbance was recorded every 24 h.

FTIR spectrum of biosynthesized SNPs revealed the presence of different distinct peaks located at 3421, 2962, 2926, 2854, 1638, 1554, 1428, 1410, 1333, 1276, 1256, 1239, 1073, 1048, 782, 467 cm^{-1} (Fig. 3). The peak at 3421cm^{-1} is ascribed to the N-H stretch vibration of primary amides of protein. The peaks at 2926 cm^{-1} and 2961 cm^{-1} could be due to the C-H stretch of the methylene groups of protein and to N-H stretching of amine salt. The absorption peak at 2854 cm^{-1} may be assigned to the C-H symmetrical stretch vibration of alkenes. The peak at 1638 cm^{-1} corresponds to the presence of amide I and amide α, which arises due to the carbonyl stretch and N-H stretch vibration while the band at 1554 cm^{-1} refers to C = C stretch corresponding to an aromatic ring. Peaks located at 1410 cm^{-1} and 1428 cm^{-1} may be related to COO^- symmetrical stretch from carboxyl groups of the amino acid residues. The peak at 1333 cm^{-1} corresponds to carbon hydrogen (CH_3) bending vibration. The peak located at 1276, 1256 and 1239 cm^{-1} represent C-O stretching of primary alcohol and *P* = O stretching, respectively. The bands at 1073 cm^{-1} and 1048 cm^{-1} refer to C-O bonds of aliphatic amines while the peaks at 782 cm^{-1} and 467 cm^{-1} can be assigned to the aromatic C-H out of plane bending vibration of aromatic primary amines.

SEM has been employed to determine the shape and morphology of biosynthesized SNPs. Figure 4 reveals SEM micrograph of SNPs obtained by the reduction of $AgNO_3$

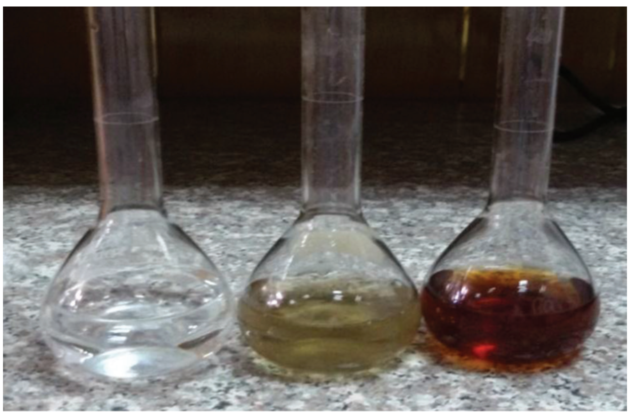

Fig. 1 **Culture flasks containing (A) 1mM $AgNO_3$ solution, (B) Fungal cell-free filtrate and (C) Mixture of fungal cell-free filtrate with 1mM $AgNO_3$.**

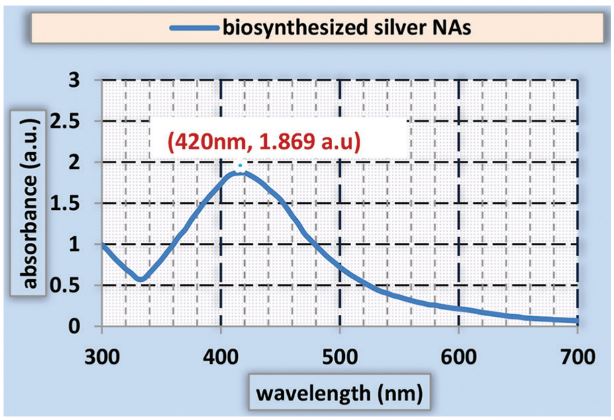

Fig. 2 **UV-Vis spectrophotometer analysis of biologically synthesized SNPs.**

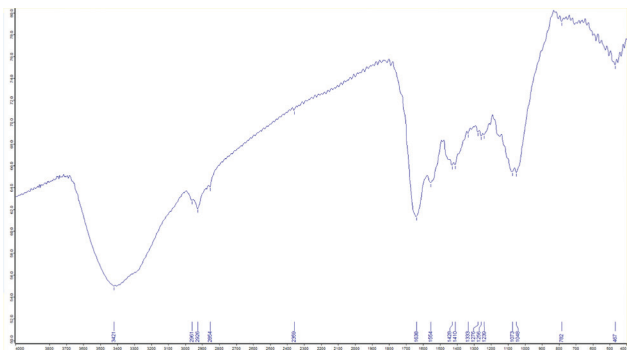

Fig. 3 **FTIR spectrum of SNPs, synthesized by *A. niger*, with distinct peaks.**

