Biosynthesis of selenium nanoparticles by Aspergillus flavus and Candida albicans and comparison of their effects with antifungal drugs

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Abstract

Biosynthesis of nanoparticles can stand as a replacement for the available chemical and physical methods by offering new procedures as green syntheses that have proved to be simple, biocompatible, safe, and cost-effective. Considering how nanoparticles with a size of 1 to 100 nanometers contain unique physical and chemical properties, recent reports are indicative of observing the antifungal qualities of selenium nanoparticles (Se-NPs). Recently, the observance of antifungal resistance towards different species of these fungi is often reported. Therefore, due to the antifungal effects of biological nanoparticles, this study aimed to investigate the exertion of these nanoparticles and evaluate their effects on the growth of fungal pathogens. Se-NPs were biosynthesized by the application of wet reduction method, which included specific concentrations of Aspergillus flavus and Candida albicans. The presence of nanoparticles was confirmed by methods such as UV-Vis spectroscopy, FT-IR analysis, and FESEM electron microscope that involved FESEM and EDAX diagram. The fungal strains were cultured in sabouraud dextrose agar medium to perform the sensitivity test based on the minimum inhibitory concentration (MIC) method in duplicate. The utilization of Se-NPs at concentrations of 1 µg/ml and below resulted in zero growth of fungal agents. However, their growth was inhibited by antifungal drugs at concentrations of 2 µg/ml and higher. Based on the obtained results, biological nanoparticles produced by fungal agents at different concentrations exhibited favorable inhibitory effects on the growth of fungal strains.

1. Introduction

Next to improving the feature of bioavailability, nanoparticles (NPs) contain a stronger ability for carrying capacity, mobility, cellular uptake, and lower toxicity compounds when being compared to the cases of microparticles. In addition, they can control and maintain the release profile of the drug that is given to the target site, as well as tailor the appointed particle to withstand detrimental pH, processing, and enzymatic conditions [1]. According to reports, Se-NPs can exhibit antimicrobial, antifungal, and anti-parasitical activities [2]. Furthermore, selenium itself contains anti oxidase effect, immune balance role, antibacterial and antifungal properties, cancer prevention functionality, growth performance, and reproduction improvement capabilities [1, 3]. The green synthesizing approach of nanoparticles can replace chemical and physical methods as a novel technique. The centralized research, which includes the usage of this advantage for nanoparticles synthesis, is simple, biocompatible, safe, and cost-effective [4, 5]. Recent studies have suggested varying biological sources for performing this synthesizing process. Certain biological organisms such as plants, fungi, or bacteria are able to convert some toxic ions into less toxic forms such as sedimentations or nanoparticles. Nanoparticles in the size of 1 to 100 nanometers can be exerted for many applications in medical sciences due to their quantum properties and unique physical and chemical properties [6-8]. These nanoparticles adhere to the cell walls and membranes and sometimes find an entrance to the inside of a cell. Moreover, they can damage cellular structures, induce the production of reactive oxygen species, and alter signal transmission mechanisms [9-11].

Observations indicate that nanoparticles can also suppress the expression of associated proteins by producing adenosine triphosphate, however, some of their specific antimicrobial mechanisms are not well understood yet [12, 13]. Recently, the number of problematic cases of drug resistance in different species of microorganisms has been exceeding. For example, various species of Candida and Aspergillus can act as opportunistic human pathogens in suppressed and defective immune systems [14, 15]. Nanoparticles have exhibited high antifungal activity in recent studies, especially throughout the cases of C. albicans. Regarding the treatment of these fungal agents, itraconazole is a widely used azole drug, to which these microorganisms have become resistant to some extent [16, 17]. Considering the antifungal effects of biological nanoparticles, the objective of this study was settled on the exertion of these nanoparticles and investigating their effects on the growth of fungal pathogens.

