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Green synthesis, Optimization and Antifungal Activity of Selenium nanoparticles using Fusarium fujikuroi MED14

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Abstract

Finding novel ways to avoid the economic impacts in agriculture brought on by phytopathogenic fungus has become necessary as a result of the constant requirement for supplying global food demand. Furthermore, the increased virulence of microbes against antimicrobial drugs has made the development of innovative compounds with broader action and less toxicity necessary. In this study, selenium nanoparticles (Se NPs) were biosynthesized by simple, cost-effective, environmentally method using *Fusarium fujikuroi* MED14. The optimum conditions for Se NPs biosynthesis were recorded at mixing of 4 discs with diameter 5mm from the biomass of fungal strain (grown on malt extract glucose yeast extract peptone) with 0.16 M of Na_2SeO_3 solution at 40°C and dark conditions. The biosynthesized Se NPs were detected at a unique absorption peak ≈246nm. The optimized Se NPs appeared to be crystalline and uniformly distributed NPs varying from 52 nm to 68 nm in diameter with surface charge of +32.3 mV. The Se NPs showed antifungal activity against *F. solani*, *Aspergillus niger*, *F. fujikuroi* MED14 and *Candida albicans* with inhibition zones 20, 15, 13, and 15 mm, respectively. Therefore, this work provides an initial basis for further research employing *F. fujikuroi* MED14 in the green synthesis of antifungal Se NPs under various trial conditions.

Keywords: Biosynthesis, optimization, selenium nanoparticles, Fusarium fujikuroi MED14, antifungal activity.

Introduction

Organizing materials at the atomic level to form nanoscale matter with special qualities that improve the properties in its applications is a crucial aspect of nanotechnology **(Buzea *et al.*, 2007)**. The size factor in nanoparticles (NPs) provides particles with new properties and enables them to be developed to different comportments. NPs within the size range of 1-100 nm have attracted a lot of interest for decades now because of their many advantages, wide range of uses, and simplicity of effective production. Recently, NPs were produced by chemical and physical processes that can be hazardous **(Sukhanova *et***

***al.*, 2018)**. Therefore, the demand to fabricate easily replicable, affordable, and ecologically appropriate techniques for the synthesis of NPs is increasing **(Saleh & Yousaf, 2018)**. A variety of techniques and the use of microorganisms have resulted from the evolution of interest in the production of NPs through biotechnology and biological systems **(Tugarova & Kamnev, 2017)**. As a result, the biotechnological processes used to produce NPs are safe and do not harm the environment.

In recent years, Selenium (Se) has received a lot of attention due to its characteristics and advantages for applications in catalysis, electronics, optics, chemistry, antioxidants, and biomedicine because of its improved photoelectric qualities, low toxicity, and biological activities **(Chaudhary *et al.*, 2014; Menon *et al.*, 2018)**. Se is an essential trace element and a constituent of selenoproteins that prevent cell damage, control thyroid function, and enhance immune system regulation **(Zhang *et al.*, 2005)**. Se is also utilized as a potent antibacterial and anticancer agent **(Romero *et al.*, 2019)**. High Se doses might have negative consequences despite their many benefits. Therefore, Se NPs have been used to overcome the high doses of Se, while preserving its biological effects, such as its anticancer action **(Li *et al.*, 2019)**. Biosynthesis techniques are increasingly being used because they are generally considered to be single-step, non-toxic, economical, safe, and clean, and they can produce nanomaterials with more precisely specified sizes and morphologies **(Fouda *et al.*, 2019)**. Moreover, the biosynthesized Se NPs remain stable for a long period of time and water dispersible because of capping by the organic molecules and nontoxic phytochemicals or natural protein molecules that maintain its polydispersion in colloidal form **(Eid *et al.*, 2020; Zhang *et al.*, 2021)**.