solution with cell-free filtrate of *A. niger* after 120 h of reaction. The morphology of NPs was spherical in shape, uniformly (monodispersed) without significant aggregation. The particle size was ranged from 15 to 50 nm.

The antifungal effect of biosynthesized SNPs was investigated against *Trichophyton interdigitale* and *Epidermophyton floccosum*. The reference antifungal drugs (miconazole and fluconazole), were used as a positive control for comparison with activity of SNPs. The obtained results, presented in Table 1, revealed that the SNPs (in the range of 0.156 to 80 µg/ml) showed significant antifungal activity against tested dermatophytes, the later exert significant variation in their susceptibility depending on fungal species. *E. foloccosum* was the most sensitive dermatophytes to SNPs, with MIC value of 0.625 µg/ml, while *T. interdigitale* showed slightly higher value than *E. floccosum* with inhibitory concentration at 1.250 µg/ml.

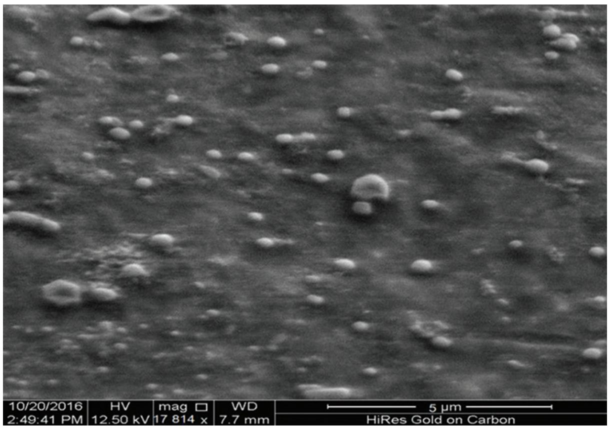

Fig. 4 **SEM micrograph of biosynthesized SNPs. The image shows size and spherical shape of monodisperse SNPs.**

Fluconazole, with an MIC range from 0.125 to 64 µg/ml, only exhibited an antifungal activity against *T. interdigitale* with relatively high MIC value about 8 µg/ml. On the other *E. foccosum* was completely resistant to all drug concentration used in our work. The lowest MIC of Miconazole, at the concentration of 1 µg/ml, was obtained against *T. interdigitale*. While *E. foccosum* was less sensitive to this antifungal agent, with MIC value reaching 4 µg/ml.

Simultaneously with the MIC of SNPs and standard antifungal drugs, their minimum fungicidal activities against the tested dermatophytes were assessed. As shown in Table 2, the obtained MFCs are considerably higher in comparison to MICs. SNPs show excellent fungicidal activity against *T. interdigitale* and *E. floccosum*, with low MFC value 3.54 µg/ml, 5 µg/ml, respectively, compared with those obtained by standard antifungal drug, Miconazole, 4 µg/ml and 8 µg/ml, respectively. The common antifungal drug, fluconazole, had no fungicidal effect on the tested dermatophytes except *T. interdigitale* with value of 32 µg/ml whereas for *E. floccosum* the MFC value were greater than the highest tested concentration (64 µg/ml), indicating that this isolate was resistant to this common antifungal drug.

The results indicated that the SNPs applied at MIC value caused significant and differential reduction in keratinase activity depending on the tested species. The effects of SNPs on keratinase enzyme activity are mentioned in Table 3.

Table 1. **Comparative MIC value of biosynthesized SNPs, fluconazole and miconazole against dermatophytes strains as proposed by CLSI (broth microdilution method)**

Tested fungal strains	MIC/Mean (µg/ml)					
	SNPs (0.156–80)	FCZ (0.125–64)	*P*-value	SNPs (0.156–80)	MCZ (0.03–16)	*P*-value
T. interdigitale	1.250	8	0.0513	1.250	1	>0.05 (Ns)
E. floccosum	0.625	>64	<0.001	0.625	4	>0.05 (Ns)

FCZ, fluconazole; MCZ, miconazole.