2. Experimental

2.1. Preparation of Se-NPs

Se-NPs were biosynthesized at a concentration range of 100 ppm by the application of two standard strains of A. flavus: TIMML-050 and C. albicans: TIMML-1306. In the following, these species were cultured in a sabouraud dextrose broth medium at 35 °C for 24 to 48h. After incubation, the supernatant that contained reducing organic proteins and enzymes (resulting from the growth and metabolism of fungal agents) was separated from the fungal culture medium through the utilization of Whatman 1 filter paper. As the next step, 800 mg of sodium selenite (Na_2SeO_4) was dissolved in 10 mL of distilled water, which was then added to 100 mL of
supernatant and incubated at 28 °C for 24h (away from light and movement) [18, 19]. Afterwards, selenium salt was reduced and the production of nanoparticles became macroscopically visible as the color of environment was changed from yellow to orange [20].

2.2. UV-Vis spectrum

We were able to confirm the presence of Se-NPs by the usage of UV-Vis spectrum recorded in the range of 290-310 nm (Fig. 1 and 2). The successful biogenic synthesis of Se-NPs was primarily assured by red color change. The formation of Se-NPs was further justified from UV-Vis spectroscopy. Fig. 1 and 2. display the typical plasmon resonance bands of Se-NPs, being observed at 290-310 nm (λmax) [21].

2.3. FT-IR and EDAX analyzes

The proper stability and dispersion of nanoparticles were attributed to their interaction with fungal proteins. During the process of nanoparticles production, it can be assumed that the probes form a coating on the nanoparticles and have them stabilized by preventing their condensation. The graphs that were obtained in the present study, displayed peaks in the ranges of 440, 518, 555, 753, 816, 1053, 1082, 1338, 1412, 1585, 1631, 2949, and 3389 cm⁻¹ for the case of *C. albicans*-Se-NPs (Fig. 3), and peaks within the ranges of 432, 538, 620, 751, 819, 1080, 1339, 1406, 2917, and 3418 cm⁻¹ for the case of *A. flavus*-Se-NPs (Fig. 4). FT-IR analysis was performed by the means of a PerkinElmer Spectrum Version 10.03.02 spectrometer device, while the samples were examined throughout the wavelength range of 4000-400 cm⁻¹. This method was exerted to determine the nanoparticles’ interaction with fungal proteins and better comprehend the roles of proteins that surround the nanoparticles as stabilizing agents. Based on the FT-IR results, the coating factor that stabilizes the nanoparticles confirms the presence of proteins. The resulting spectra displayed different functional groups including tensile O-H bonds, amide bonds, N-H-, C-O, and C-OH tensile vibrations [22].

2.4. FESEM/EDAX/PSA

According to the provided EDAX diagram, the presence of selenium structure as an element was approved (Fig. 5 (c)). The presence of Se-NPs with an almost spherical shape and a size of approximately 64 nm within the scale of 200 nm was proved by FESEM/PSA images (Fig. 5 (a, b)).

2.5. Disk Diffusion Agar test

The desired fungi's standard and resistant strains were cultured on a plate that contained sabouraud dextrose agar (SDA) medium. Then, drug-saturated disks (itraconazole and amphotericin B), along with the disks that were saturated with biosynthesized Se-NPs, were placed on the surface of agar medium by the usage of disk diffusion agar (DDA) method to be incubated at 35 °C for 18-24 h. The results of resistance and sensitivity to microorganisms were qualitatively examined on the surface of agar medium and as it was expected, the microorganism were observed to be somewhat sensitive towards the nanoparticle-saturated disks and formed an Aura of small lack of growth.