The microbial production of Se NPs has been characterized by a unique and complicated nanostructures arrangement of Se atoms **(Shoeibi *et al.*, 2017)**. In addition, larger surface-to-volume ratios, surface energies, and geometric limitations that expand the region of contact are factors that define the characteristics of NPs **(Shah *et al.*, 2015)**. The majority of microorganisms are classified as fungi, and they are employed in a wide range of scientific fields for manufacture of food items, enzymes, organic acids, biofuels, nanotechnology, and bioremediation and biodeinking **(Youssef *et al.*, 2019; Hasanin *et al.*, 2020)**. Fungi have the capacity to synthesize a diverse range of protein molecules and can change active ions into less harmful ones by enzymatic conversion **(Dhillon *et al.*, 2012)**. Additionally, it is known that fungi have a high tolerance for collecting heavy metals and other microelements in their mycelium **(Zare *et al.*, 2013)**. Dispersed Se NPs, resulting from this transition, might develop extracellularly, inside the cytoplasm, or within the periplasm. Due to the remarkable scalability, controllability, and affordability of the NP production process, the fungi are referred to as a "biofactory". The fungus-based bio-inspired approach is more cost-effective and reproducible than the traditional biosynthesis methods because bacterial biosynthesis necessitates expensive, sophisticated equipment for the separation and purification of NPs, while plant-based biosynthesis is subject to seasonal and geographic variations **(Islam *et al.*, 2022)**. Although certain microorganisms have been utilized in the production of Se NPs, only a few fungal species have been identified as the synthesizers of Se NPs **(El-Sayed *et al.*, 2020)**. Gram-negative and Gram-positive bacteria can both be effectively inhibited by Se NPs. Additionally, it has been noted that Se NPs have antifungal action and prevent spore germination **(Shubharani *et al.*, 2019)**.

In this study, the optimal parameters for the reduction of Na_2SeO_3 to its nano-elemental form using *Fusarium fujikuroi* MED14 were investigated, along with an evaluation of its antifungal activity against several pathogenic fungal strains.

Materials and methods

Fungal strain

Fungal strains including *F. fujikuroi* MED14 (AC: PP794203), *F. solani* DSM 62413, *Aspergillus niger* van Tiegh niger, and *Candida albicans* ATCC10231 were obtained from the Microbiology Lab, Botany and Microbiology Department, Faculty of Science, Damietta University. *F. fujikuroi* MED14 was subcultured on Czapek Dox agar plates (pH6) and incubated for 5-7 days at 28 ± 2°C for further use.

Testing for extracellular green synthesis of Se NPs using F. fujikuroi MED14

The green synthesis of Se NPs using *F. fujikuroi* MED14 was performed according to **Abbas & Abou Baker (2020)** with some modifications. In 250 mL Erlenmeyer flasks, 4 discs (5 mm) of 7-day-old *F. fujikuroi* MED14 culture were added to 100 mL of 0.16 M Na_2SeO_3 solution. The flasks were incubated for 2 days at 40°C in dark conditions. After incubation, the red precipitate of Se NPs was collected and washed three times with distilled water by centrifugation at 8000 rpm for 20 minutes. Finally, the Se NPs were dried in an oven at 40-50°C.

Optimal investigations of Se NPs production

The optimal conditions for the myco-synthesis of Se NPs were studied using 4 discs (5 mm) of *F. fujikuroi* MED14 growth culture (7days old) that were grown on different culture media (pH6) including potato dextrose agar (PDA), Czapek Dox agar, malt extract agar (MEA), malt extract glucose yeast extract peptone (MGYP) agar, yeast extract peptone agar (YEP), peptone water agar (PW), yeast extract water agar (YE), and yeast extract sucrose agar (YES) **(Safaei *et al.*, 2022)**.

Different number of fungal culture discs (1-9 discs, 5mm in diameter) of 7 days old fungal culture were tested for the optimal synthesis of Se NPs **(Saleh & Yousaf, 2018)**.

Various Na_2SeO_3 concentrations (0.01-0.19M, pH6) were applied to determine the optimal concentration for Se NPs production **(Mohamed & El-Zahed, 2024)**.

The experiments also investigated the Se NPs formation at different temperatures (20–60°C) at 150 rpm in dark conditions **(Saleh & Yousaf, 2018)**.

Characterization of the optimized Se NPs

The spectrum of the optimized Se NPs was detected by using UV-Vis spectrophotometry (Double beam spectrum UV–Vis spectrophotometer V-760, JASCO, UK), and Fourier transform infrared spectroscopy (FT/IR-4100typeA). The Se NPs was analyzed using Malvern Zetasizer Nano-ZS90 (Malvern, UK), and transmission electron microscopy (TEM, JEM-2100, Japan).