Table 2. **Comparative MFC value of biosynthesized SNPs, fluconazole and miconazole against dermatophytes strains as proposed by CLSI (broth microdilution method)**

Tested fungal strains	MFC/Mean (µg/ml)					
	SNPs (0.156–80)	FCZ (0.125–64)	*P*-value	SNPs (0.156–80)	MCZ (0.03–16)	*P*-value
T. interdigitale	3.54	32	< 0.001	3.54*	4	>0.05 (Ns)
E. floccosum	5	>64	<0.001	5	8	>0.05 (Ns)

Table 3. **Effect of biosynthesized SNPs on Keratinase activity of the tested dermatophytes as compared with miconazole and fluconazole as reference antifungal drugs**

Dermatophytes	Keratinase activity (KU)						
	Control (Mean ± SD)	SNPs		Miconazole		Fluconazole	
		Activity	Reduction%	Activity (Mean ± SD)	Reduction%	Activity (Mean ± SD)	Reduction%
T. interdigitale	9.47 ± 0.65	6.267 ± 1.25	33.82	7.77 ± 1.24	17.97	6.6 ± 0.656	30.23
E. floccosum	11.03 ± 2.15	5.83 ± 1.457*	47.41	8.7 ± 0.67	26.01	0	0

*Significant ($P < 0.05$) in compared with control.

The maximum reduction in activity was recorded with *E. floccosum* (47.41%), which had significant values compared with control at $P < 0.05$, while treatment with Miconazole resulted in lower enzyme reduction (26.01%). Although the SNPs inhibited the keratinase activity of *T. interdigitale* (33.82%), the effect was insignificant; a similar effect was demonstrated by both miconazole and fluconazole (17.97%, 30.23%, respectively).

Our results showed that fungal exposure to biosynthesized SNPs caused drastic changes in the mycelial morphology of all strains of dermatophytes compared with untreated controls. The microscopic observation was showed that SNPs clearly damaged the hyphae with severe distortion. It could be clearly seen that SNPs either attached to the mycelium wall or penetrated the mycelial filaments which visualized as black aggregations. The vast majority of hypha were swelling (Fig. 5) and often appeared wider than normal hyphae with presence of large vacuoles inside them. Observation of *T. interdigitale* under light microscope, after exposure to SNPs, showed collapsed hyphae, wall disorganization and loss of integrity of their biological membranes indicating extensive cellular death (Fig. 6). The presence of chlamydospores was abundant in all the tested dermatophytes (Fig. 7).

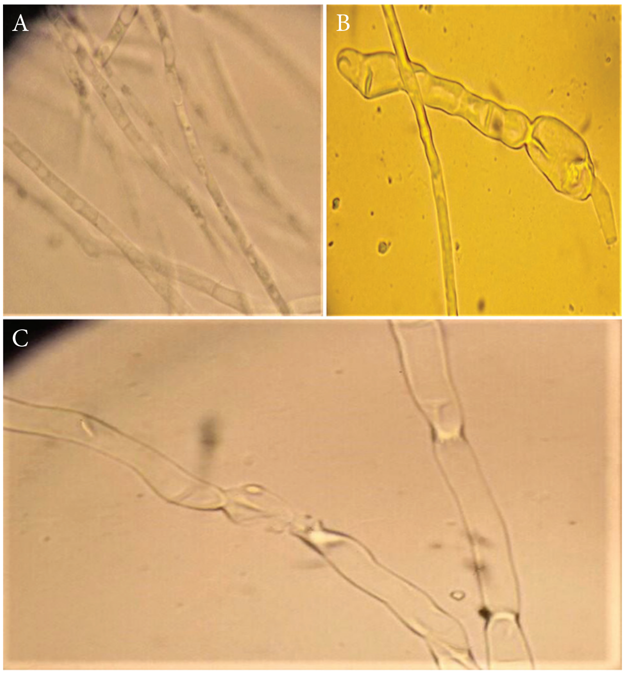

Fig. 6 **Effect of SNPs on mycelial growth of *T.interdigitale* (A) Normal mycelia in control sample (B) & (C) deformed and damaged hyphae with obvious cracks on the cells wall.**

