

SELENIUM NANOPARTICLES AS ANTIFUNGAL AGENTS: A CRITICAL REVIEW OF BIOSYNTHESIS, CHARACTERIZATION, MECHANISMS AND APPLICATIONS

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Review



ABSTRACT

Selenium nanoparticles (SeNPs) show significant promise as antifungal agents because of their unique physicochemical properties, high bioavailability, and lower toxicity compared with conventional selenium forms. This critical review comprehensively analyzes the extensive literature on SeNPs biosynthesis, characterization, precise mechanisms of action, and diverse in vivo applications. The review synthesizes data from chemical, physical, and biological synthesis approaches using fungi, bacteria, actinobacteria, and various plant extracts. Key characterization techniques (UV-Vis, TEM, SEM, XRD, DLS, and zeta potential) are critically evaluated to confirm nanoparticle formation, morphology, size, crystallinity, and colloidal stability. SeNPs have been reported to exert strong antifungal effects partly through inducing oxidative stress through the generation of reactive oxygen species (ROS), disrupting fungal cell membranes, and interfering with essential cellular processes. The crucial influence of particle size, surface charge, and surface coating on antifungal efficacy is thoroughly discussed. In vivo studies indicate that SeNPs can reduce fungal disease severity in plants by up to 90%, improve growth, and enhance antioxidant parameters in animal models. However, critical appraisal reveals recurring methodological shortcomings, including incomplete physicochemical characterization, lack of accurate concentration measurements (ICP-MS), and limited safety assessments. The review concludes with evidence-based recommendations for standardized protocols to facilitate the translation of SeNPs into reliable agricultural and medical applications.

Keywords: Selenium nanoparticles, antifungal, biosynthesis, characterization, mechanisms, critical review

INTRODUCTION

Selenium (Se) is a metalloid that was once thought to be toxic until it was found to play vital roles in certain organisms, including mammals, algae, and bacteria (Ahrwar *et al.*, 2020). It is also considered an essential trace element for maintaining physiological functions, growth, and general health, and it plays a crucial role in these processes (Boghdady *et al.*, 2017 & El-Gazzar & Ismail, 2020). This role is mediated through its incorporation into important proteins (selenoproteins) such as glutathione peroxidase and thioredoxin reductase, which are involved in antioxidant defense (protecting cell membranes from oxidative damage), detoxification, metabolism, regulation of thyroid function, and modulation of the immune system (Elshahawy *et al.*, 2018 & Fisher *et al.*, 2012). In recent years, selenium nanoparticles have attracted increasing attention due to their unique properties and advantages in catalytic, electronic, optical, and biomedical applications (Lebaka *et al.*, 2021), owing to their high absorption capacity, high catalytic efficiency, and significant surface activity (Martinez-Esquivas *et al.*, 2021). The nanoscale form of selenium is characterized by significantly lower toxicity—up to three times lower than organic selenium and seven times lower than inorganic selenium—higher bioavailability, increased therapeutic efficacy, improved chemical stability, and gradual release compared to other chemical forms of selenium such as selenite, selenomethionine, and selenocysteine (Najjar & Abu-Khalaf, 2021). Studies have also indicated that it possesses antioxidant, anticancer, antibacterial, antiviral, and antiparasitic properties (Nargund *et al.*, 2020).

The synthesis of selenium nanoparticles involves three main approaches: chemical methods such as chemical reduction (with ascorbic acid or hydrazine), sol-gel techniques, and hydrothermal processes; physical methods such as laser ablation, vapor deposition, and microwave-assisted synthesis; and biological methods utilizing microorganisms (such as bacteria: *Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumoniae*; fungi and yeast), algae, cyanobacteria, and plant extracts (such as citrus extracts containing ascorbic acid as a reducing agent (Periakaruppan *et al.*, 2023). Chemical and physical methods provide controllable synthesis but have significant drawbacks, including the use of toxic raw materials, energy-intensive equipment, the production of hazardous waste, and poor biocompatibility (Sharma *et al.*, 2009). In contrast, biosynthesis (particularly extracellular biosynthesis due to ease of extraction) is favored as a practical, low-cost, and environmentally friendly alternative. It produces biocompatible nanoparticles that are naturally stabilized by organic molecules and characterized