Standard strains of *C. albicans*: TIMML-1306,1291,491,183, *C. tropicalis*: TIMML-1316, *C. krusei*: TIMML-1321, *C. glabrata*: TIMML-368, *C. parapsilosis*: ATCC-2201, *A. flavus*: TIMML-050 and *A. fumigatus*: TIMML-025 was cultivated on SDA and incubated at 35°C for 24 h in order to prepare fungal suspensions for antifungal susceptibility testing. To evaluate the effect of antifungal drugs on the growth of desired colonies, we dissolved 3.2 mg of pure itraconazole powder in 2.4 mL of DMSO (concentration = 10X) to force the appointed working concentration in the RPMI environment to reach the concentration of X. To prepare the specified concentration of pure amphotericin B powder, 3.2 mg of pure drug powder was dissolved in 1 mL of DMSO; this particular amount of concentration in this case is 100X, which acts as the drug stock in the course of arranging the drug plate in RPMI-1640 medium. Once this value was forced to reach the concentration of X, which is, it was diluted in a ratio of 1 to 100 and added to the wells.

Pure drug powder was obtained from itraconazole (Janssen, Beere, Belgium), anidulafungin (Pfizer, Sandwich, United Kingdom), and amphotericin B (Bristol-Mers-Squib, Woerden, The Netherlands). The antifungal susceptibility testing was performed by the application of clinical & laboratory standards institute (CLSI) M38-A2. The Minimum Inhibitory Concentration (MIC) method was carried out in duplicate on fungal species' leachates that involved the usage of different and serial concentrations of itraconazole, amphotericin B, and anidulafungin, along with biosynthesized nanoparticles using 96 wells plates. Within a number range of 10 to
According to CLSI standards, *Candida* contains a minimum growth inhibitory concentration of any well that would reach a stunted growth of up to 80%. However, in the case of *Aspergillus* species, the minimum inhibitory concentration is represented by the well that is 100% stunted. The susceptibility test was performed on the leachates of fungal species through different and serial concentrations of itraconazole and nanoparticles. The results were evaluated and compared subsequent to the incubation process at 35 °C for 24 to 48h [23].

### 3. Results

The utilization of Se-NPs at concentrations of 1 µg/ ml and below resulted in zero growth of fungal agents, however, their growth was inhibited by antifungal drugs at concentrations of 2 mg/mL and higher. Tab. 1 represents the performed comparison between the inhibitory effects of different concentrations of itraconazole with biosynthesized Se-NPs on the growth of standard strains of *C. glabrata*, *C. albicans*, *C. tropicalis* and *C. krusei*. As it is indicated, *C. albicans*: TIMML-1306 displayed a resistant behavior towards itraconazole in concentrations up to 64 µg/ ml, however, biosynthesized Se-NPs were effective in lower concentrations than 1 µg/ ml and also caused the occurrence of fungal inhibition. In regards to the case of *C. glabrata*: TIMML-368, *C. tropicalis*: TIMML-1316 and *krusei*: TIMML-1321 itraconazole was observed to be effective at concentrations of 4 µg/ ml and 1 µg/ ml, respectively, while Se-NPs were able to prevent the growth of these fungi at the concentrations of 0.5 µg/ ml and 0.125 µg/ ml.

Tab. 2 demonstrates the evaluation results of comparing the inhibitory effects of antifungal drug itraconazole and biosynthesized Se-NPs on the growth of *A. fumigatus*: TIMML-025 and *A. flavus*: TIMML-050. Accordingly, it was observed that the fungal species *A. flavus* was 100% resistant towards all of the itraconazole concentrations, while displaying a satisfying sensitivity to Se-NPs at a concentration of 1 µg/ ml.

Tab. 3 exhibits the evaluation outcomes of comparing the inhibitory effects of antifungal drugs on amphotericin B and anidulafungin in regards to the strains of *C. albicans*. In this case, the application of Se-NPs at concentrations of 0.5 µg/ ml and below resulted in zero growth of fungal agents.

Tab. 4 contains the performed evaluation on the inhibitory effects of itraconazole on *C. albicans*: TIMML-1291 and *C. parapsilosis*: ATCC-2201, as well as the examination results of funguses’ response to the combination of drug and Se-NPs and their response to the solo usage of Se-NPs. The response of *C. albicans* and *C. parapsilosis* to the combination of drug and Se-NPs seemed to be acceptable and at concentrations of 2 µg/ ml and 1 µg/ ml, there were signs of sensitivity observed along with the lack of any fungi growth. These are sensitive towards all of the Se-NPs concentrations that were applied without being combined with any drugs.