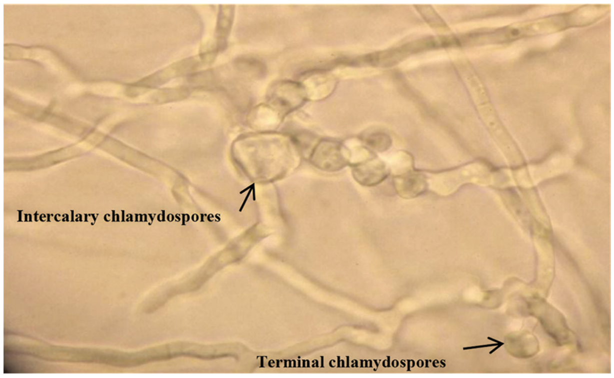

Fig. 7 **Effect of SNPs on mycelial growth of *E.floccosum* (A) normal mycelia in control sample (without treatment). (B) Distorted mycelium due to effect of SNPs, it exhibited a terminal and an intercalary chlamydospores in the middle of hyphae (or perhaps the hyphae itself running through it) X1000.**

Discussion

SNPs had been utilized in various aspects like energy production, optical receptors, consumer product, tissue engineering, biolabelling and antimicrobial agents.[20] Application of SNPs in these fields is dependent on the ability to synthesize particles with different chemical composition, shape, size and monodispersity. Development of simple and ecofriendly method would help in developing further interest in the synthesis and application of metallic NPs. In this respect, nature has provided exciting possibilities of utilizing biological systems such as microorganism for this purpose. In general, fungi tolerate higher metal concentrations than bacteria and secrete abundant extracellular redox proteins to reduce soluble metal ions to their insoluble form and eventually to nanocrystals.[21] Therefore, we have successfully demonstrated an easy, rapid and efficient route for extracellular synthesis of SNPs by employing the cell-free filtrate of *A. niger*. This type of synthesis has preference on the intracellular synthesis as the later demands additional steps of releasing the NPs from the biomass by certain chemical methods or ultrasound treatment and purification of it. It was observed that after addition of the silver ion into the flask containing the cell filtrate, the color of the medium changed from pale yellow to brown, which indicates the formation of colloidal SNPs in the medium. The brown color of the medium could be due to the excitation of surface plasmon vibrations and typical of the SNPs.[22] The results of UV-Visible spectrophotometer showed strong and characteristic surface plasmon resonance centered at 420 nm. Many studies confirmed that the fungal cell filtrate treated with $AgNO_3$ solution gave a peak around 420 nm,[23,24] which supports our finding and indicating the biosynthesis of SNPs by *A. niger*. FTIR spectrum indicated that *A. niger* cell-free filtrate contains active biomolecules responsible for the biotransformation of silver ions to SNPs. The IR spectra reveal the presence of NH group as well as the carbonyl group, which attributed to the peptide linkage of the fungal filtrate and many other functional groups resulted from amino acid residue and peptide protein. Thus, the presence of the signature peaks of amino acids supports the presence of proteins in cell-free filtrate and revealed that secondary structure of proteins have not been

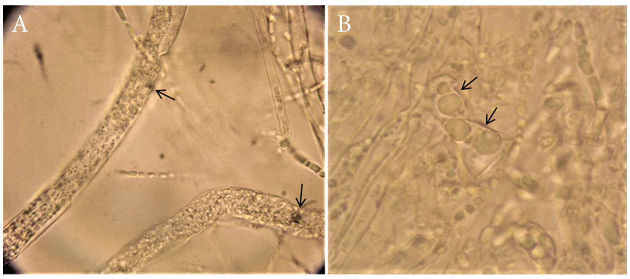

Fig. 5 **Fungal mycelium incubated with SNPs (A) Swelling hyphae which appear wider than the normal with presence of SNPs aggregates.(B) large vacuoles inside the hyphae.**

affected as a consequence of reaction with silver ions or binding with SNPs. In addition, these results confirmed that amino acid residues and peptides of proteins have a stronger ability to bind with metal and capping it to prevent agglomeration of the particles and stabilizing in the medium.[14] These findings resemble with the results of Gole et al.[25] The nanostructural studies of SEM micrograph showed SNPs to be spherical in shape and are uniformly distributed (mono dispersed) without significant agglomeration. The monodispersity of NPs attributed to the capping agents which provide stability of NPs and prevent agglomeration of it. These results were compatible with Elgorban et al.[26] who obtained spherical SNPs by extracellular synthesis of SNPs using *Aspergillus versicolor*.