Evaluation of the antifungal activity of optimized green synthesized Se NPs

The selected fungi and yeast for the antifungal activity test including *F. fujikuroi* MED14, *F. solani* DSM 62413, *A. niger* van Tiegh niger, and *Candida albicans* ATCC10231 were refreshed by subculturing under sterile conditions on PDA agar and yeast extract peptone dextrose (YEPD) agar plates, respectively. The agar well diffusion method was used to investigate the antifungal action of Se NPs **(CLSI, 2009)**. One centimeter disc of the propagule of the pathogenic fungi and yeast was deposited into the cooled molted agar media, separately and subsequently, the flasks were homogenized well by vortex shaker, then poured into petri dishes (9 cm). After the complete solidification, wells were aseptically punched (5 mm) using a sterile corkborer. 200µg/ml from Se NPs were prepared and added to the wells. The plates were incubated at 30°C for 5 days or at 37°C for 24 h for testing fungi or yeast, respectively. After the incubation period, the plates were examined, and zones of inhibition were observed and measured around the pores in millimeters. Three replicates and average readings were recorded.

Statistical analysis

The data were subjected to statistical analysis using SPSS version 18. The data were reported as means ± standard deviations (SD). The significance level of $p < 0.05$ was used.

Results

Extracellular green synthesis of Se NPs

Fusarium fujikuroi MED14 successfully green biosynthesized Se NPs within 48 h as observed by color change from colorless to a red-orange color (Figure 1). Additionally, the UV–Vis spectrum confirmed the formation of biosynthesized Se NPs with a unique wavelength peak at 246nm.

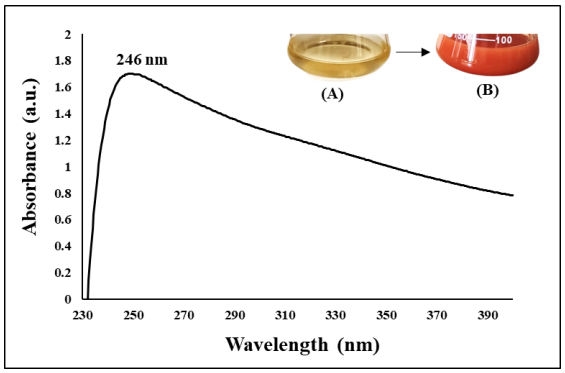

Figure 1. UV-Vis spectrum revealed color change of the biosynthesized Se NPs. (A) Intiated color of the reaction mixture. (B) Changed color at the end of green biosynthesis.

Optimization of Se NPs production

Different parameters were evaluated to optimize the biosynthesize of Se NPs including different culture media, number of discs from the biomass of *F. fujikuroi* MED14, concentration of Na_2SeO_3, different temperatures, incubation periods, and pH values (Figure 2). Biosynthesized Se NPs were distinctive using fungal discs that grew on the MGYP medium compared to other culture media which showed the highest maximum peak at earlier wavelength. 4 discs from the biomass of *F. fujikuroi* MED14, 0.16 M of Na_2SeO_3 and 2 days incubation time were the best conditions for the Se NPs production. It was observed that the progress of the Se NPs biosynthesis was increased gradually by increasing the temperature until 40°C, then begin decay at higher temperature (50-60°C).

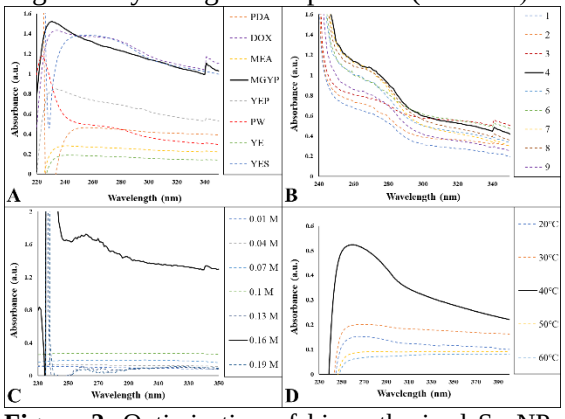

Figure 2. Optimization of biosynthesized Se NPs production. (A) Different media. (B) Number of *F. fujikuroi* MED14 discs. (C) Concentrations of Na_2SeO_3. (D) Temperatures.