### 4. Discussion

Based on the results, the occurrence of a favorable inhibitory effect on the growth of fungal species was observed, along with the capability of nanoparticles in preventing their growth in several wells. In addition, the usage of only nanoparticles can lead to the inhibition of fungal agent’s growth in several desired wells. In conformity to the comparaison results, the performance of these nanoparticles in some cases was more effective than the mentioned drugs, especially itraconazole, in such a way that they were able to inhibit the growth of fungi in the wells even at lower concentrations. Se-NPs were biosynthesized with the help of fungal agents. According to the results, these resistant strains are more responding towards the higher dosage of antifungal drugs than the biosynthesized Se-NPs, which can be very challenging throughout the treatment of patients. This study suggests that the utilization of Se-NPs, which were biosynthesized with fungal microorganisms, can be considered as a growth inhibitor in human pathogenic microorganisms. The work of Shoeibi *et al.* has used a similar method for the biosynthesis of Se-NPs with Bacillus bacterial species [24]. The first sign of nanoparticles formation is the inducement of an alteration in the yellow color of selenium towards orange, which has been also mentioned in the research of Shu Bharani *et al.* [2, 25, 26].

These nanoparticles can function as growth inhibitors in human pathogenic microorganisms that are involved throughout the delivery of drugs into the cells. They can also pass through cell membrane pores alone and find an easier transmission into the
cells. This process may be associated with the decreased expression of drug resistance genes either in the stage of cells transcription or the production of new products in the translation. According to the report of Liu et al., the impact of nanoparticles on cells and projects can result in strengthening the host immune system [27, 28].

Due to the increasing drug resistance towards the antifungal drugs that are used against fungal diseases, it is quiet necessary to consider alternatives for suppressing this problem. Also, the observance of drug resistance in other pathogenic microorganisms throughout various diseases further reveals the importance of this issue and how the study of nanoparticles along with their applications and extensive unique properties should be taken more seriously [29, 30]. Considering how chemotherapy stands as the most effective method of cancer treatment, S Dawar et al. in a survey of resistance to chemotherapy in cancer patients have foreseen the obstacle of facing drug resistance in the future; therefore, the application of nanoparticle systems can be new and useful in the treatment of cancer patients [31].

According to previous studies, there has been no reports of side effects in humans that had used these biosynthesized particles. The research of Ziaei regarding the impact of nanoparticles on biological and ecological systems indicate that there is currently no evidence of any adverse effects of nanoparticles used in products on humans [32].

Therefore, due to the available successful studies and positive results, it is possible to consider the evaluation of inhibitory effect of nanoparticles and chemical antifungal drugs on the growth of different fungal species during additional experiments and compare the usage of each alone to finally achieve an effective and prescriptive treatment for patients [33]. In conformity to the work of Yakhchi et al., nano liposomes can provide increased drug stability, reasonable release control, and easy passage through the cell membrane due to containing plant extracts with appropriate physical and chemical properties; therefore, they can be suggested as a promising anti-inflammatory agent with high antifungal effects and low side effects [34].

One of the most important potential benefits of concomitant usage of nanoparticles and drugs is to facilitate the passage of medication into the fungal cell membrane that is destroyed by nanoparticles, which helps in controlling fungal infections [35, 36]. It has been indicated by Menezes et al. that the application of nanoparticles in pregnant women can reduce the risks of taking various drugs during pregnancy and also lower the risks of death [37]. Zhang et al. have also reported similar observations subsequent to studying the facilitation of drug passage and nanoparticles according to their size [38].