In the present study, the MIC of the SNPs on 2 isolates of dermatophyte species was investigated. To our knowledge, this is the first study that applies SNPs successfully to *T. interdigitale*. Several authors obtained higher or lower MIC and MFC values against *E. floccosum* than those reported in the present study.[27,28] This variation in SNP toxicity level found for the fungal species in this research compared with the same species demonstrated by other authors may be attributed to the difference in susceptibility pattern of the strains or may be explained by differences in the method used for NPs synthesis and subsequent stabilization. Our data are in agreement with those reports proved that fluconazole had less antifungal activity against dermatophytes.[29,30] Prolonged usage of azole agents as well as emergence of fungal spp. that have decreased susceptibility or intrinsic to these drugs have resulted in increased resistance and treatment failure.[31]

The SNPs exhibit efficient antimicrobial property due to their extremely large surface area, which enable better contact with microorganisms.[4] It is suppose that fungi carry a negative charge while the NPs release ions carry a positive charge, thus an electrostatic attraction between the NPs and microbe will be created. As a result, the microbe will oxidize and killed.[32]

Keratinases are key proteolytic enzymes produced by dermaptophytes; in the past few decades, a number of research projects have focused on the activities of keratinases and their role in the virulence of dermatophytes such as *Trichophyton*.[19] It was clear that presence of SNPs in the growth medium act as an enzyme inhibitor, and their activity was decreased significantly in some of tested dermatophytes. Our finding agrees with Ouf et al.[28] A number of hypotheses have been proposed to explain the mechanism of keratinase inhibition by SNPs; it has been suggested that silver ions (Ag^+), which released from NPS, can interact with sulphur-containing proteins; or may attach to the sensing surface of C-terminal residue of amines in keratinase enzyme, all these events lead to modification and enzyme inactivation.[33,34] The present experiments indicate a possibility of using the biosynthesized SNPs as a very useful agent to reduce the keratinolytic activity of dermatophytes.

The biosynthesized SNPs induced considerable morphological changes in the hyphae of the tested dermatophytes. Swelling of hyphal cells, as determined by microscopical examination, is indicative of alterations in the cell wall structure. In addition, the presence of large vacuoles inside hyphae was detected, altered vacuolar morphology and physiology have been associated with impairment of hyphal growth and virulence in different human pathogens such as *Candida albicans*.[35] It is evident that chlamydospores were abundant in SNPs-treated dermatophytes. A fungus can survive unfavourable environmental conditions by forming chlamydospores.[36] Therefore, excessive production of chlamydospores was induced by the presence of SNPs in the growth medium. Observation under light microscope revealed that most hyphae undergo wall disorganization and loss of integrity of their biological membranes indicating extensive cellular death after exposure to SNPs. Nalwade and Jadhav[37] observed that such morphological changes become evident upon interaction between SNPs and negatively charged cell membrane of the microorganisms and can be characterized by shrinkage of the cytoplasm and membrane detachment, which leads finally to the rupture of the cell wall. Finally, all these modifications in the cytological structure of fungi may be related to the interference of the SNPs with the vital events responsible for synthesis or maintenance of fungal cells. These findings demonstrated that biosynthesized SNPs was effective in restricting the fungal growth of filamentous fungi.

Conclusion

The obtained results established the fact that SNPs can be synthesized in ecofriendly, inexpensive and promising technique by fungal strain of *A. niger*. The biosynthesized NPs exhibited a potent antifungal activity against the tested dermatophytes at very low concentration. The results also revealed that SNPs caused different mycelial deformation. In addition, it reduces the keratinase activity. The significance of this work is that this is the first study concerning the effect of biosynthesized SNPs against *T. interdigitale*. Finally, it can be expected that biosynthesized SNPs represent a promising antifungal agent to compact dermatophytes infection.

Conflict of Interest

None.

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