Characterization of the optimized green synthesized Se NPs

The Se NPs FTIR spectrum was studied and shown in Figure 3. The emergence of significant broadly peaks around 3429cm^{-1} indicating hydroxyl (O–H) group. Moreover, the FTIR spectra showed bands at 2937cm^{-1} which indicated C–H stretching. The peaks at 2358cm^{-1} are observed that correlate to the presence of proteins. Bands at 1645cm^{-1} which was associated with proteins' amide I (N–C=O-stretching mode). The more complex Amide band is located close to 1566 &1410cm^{-1} correspond to amide II (N–H bending mode) and amide III. Interestingly, Se stretching vibration (C–Se) significantly appeared as broadly peak at 606cm^{-1}.

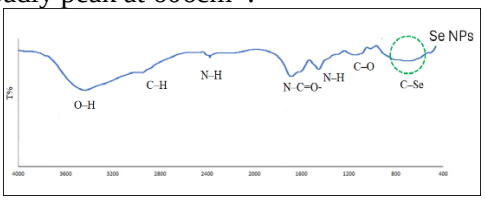

Figure 3. FTIR spectrum pattern of green synthesized Se NPs.

In the present study, Zeta potential analysis was tested and indicated to the presence of an intensive positive net surface charge at +32.3 mV (Figure 4).

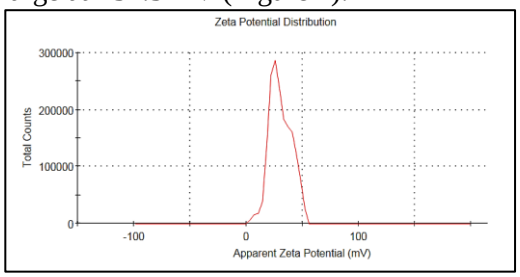

Figure 4. Zeta potential of Se NPs.

TEM images clearly show the spherical form and crystalline structure of uniformly distributed NPs that varied in size from 52 nm to 68 nm as shown in Figure 5.

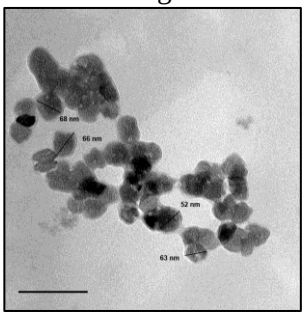

Figure 5. Transmission electron microscope of Se NPs. Bars scale = 200 nm.

Antifungal activity of Se NPs

The biosynthesized Se NPs using *F. fujikuroi* MED14 showed a significant antifungal effect against *F. solani* with inhibition zone of 20 mm (Figure 6). Also, moderate antifungal inhibition zones were also recorded against *A. niger* and *C. albicans* both measuring 15 mm for both. While low antifungal activity was demonstrated against *F. fujikuroi* MED14 with inhibition zone of 13 mm.

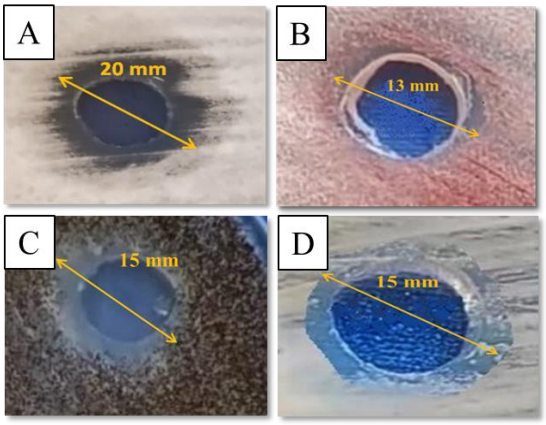

Figure 6. Antifungal activity of biosynthesized Se NPs using *F. fujikuroi* MED14 against *F. solani*; (A), *F. fujikuroi* MED14; (B), *A. niger*; (C), and *C. albicans* (D).

Discussion

It is thought that fungi are an exceptional source for the extracellular production of nanomaterials. Finding innovative fungal strains with compelling biological potential is a bigwig problem. On the other hand, *Fusarium*, *Aspergillus*, and *Candida* were reported as multidrug-resistant genus that are found all over the world and can infect plants and humans with a variety of diseases **(Arendrup & Patterson, 2017; Al-Hatmi *et al.*, 2018; Poulsen *et al.*, 2021)**. In the current investigation, *F. fujikuroi* MED14 (AC: PP794203) was selected to green synthesize and optimize Se NPs while *F. fujikuroi* MED14, *F. solani* DSM 62413, *A. niger* van Tiegh niger, and *C. albicans* ATCC10231 strains were used to investigate the inhibitory activity of the optimized fungus-based biosynthesized Se NPs.