by lower toxicity and long-term stability due to their colloidal nature (Srivastava *et al.*, 2016 & Dhanjal & Cameotra, 2010), making it possible to incorporate and apply them in edible coatings for product preservation (Forootanfar *et al.*, 2014). Globally, plant pathogenic fungi are among the most significant and common pathogens affecting valuable crops, leading to deterioration in quality and a significant reduction in yield (estimated at 20–40% annually), posing a major threat to food security (Khanna *et al.*, 2022 & Menon, Agarwal, *et al.*, 2020). For example, beans are subject to biotic stress caused by the fungus *Rhizoctonia solani*, which causes root rot (Menon, Ks, *et al.*, 2018), while the fungus *Botrytis cinerea*, the causative agent of gray mold, causes significant post-harvest damage to more than 200 crops (Sadeghian *et al.*, 2012). In addition to plants, the significance of fungal infections in humans and animals has increased over the past two decades, with fungi from various families causing serious diseases (Shi *et al.*, 2011). Traditionally, synthetic fungicides are used to control these fungi; however, their overuse and misuse lead to harmful environmental problems, including toxicity to non-target organisms, the development of resistance in pathogenic fungi to conventional drugs, and environmental pollution (Shirsat *et al.*, 2015). Scientific research institutions are therefore continuing their intensive efforts to explore innovative molecular frameworks and develop safe and effective therapeutic alternatives to combat bacterial and fungal diseases (Zhang, Wang, & Xu, 2007). In this context, nanotechnology plays a promising role in combating plant diseases, as the creation of materials in nanoscale form has numerous applications in the field of plant protection (Cheng *et al.*, 2020 & Dworecka-Kaszak *et al.*, 2020). Nano-fungicides are of particular interest due to their low toxicity at low doses, high solubility, targeted delivery, and controlled release, making them a potential alternative to conventional fungicides for achieving sustainability (Fernández-Lázaro *et al.*, 2020) & (Ghareeb *et al.*, 2025). Studies have suggested that the use of nutrients in their nanoscale form may (such as selenium) plays a protective role against plant pathogens (Joshua *et al.*, 2016). Selenium compounds (such as sodium selenite) and their nanoparticles have demonstrated clear antifungal properties, inhibiting fungal growth and spore germination, as well as preventing the contamination of food and feed with mycotoxins (Rudrappa *et al.*, 2022). The antifungal effect has been suggested to involve multiple mechanisms, including: selenium's ability to replace sulfur in cellular proteins, causing changes in sulfur-containing amino acids (cysteine and methionine); the generation of reactive oxygen species that cause DNA strand breaks; enhanced scavenging of these reactive oxygen species; induction of defensive callus formation; and stimulation of antifungal pathways in plants (Zhang, Wang, & Xu, 2007). Although selenium

compounds (such as selenium sulfide) have long been used as antifungals, research is still ongoing to investigate the antifungal activity of selenium nanoparticles (Cheng et al., 2020). Furthermore, bio-produced nanoparticles are characterized by their low cytotoxicity toward mammalian cells, making them promising candidates for medical applications as drug carriers (Dworecka-Kaszak et al., 2020& (Lin et al., 2021), as well as having attractive applications within the context of sustainable agriculture for combating fungal diseases (Singh, 2014).

MATERIALS AND METHODS

Literature Search Strategy

A systematic literature search was performed using PubMed, Scopus, and Web of Science for the period 2015–2025. The following keywords were used: "selenium nanoparticles" OR "SeNPs" OR "nano-selenium" AND "antifungal" OR "antifungal activity" AND "biosynthesis" OR "green synthesis" AND "fungi" OR "fungal pathogens".

Studies were included if they were (1) original peer-reviewed articles published in English, (2) reported the synthesis and physicochemical characterization of SeNPs, and (3) evaluated antifungal activity with quantifiable parameters (e.g., MIC, MFC). Review articles, studies without antifungal assessment, and those with incomplete or unclear methodologies were excluded.

The initial literature search yielded 243 records. After removing 49 duplicates, 194 articles were screened based on titles and abstracts. Of these, 109 were excluded as they were either not related to selenium nanoparticles, did not address antifungal activity, or were review articles. The remaining 85 full-text articles were assessed for eligibility. A total of 73 articles were excluded because they lacked MIC/MFC data, had unclear methodologies, or were not written in English. Ultimately, 12 studies met all inclusion criteria and were included in the final critical analysis. A PRISMA-style flow diagram summarizing the selection process is presented in Figure 1.