It is mentioned in the research of Wang et al. on the future of nanoparticles that due to the predicted occurrence of drug resistance towards antibiotics, the treatment of infections will become complicated and therefore, nanoparticles could stand as a viable alternative [39]. Q.L Feng studies have also indicated that the production of bio particles and their application instead of chemical methods can offer a faster and cheaper approach than chemical procedures with less harmful conditions for humans and the environment [40].

5. Conclusion

Based on the results, biological Se-NPs produced by fungal agents at different concentrations exhibited favorable inhibitory effects on the growth of fungal strains. Therefore, the use of these agents can reduce the development of antifungal resistance in the future.

Declarations

If any of the sections are not relevant to your manuscript, please include the heading and write 'Not applicable' for that section.

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Conflicts of interest/Competing interests

The authors declare that they have no conflicts of interest.

Availability of data and material
The data used to support the findings of this study are available from the corresponding author upon request.

**Code availability**

Not applicable

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**Tables**

**Tab. 1.** MIC results of the effect of itraconazole (ITC) and Se-NPs on the growth of *Candida* strains.
### Tab. 2. MIC results of the effect of ITC and Se-NPs on the growth of *Aspergillus* strains.

|                | PC  | NC  | 0.625 µg/ml | 0.125 µg/ml | 0.5 µg/ml | 1 µg/ml | 2 µg/ml | 4 µg/ml | 8 µg/ml | 16 µg/ml | 32 µg/ml | 64 µg/ml |
|----------------|-----|-----|-------------|-------------|-----------|---------|---------|---------|---------|----------|----------|----------|
| **Candida strain** |     |     |             |             |           |         |         |         |         |          |          |          |
| Itraconazole    | +   | -   | R           | R           | R         | R       | R       | R       | S*      | S         | S         | S         |
| C. glabrata TIMML-368 |     |     |             |             |           |         |         |         |         |          |          |          |
| +   | -   | R   | R           | R           | R         | R       | R       | R       | R       | S*       | S         | S         |
| C. albicans TIMML-1306 |     |     |             |             |           |         |         |         |         |          |          |          |
| +   | -   | R   | R           | S           | S*       | S       | S       | S       | S       | S         | S         | S         |
| C. tropicalis TIMML-1316 |     |     |             |             |           |         |         |         |         |          |          |          |
| +   | -   | R   | R           | S           | S*       | S       | S       | S       | S       | S         | S         | S         |
| C. krusei TIMML-1321 |     |     |             |             |           |         |         |         |         |          |          |          |
| SeNPs          | +   | -   | R           | R           | S*       | S       | S       | S       | S       | S         | S         | S         |
| C. glabrata TIMML-368 |     |     |             |             |           |         |         |         |         |          |          |          |
| +   | -   | R   | R           | R           | S*       | S       | S       | S       | S       | S         | S         | S         |
| C. albicans TIMML-1306 |     |     |             |             |           |         |         |         |         |          |          |          |
| +   | -   | R   | S*          | S           | S       | S       | S       | S       | S       | S         | S         | S         |
| C. tropicalis TIMML-1316 |     |     |             |             |           |         |         |         |         |          |          |          |
| +   | -   | R   | S*          | S           | S       | S       | S       | S       | S       | S         | S         | S         |
| C. krusei TIMML-1321 |     |     |             |             |           |         |         |         |         |          |          |          |
|               | 1/1024 | 1/512 | 1/256 | 1/128 | 1/64 | 1/32 | 1/16 | 1/8 | 1/4 | 1/2 |
| Aspergillus strain | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  |
|-------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|                   | 64  | 32  | 16  | 8   | 4   | 2   | 1   | 0.5 | 0.125 | 0.625 | NC  | PC  |
| A. fumigatus      | S   | S   | S   | S   | S   | S   | S*  | R   | R   | R   | R   | -   | +   |
| TIMML-025         |     |     |     |     |     |     |     |     |     |     |     |     |     |
|                   | S   | S   | S   | S   | S   | S   | S*  | R   | R   | R   | R   | -   | +   |
| A. flavus         | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | -   | +   |
| TIMML-050         |     |     |     |     |     |     |     |     |     |     |     |     |     |
|                   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | -   | +   |
| A. fumigatus      | S   | S   | S   | S   | S   | S   | S   | R*  | R   | R   | R   | -   | +   |
| TIMML-025         |     |     |     |     |     |     |     |     |     |     |     |     |     |
|                   | S   | S   | S   | S   | S   | S   | S   | S   | R*  | R   | R   | -   | +   |
| A. flavus         | S   | S   | S   | S   | S   | S   | S   | S*  | R   | R   | R   | -   | +   |
| TIMML-050         |     |     |     |     |     |     |     |     |     |     |     |     |     |
|                   | S   | S   | S   | S   | S   | S   | S   | S*  | R   | R   | R   | -   | +   |
|                   | 1/2 | 1/4 | 1/8 | 1/16| 1/32| 1/64| 1/128| 1/256| 1/512 | 1/1024 |     |     |