Fusarium fujikuroi MED14 was able to produce distinct Se NPs as a first study to demonstrate this green synthesis using this fungal strain. The most distinctive optical property of metallic nanostructure of biosynthesized Se NPs was distinguished by changing in color from colorless solution to a red orange color known as a "brick". The stimulation of the surface plasmon vibration of the Se NPs generated the brick red hue, indicating the production of elemental nano Se **(Hassanien *et al.*, 2019)**. In this study, UV–Vis spectrum confirmed the biosynthesis of Se NPs with an adsorption peak at 246nm. **Safaei *et al.* (2022)** used a green method to biosynthesize Se NPs using the *Halomonas elongata* bacterium that revealed an absorption peak at 267nm. While **Nassar *et al.* (2023)** used wide range of endophytic fungal strains including *Penicillium verhagenii*, *A. niger*, *Alternaria alternata* and *Penicillium* sp. that showed absorption peaks of Se NPs at 270, 265, 265 and 280nm, respectively.

Different culture media, number of discs from the biomass of *F. fujikuroi* MED14, concentration of Na_2SeO_3, temperatures, incubation periods, and pH were investigated and studied to optimize the biosynthesis of Se NPs. The optimization processes confirmed that fungal discs that grew on the MGYP medium, 4 discs from the biomass of *F. fujikuroi* MED14, 0.16 M of Na_2SeO_3 and 2 days incubation time were the best conditions for the Se NPs formation. Se NPs biosynthesis increased gradually by increasing the temperature until 40°C, then begin decay at higher temperature which might be due to the denaturation of enzymatic systems of the fungus **(Mohamed & El-Zahed, 2024)**. The obtained results matched with **Abbas & Abou Baker (2020)** who used MGYP for Se NPs biosynthesis which was inoculated with 5mm discs taken from *F. semitectum* (7 days-old). According to **Diko *et al.* (2020)** showed that the optimal concentration of SeO_2 for synthesis Se NPs using *Trichoderma* sp. was 2 mM and incubation at 30°C. On the other hand, **Ahamad Tarmizi *et al.* (2023)** reported that the absorption peak increased in intensity at using 100mM sodium selenite and incubation for 24h at 37°C.

One of the common issues that reduces the biological potential of NPs is their aggregation. By stopping NPs from aggregating, the outer capping agents control the size and shape of the particles. The Se NPs FTIR spectrum was studied and found to be similar to the documented results by **Gharieb *et al.* (2023)**. Different peaks are observed that

correlate to the presence of proteins, amide I, amide II and amide III. The stabilization of metal ions and the synthesis of reduction were carried out by the amide groups, which indicated the existence of enzymes and proteins **(Prasad & Selvaraj, 2014)**. Therefore, based on this data, it is possible that the proteins created a capping agent on top of the Se NPs, which may have contributed to their stability.

Zeta potential must be used to investigate the stability and size distribution of the NPs. Colloid stability is significantly influenced by the surface charge, which may be studied using zeta potential data; a comparatively low zeta potential may be indicative of nanoparticle agglomeration. The current study confirmed the positive net surface charge of Se NPs (+32.3 mV), so this is evidence of the high stability of Se NPs. On the other hand, previous study recorded the negative charge of the biosynthesized Se NPs which was around -20 mV as reported by **Hussein *et al*. (2022)** study who used different endophytic fungi for Se NPs biosynthesis including *Aspergillus quadrilineatus*, *A. ochraceus*, *A. terreus*, and *F. equiseti*.

The TEM method is an imperative instrument to evaluate the size and shape of produced NPs. TEM images clearly show the crystalline and uniformly distributed NPs varying from 80 nm to 90 nm in diameter. According to **Gharieb *et al*. (2023)** the size of individual synthesized Se NPs by *F. oxysporum* ranged between 60–97 nm. In addition, the study carried out by **Abbas & Abou Baker (2020)** on bio-Se NPs by *F. semitectum*, showed that its diameter ranged from 32.80 nm to 103.82 nm.