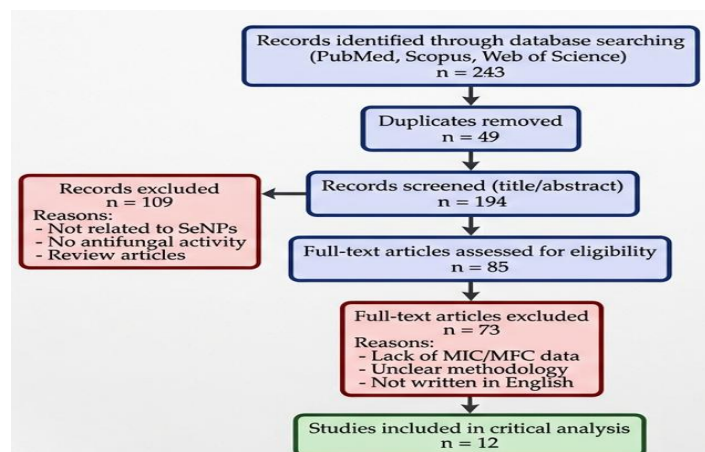


Figure 1 PRISMA-style flow diagram of the study selection process

RESULTS AND DISCUSSION

Methods for the preparation of Selenium Nanoparticles

Selenium nanoparticles (SeNPs) have been synthesized using diverse approaches, broadly categorized as chemical, physical, and biological methods. Each approach offers distinct advantages and limitations that critically influence the physicochemical properties and antifungal efficacy of the resulting nanoparticles.

Chemical and Physical Methods

Chemical reduction remains the most widely used approach, typically employing sodium selenite (Na_2SeO_3) as a selenium precursor and ascorbic acid as a reducing agent. Alagawany et al. (2021) reported rapid synthesis at room temperature within 30 minutes, yielding SeNPs that were collected by centrifugation. However, chemical methods often require additional stabilizers to prevent particle aggregation; for instance, Kang et al. (2023) incorporated chitosan as a stabilizer, achieving colloidal stability through extended stirring (3 hours at 25°C). While chemical reduction offers rapid synthesis and precise control over particle size, it involves toxic reagents and generates hazardous waste, limiting its biocompatibility and environmental sustainability (Sharma et al., 2009).

Physical methods, such as high-energy ball milling, have been used to produce ultrafine SeNPs (~24 nm), as calculated using the Scherrer equation (Bazina et al., 2025). Although physical approaches avoid chemical contaminants, they are energy-intensive and less scalable for routine biological applications.

Biological Synthesis (Mycogenic)

Fungal mediated synthesis has gained attention due to the high protein content of fungal filtrates, which serve as both reducing and capping agents. Using *Penicillium citrinum*, Gharieb, Omara, and Soliman (2025) produced spherical SeNPs ranging from 50–80 nm after fermentation, filtration, and centrifugation. *Fusarium oxysporum* demonstrated high efficiency, converting 85.2% of selenite under optimized conditions (Czapek's Dox medium, 30°C, pH 7) (Gharieb, Hassan, & Soliman, 2024). Similarly, *Fusarium fujikuroi* MED14 yielded SeNPs following incubation with Na_2SeO_3 at 40°C for two days (El-Fallalet et al., 2024). Interestingly, the oyster mushroom *Pleurotus ostreatus* produced SeNPs through a simple one step incubation (Sonbol et al., 2025). Mycogenic synthesis is eco friendly and cost effective (Srivastava et al., 2016; Dhanjal & Cameotra, 2010) but often requires longer incubation times (24–72 hours) and yields relatively larger particles (50–80 nm) compared with chemical methods, which typically produce smaller particles within minutes to hours (Alagawany et al., 2021; Kang et al., 2023).