Tab. 3. MIC results effect of amphotericin B (AMB), anidulafungin (AFG) and Se-NPs on growth of *Candida* strains.
|                    | Candida Strains | PC  | NC  | 0.625 µg/ml | 0.125 µg/ml | 0.5 µg/ml | 1 µg/ml | 2 µg/ml | 4 µg/ml | 8 µg/ml | 16 µg/ml | 32 µg/ml | 64 µg/ml |
|--------------------|-----------------|-----|-----|-------------|-------------|-----------|---------|---------|---------|---------|---------|---------|---------|
| **Amphotericin B** | +               | - R | R   | R           | R           | S*        | S       | S       | S       | S       | S       | S       |
|                    |                 | +   | R   | R           | R           | S*        | S       | S       | S       | S       | S       | S       |
| **SeNPs**         | +               | - R | S*  | S           | S           | S         | S       | S       | S       | S       | S       | S       |
|                    |                 | +   | R   | S*          | S           | S         | S       | S       | S       | S       | S       | S       |
| **Anidulafungin** | +               | - R | R   | R           | R           | S*        | S       | S       | S       | S       | S       | S       |
|                    |                 | +   | R   | R           | R           | S*        | S       | S       | S       | S       | S       | S       |
| **SeNPs**         | +               | - R | R   | S*          | S           | S         | S       | S       | S       | S       | S       | S       |
|                    |                 | +   | R   | S*          | S           | S         | S       | S       | S       | S       | S       | S       |

**Tab. 4.** Comparison results of the effect of ITC and Se-NPs in both cases of being combined and used separately
|     | 12 | 11 | 10 | 9  | 8  | 7  | 6  | 5  | 4  | 3  | 2  | 1  | Candida Strains |
|-----|----|----|----|----|----|----|----|----|----|----|----|----|-----------------|
| PC  | NC | 0.625 µg/ml | 0.125 µg/ml | 0.5 µg/ml | 1 µg/ml | 2 µg/ml | 4 µg/ml | 8 µg/ml | 16 µg/ml | 32 µg/ml | 64 µg/ml | Itraconazole + | - | R | R | R | R | R | R | R | S* | S | S | C. albicans TIMML-1291 |
|     |    |   |    |    |    |    |    |    |    |     |     | 50% Itraconazole + 50% SeNPs |
|     |    |    |    |    |    |    |    |    |    |     |     | 100% SeNPs |
|     |    |    |    |    |    |    |    |    |    |     |     | Itraconazole + - - S | S | S | S | S | S | S | S | S | S | S | C. albicans TIMML-1291 |
|     |    |    |    |    |    |    |    |    |    |     |     | C. parapsilosis ATCC-2201 |
|     |    |    |    |    |    |    |    |    |    |     |     | 1/1024 | 1/512 | 1/256 | 1/128 | 1/64 | 1/32 | 1/16 | 1/8 | 1/4 | 1/2 |