Surprisingly, it was found that *in vitro*, fungus-based Se NPs had stronger antifungal activity against *F. fujikuroi* MED14 followed by *A. niger*, and *C. albicans*. Numerous investigations revealed that the mechanism leading to the fungicidal effects of Se ions included their absorption and accumulation by the fungal cell. Consequently, it allowed the cytoplasm membrane to contract and prevented the essential functions of the cell **(Mohammadlou *et al*., 2017; Eskandari-Nojedehi *et al*., 2018)**. Se NPs showed potential effect against *F. oxysporum* and *Colletotrichum gloeosporioides* at concentration from 0.25 to 1.7mg/ml reported by **Lazcano-Ramírez *et al*. (2023)**. Also, it was demonstrated that Se NPs had antifungal properties at different concentrations 50–150µg/ml against several *Fusarium* species **(El-Saadony *et al*., 2021). Ali *et al*. (2020)** showed high anticandidal activity against *C. albicans* about range 20.33mm at different concentrations. Furthermore, the results of **Kazempour *et al*. (2013)** study reported that the MICs of biosynthesized Se NPs using *Klebsiella pneumoniae* against *A. niger* and *C. albicans* were 250µg/ml and 2000µg/ml, respectively.

Conclusions

Se NPs was obtained via bioformation using *F. fujikuroi* MED14, reported for the first time. The green synthesis involved the presence of proteins as capping and stabilizing agents as showed in the FTIR analysis. The surface positive charge of Se NPs (+32.3 mV) also contributed to the stabilizing of the NPs. The biosynthesized Se NPs demonstrated antifungal effect against several phytopathogenic fungi strains including *F. solani A. niger, F. fujikuroi* and *C. albicans* at 200µg/ml. In summary, Se NPs are recommended for application in various sectors including pharmaceutical, biological and agricultural fields due to their antifungal activity. Future efforts should focus on reducing the concentration required and improving their efficiency.

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الملخص العربي

عنوان البحث: التخليق الأخضر والتحسين والنشاط المضاد للفطريات لجسيمات النانوسيلينيوم والمصنعة باستخدام فطر فيوزاريوم فوجيكيوري MED14

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أصبح من الضروري إيجاد طرق جديدة لتجنب التأثرات الاقتصادية السلبية في الزراعة الناجمة عن الفطريات المسببة للأمراض النباتية نتيجة للمتطلبات المستمرة لتلبية الطلب العالمي على الغذاء. وعلاوة على ذلك، فإن زيادة مقاومة الميكروبات ضد الأدوية المضادة الحالية جعلت من الجدير بالأهمية تطوير مركبات مبتكرة ذات نطاق أوسع وسمية أقل. في هذه الدراسة، تم التصنيع الحيوي لجسيمات النانوسيلينيوم بطريقة بسيطة وفعالة وقليلة التكلفة وصديقة للبيئة باستخدام فطر فيوزاريوم فوجيكيوري MED14. تم تسجيل الظروف المثلى لتصنيع جسيمات النانوسيلينيوم عند خلط ٤ أقراص بقطر ٥ مم من الكتلة الحيوية للسلالة الفطرية والمنماة على بيئة غذائية مكونة من مستخلص الشعير ومستخلص الخميرة والجلوكوز والبيتون مع ٠,١٦ مول من محلول سيلينات الصوديوم عند ٤٠ درجة مئوية في الظلام. تم الكشف عن جسيمات النانوسيلينيوم المصنعة حيوياً والتأكد من تصنيعها باستخدام جهاز قياس الطيف المرئي والأشعة فوق البنفسجية والذي أظهر ذروة امتصاص فريدة عند ٢٤٦ نانومتر. وقد ظهرت جسيمات النانوسيلينيوم المُحسَّنة على هيئة جسيمات بلورية وموزعة بشكل جيد ومتجانس وقد تراوح قطرها ما بين ٨٠ إلى ٩٠ نانومتر وصاحبها وجود شحنة سطحية موجبة تبلغ +٣٢,٣ م فولت. أظهرت جسيمات النانوسيلينيوم نشاطًا مضادًا للفطريات ضد فيوزاريوم سولاني وأسبرجيلس نايجر و فيوزاريوم فوجيكيوري وكانديدا ألبيكانس مع مناطق تثبيط تصل إلى ٢٠ و١٥ و١٣ و١٥ ملليمترعلى التوالي. لذلك، يوفر هذا العمل أساسًا أوليًا لمزيد من البحث في التصنيع الأخضر لجسيمات النانوسيلينيوم باستخدام فطر فيوزاريوم فوجيكيوري MED14 وذات المقاومة المضادة للعديد من الفطريات الممرضة في ظل ظروف تجريبية مختلفة.