Bacterial Synthesis

Bacterial systems offer faster reduction kinetics and greater reproducibility. *Bacillus megaterium* reduced selenious acid within 48 hours, yielding SeNPs collected by centrifugation and resuspended in DMSO for antifungal assays (Hashem et al., 2021). *Paenibacillus polymyxa* required 2–5 days for visible colour change (Yuan et al., 2025), whereas *Klebsiella pneumoniae* achieved synthesis within 24 hours, producing particles below 250 nm (Fesharaki et al., 2010). *Lactobacillus acidophilus* (El-Saadony et al., 2021), *Bacillus cereus* (Taha et al., 2023), *Bacillus* sp. MSh 1 (Shakibaei et al., 2015), *Halomonas elongata* (Safaei et al., 2022), *Escherichia coli* (Rasha & Neihaya, 2025), and *Paenibacillus terreus* (Nile et al., 2023) have all demonstrated selenite reduction capability, with particle sizes ranging from 41 to 250 nm. Bacterial synthesis is rapid and reproducible (Hashem et al., 2021; Fesharaki et al., 2010) but may require complex purification steps, including centrifugation, washing, and sonication, to separate nanoparticles from bacterial cells (Shakibaei et al., 2015; Nile et al., 2023).

Actinobacteria and Plant Mediated Synthesis

The marine actinobacterium *Streptomyces vinaceus drappus* AMG31 produced SeNPs within 24 hours under alkaline conditions (pH 8), with particle collection by centrifugation and drying at 100°C (Ghareeb et al., 2025). This approach combines the eco friendliness of biological synthesis with the robustness of bacterial systems.

Plant mediated synthesis has emerged as a particularly attractive green approach. Using methanolic extracts of *Amphipterygium glaucum* and *Calendula officinalis*, Lazcano-Ramirez et al. (2023) produced SeNPs with variable sizes depending on the extract composition. *Melia azedarach* leaf extract yielded brick red SeNPs after heating at 80°C (A Shahbaz et al., 2023). Notably, lemon and orange peel extracts, rich in ascorbic acid, produced ultrafine particles (~42 nm) under mild conditions (40°C, pH 4) (Desouky et al., 2025). Pomegranate peel extract (Salem et al., 2022) and extracts from *Allium paradoxum*, *Crocus caspius*, *Pistacia vera*, *Vicia faba*, and *Heracleum persicum* (Nosratabadi et al., 2023) have also been successfully employed. Plant based synthesis is simple, low cost, and environmentally sustainable, but batch to batch variability in extract composition remains a significant challenge.

Lactic Acid Bacteria and Cyanobacteria

Lactic acid bacteria, including *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Streptococcus thermophilus*, reduced selenite to SeNPs (45–275 nm) within 72 hours in MRS medium (Zommara et al., 2025). The cyanobacterium *Desmonostoc alborizicum* also demonstrated efficient SeNP production (Nowruzi et al., 2023). These approaches are particularly attractive for food and feed applications due to the generally recognised as safe (GRAS) status of lactic acid bacteria.

Radiation Assisted Synthesis

Gamma radiation induced polymerisation using PVP as a coating agent produced boron/selenium nanocomposites with enhanced stability (Gouda et al., 2025). Although highly efficient, this method requires specialised equipment and safety precautions, limiting its widespread adoption.

Ready Made Nanoparticles

Some studies utilised commercially available SeNPs dispersed by ultrasound and incorporated into acrylic resins for antifungal applications (Rostamzadeh et al., 2024). While convenient, this approach provides limited control over particle properties and does not address the synthesis related challenges. Despite the diversity of synthesis platforms, direct comparisons remain challenging due to the

lack of standardised protocols. For example, reaction temperatures, pH, and precursor concentrations vary considerably across studies, which may account for the wide range of particle sizes (41–275 nm) and zeta potential values (−37.9 to +32.3 mV) reported in the literature. Future studies should adopt unified synthesis conditions to enable meaningful cross-study comparisons.

Critical Synopsis

The reviewed literature reveals that no single synthesis platform is universally optimal; rather, the choice depends on the intended application. Chemical and

physical methods offer rapid production and size control but suffer from toxicity and energy concerns. Biological methods, particularly plant based and mycogenic routes, are environmentally sustainable and produce biocompatible nanoparticles but require longer synthesis times and face reproducibility challenges. Notably, smaller particles (<50 nm) generally exhibit superior antifungal activity, emphasising the need for synthesis protocols that achieve ultrafine sizes while maintaining colloidal stability. Future research should focus on optimising biological synthesis parameters to achieve rapid, reproducible, and scalable production of small, stable SeNPs with consistent antifungal efficacy.

Table 2 Comparative overview of synthesis platforms for selenium nanoparticles (SeNPs).

Synthesis Platform	Particle Size Range	Synthesis Time	Advantages	Limitations
Chemical reduction	~20–50 nm	30 min – 3 h	Rapid, precise control	Toxic reagents, waste
Physical (ball milling)	~24 nm	Variable	No chemical contaminants	Energy-intensive
Mycogenic (fungi)	50–80 nm	24–72 h	Eco-friendly, capping agents	Longer time, larger particles
Bacterial	41–250 nm	24–72 h	Reproducible, fast	Purification steps
Plant extracts	~42 nm	1–48 h	Low-cost, sustainable	Batch variability
Lactic acid bacteria	45–275 nm	72 h	GRAS status, food applications	Longer time
Radiation-assisted	Variable	Hours	High stability	Specialised equipment
Ready-made commercial	Variable	Instant	Convenient	Limited control

Note. GRAS = Generally Recognized as Safe. All values represent direct comparisons extracted from synthesis parameters.

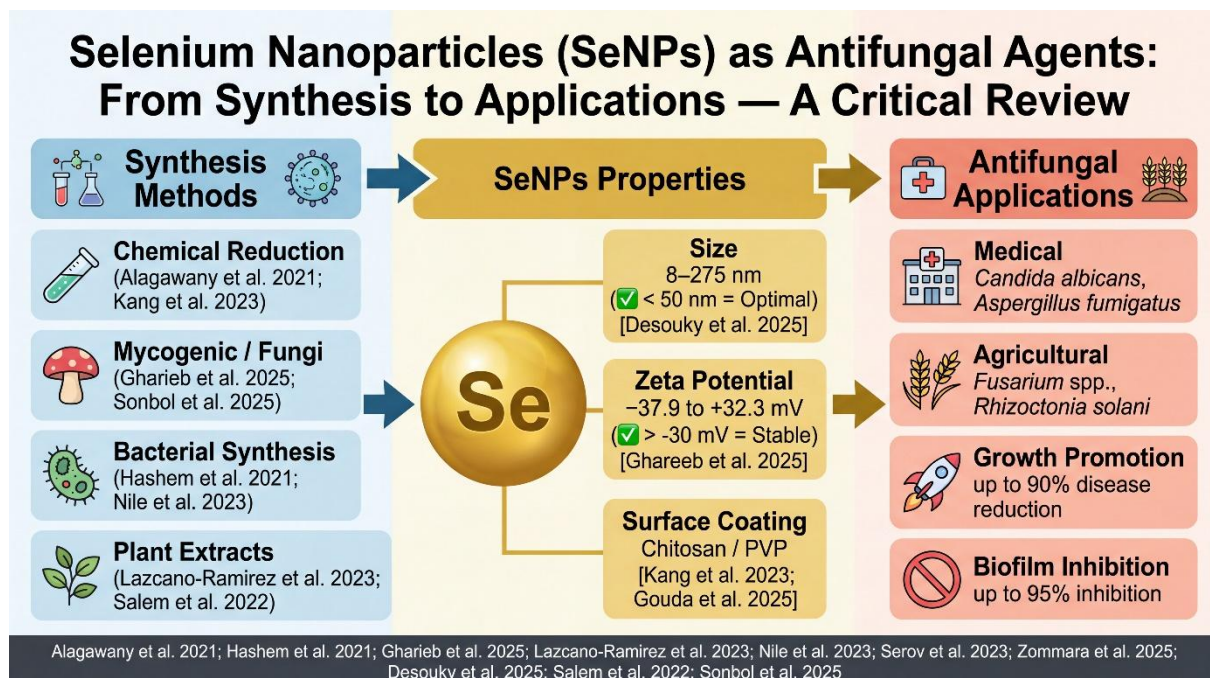


Figure 2 Summary of SeNPs synthesis routes, key physicochemical properties, and antifungal applications

Analytical methodologies for the evaluation of selenium nanoparticles (SeNPs)

Accurate characterization of selenium nanoparticles (SeNPs) relies on an integrated hierarchy of complementary techniques designed to verify identity, measure physicochemical properties, and assess colloidal stability. This section presents the methodological framework for these techniques, highlighting their role in establishing the structure-function relationship and explaining the wide variation in reported data across studies.

1. Initial Spectroscopic Analyses: Confirmation of formation and identification of active components; this set of rapid techniques serves as an initial and decisive confirmatory step.

Ultraviolet-Visible (UV-Vis) Spectroscopy: This is the primary method for detecting SeNP formation by monitoring the characteristic absorption band associated with Surface Plasmon Resonance (SPR). The peak position ranges from 205 nm (Sonbol et al., 2025) to 435 nm (Hashem et al., 2021), reflecting variations in particle size and the nature of the surrounding medium. The stability of the colloidal suspension is inferred from the stability of the peak's intensity and position over time (Ghareeb, Omara, & Soliman, 2025; Nile et al., 2023).

Fourier Transform Infrared Spectroscopy (FTIR): reveals the characteristic features of the functional groups of biological or chemical compounds involved in

reduction and immobilization processes. Typical spectral bands are attributed to compounds such as proteins and polyphenols involved in biosynthesis. It is also used to study binding interactions in hybrid nanomaterials. ((Ghareeb et al., 2025) & Tayel et al., 2025).

2. Advanced structural and morphological analysis techniques: determination of shape, size, and crystallinity

These techniques provide indispensable insights into the fundamental physical characteristics of particles.

Transmission electron microscopy (TEM): This is considered the gold standard for determining fine morphology, primary size distribution, and the degree of particle aggregation. A comprehensive analysis of the images reveals a wide range of reported sizes, from ultrafine particles (4–25 nm) (46) to larger particles (45–275 nm) (Zommara et al., 2025); this diversity directly reflects differences in sources and synthesis methods.

Scanning electron microscopy (SEM): Complements TEM by assessing surface topography and the extent of particle aggregation. It is also the preferred tool for visualizing and evaluating the direct interaction between nanoparticles and target biological surfaces, such as fungal hyphae (Taha et al., 2023; Nile et al., 2023). Some applied studies use SEM exclusively for this purpose without providing a basic morphological characterization of the particles themselves (Rostamzadeh et al., 2024).

X-ray diffraction (XRD): XRD is used to determine the crystalline phase and degree of crystallization. The patterns show sharp peaks characteristic of the trigonal selenium structure. The average crystal size is calculated using the Scherrer equation, which differs from the total size measured by microscopy (Ulloa *et al.*, 2023; Hashem *et al.*, 2021).

3. Assessment of colloidal stability: behavior in solution and surface interactions. These tests are crucial for predicting particle performance in liquid application media.

Dynamic Light Scattering (DLS) and Zeta Potential Measurement: DLS measures the hydrodynamic size of particles (core plus solvent and ionic shell) and the Particle Size Distribution Index (PDI). Zeta potential, on the other hand, reflects

the net surface charge, and high absolute values ($> \pm 30$ mV) indicate good colloidal stability due to electrostatic repulsion (Alagawany *et al.*, 2021).

Translational Considerations for Synthesis Platforms

Beyond physicochemical properties, the practical applicability of SeNPs depends on several translational factors. Table 2 provides a comparative evaluation of each synthesis platform based on scalability, reproducibility, cost, long-term stability, and regulatory considerations. This assessment highlights the trade-offs between different methods and identifies key gaps—particularly regarding regulatory data and long-term stability—that must be addressed to facilitate the translation of SeNP-based antifungals from laboratory to commercial

Table 2 Comparative evaluation of synthesis platforms for SeNPs based on translational and regulatory factors

Synthesis Platform	Scalability	Reproducibility	Cost	Stability	Regulatory Considerations	References
Chemical reduction	High	High	Moderate	High (with coating)	Moderate	Alagawany <i>et al.</i> , 2021; Kang <i>et al.</i> , 2023
Physical (ball milling)	Moderate	High	High	Not reported	Not reported	Bazina <i>et al.</i> , 2025
Mycogenic (fungi)	Low to Moderate	Low to Moderate	Low	Moderate	Not reported	Gharieb, Omara, & Soliman, 2025; Gharieb, Hassan, & Soliman, 2024; El-Fallal <i>et al.</i> , 2024
Bacterial	Low to Moderate	Low to Moderate	Low	Moderate	Not reported	Hashem <i>et al.</i> , 2021; Fesharaki <i>et al.</i> , 2010; Nile <i>et al.</i> , 2023
Plant extracts	Moderate	Moderate	Low	Variable	Not reported	Desouky <i>et al.</i> , 2025; Salem <i>et al.</i> , 2022; Lazcano-Ramirez <i>et al.</i> , 2023
Lactic acid bacteria	Moderate	Moderate	Low	Moderate	High (GRAS)	Zommara <i>et al.</i> , 2025
Radiation-assisted	Low	High	High	High	Not reported	Gouda <i>et al.</i> , 2025
Ready-made commercial	High	High	High	High	Not reported	Rostamzadeh <i>et al.</i> , 2024

Note. GRAS = Generally Recognized as Safe; SeNPs = Selenium Nanoparticles. Evaluations are qualitative, based on synthesis parameters and industrial translation potential reported in the cited literature

Notably, several studies relied on partial characterisation, omitting key parameters such as zeta potential or DLS (observed in 6 out of 12 studies). This limits the ability to correlate surface charge with antifungal efficacy and to assess colloidal stability—a critical factor for practical applications.

The influence of the physicochemical properties of selenium nanoparticles on their antifungal efficacy

The antifungal activity of selenium nanoparticles (SeNPs) is not merely due to their nanoscale nature but is governed by causal relationships among three physicochemical variables: size, specific surface area, and surface charge (zeta potential).

First: Size and surface area – determinants of penetration and oxidative stress

Particle size appears to be one of the most well-documented variable explaining efficacy, as studies demonstrate that smaller particles have been associated with higher efficacy for the following reasons:

A. Increased surface area to volume ratio

The smaller the size, the higher the surface area-to-volume ratio, which increases biological interaction with the cell wall and membrane (Lazcano-Ramirez *et al.*, 2023). Ultrafine particles were found to be more effective than larger ones in some studies in inhibiting *Fusarium oxysporum* and *Colletotrichum gloeosporioides*, a result directly attributed to their small size and high surface area.

B. Enhanced cellular penetration and ROS production: Smaller particles may penetrate the cell wall, enter organelles, and contribute to cellular damage, as suggested by electron microscopy studies, and cause extensive damage. Ultrafine particles (11 nm, 9.41 nm) penetrate cellular structures more effectively and cause functional disruption, increasing reactive oxygen species (ROS) production by 20–30% compared with larger particles (150–275 nm) (Safaei *et al.*, 2022&Zommara *et al.*, 2025)

C. Complete inhibition at the lower size ranges: Reducing the size from 42.28 nm to 32.7 nm increased the inhibition rate from 69% to 100% at the same concentration of 0.5 ppm (Desouky *et al.*, 2025).

D. Limited effect of larger particles: Larger particles (150–275 nm) cause less penetrating local structural damage, whereas smaller particles (45–100 nm) excel in cellular uptake and ROS production (Zommara *et al.*, 2025).

Second: Surface charge – between colloidal stability and cellular adhesion

Surface charge plays a dual role
it controls the physical stability of the nanoparticle suspension and regulates electrostatic interactions with the cell membrane. A. Negative charge: Stability and distribution:

A- negative charge (e.g., -37.9 mV, -31.4 mV, -26.21 mV) confers high colloidal stability and prevents agglomeration, thereby improving distribution and bioactivity (Ghareeb *et al.*, 2025) Surface groups (OH, COO⁻) are the source of this charge and stability (EL-Sayed *et al.*, 2024). A high negative charge (-37.44 mV) increases the solubility and bioavailability of drug compounds (Wang *et al.*, 2023).

B. Positive charge: Adhesion and penetration: The positive charge (+32.3 mV, +31.7 mV) enables particles to adhere to and penetrate negatively charged fungal cell walls, thereby enhancing efficacy (El-Fallal *et al.*, 2024). C. Synergy between size and charge: The combination of small nanoscale size and a negatively charged surface is directly linked to disruption of membrane integrity and internal organelles in fungal hyphae (EL-Sayed *et al.*, 2024). Improving the charge (from -19 mV to -7.24 mV) while reducing the size (42.28 $\leftarrow$ 32.7 nm) increased inhibition to 100% (Desouky *et al.*, 2025).

Third: Surface modification – enhancement or suppression, depending on the nature of the coating

The encapsulation or grafting of SeNPs produces varying effects depending on the extent of the active surface:

A. Negative effect (masking of the active surface): Incorporating SeNPs into the nanocystan matrix formed physical bonds between the hydroxyl/amine groups and the SeNP surface, reducing the availability of the active surface and lowering inhibition rates compared with individual SeNPs (Gharieb, Omara, & Soliman, 2025).

B. Positive effect (stabilisation and enhancement):

C- The formation of the nanocomposite (NCT/AmE/SeNPs) increased particle size (132.71–149.37 nm) while maintaining a stable surface charge, thereby enhancing efficacy and outperforming SeNPs alone in inhibition diameters and MFC values (Tayel *et al.*, 2025).

D- Coating SeNPs with nanokitosan reduced the size (42.28 $\leftarrow$ 32.7 nm), improved the charge (-7.24 mV), and increased inhibition to 100% (Desouky *et al.*, 2025).

E- Coating with PVP prevents agglomeration and improves stability and bioavailability, Although the effect of particle size on antifungal activity has been documented, the relationship between a slight reduction in size (from ~42 nm to

~33 nm) and a significant increase in inhibition (from 69% to 100% at the same concentration) has not been systematically investigated, particularly regarding the role of different stabilizers (such as PVP compared to chitosan) in achieving this sharp improvement in efficacy. This calls for mechanistic and comparative studies to identify the critical factors in the design of highly effective nanoparticles (Gouda *et al.*, 2025).

While oxidative stress and membrane disruption are consistently invoked as antifungal mechanisms, direct experimental evidence remains limited. Many studies infer ROS production indirectly via lipid peroxidation or antioxidant enzyme assays, without direct measurement of ROS in fungal cells. Furthermore, the distinction between primary and secondary effects is often unclear, and some proposed mechanisms are extrapolated from studies on other selenium compounds rather than from SeNP-specific investigations.

Antifungal activity of selenium nanoparticles: in laboratory and field studies

The antifungal efficacy of chemically or biologically derived selenium nanoparticles (SeNPs) against a wide range of pathogenic fungi was evaluated using various laboratory protocols. The chemical nanoparticles (Che-SeNPs) were reported to show activity against *Candida* spp., with inhibition zones of 11–17 mm, minimum inhibitory concentrations (MICs) of 70–100 µg/ml, and minimum fungicidal concentrations (MFCs) of 140–200 µg/ml (Alagawany *et al.*, 2021). SeNPs also showed high efficacy against other fungi in the cited studies, such as *Aspergillus niger* and *Candida albicans*, with inhibition zones of 17–33 mm, MICs of 6.25–100 µg/ml, and MFCs of 50–300 µg/ml ((Ghareeb *et al.*, 2025)). In other studies, methods such as agar dilution and colony diameter measurement were used, with SeNPs achieving MICs of 0.0625 mM against *Rhizoctonia solani* (Hashem *et al.*, 2021), 70 µg/ml against *C. albicans*, and 100 µg/ml against *Aspergillus fumigatus* (Shakibaei *et al.*, 2015). BioSeNPs also exhibited activity against six *Fusarium* species, with MICs of 20–40 µg/ml and inhibition zones of 18–33 mm (El-Saadony *et al.*, 2021).

Selenium-loaded nanocomposites were found to exhibit enhanced efficacy in some studies. The NCT/AmE/SeNPs composite inhibited *Botrytis cinerea*, with a zone diameter of 25.6 mm and an MFC of 27.5 mg/l (Tayel *et al.*, 2025), whereas the NCT/PPE/SeNPs compound inhibited *Penicillium digitatum*, with diameters ranging from 30.1–31.4 mm (Salem *et al.*, 2022). Irradiated boron/selenium nanoparticles also showed high activity against *Alternaria alternata*, with an inhibition zone diameter of 49 mm and an MIC of 3.125/0.938 mg/ml (Gouda *et al.*, 2025). In contrast, some studies indicated that larger particles or certain extracts were less effective (Lazcano-Ramirez *et al.*, 2023), and that bio-particles alone may not exhibit activity even at high concentrations (Nile *et al.*, 2023).

Regarding the mechanisms of inhibition, microscopic examinations (TEM, SEM) showed that treatment with SeNPs caused severe morphological abnormalities in fungal hyphae, including membrane rupture and destruction of internal organelles (EL-Sayed *et al.*, 2024). Complete inhibition (100%) of mycotoxin (aflatoxin) production was also observed at a concentration of 5,000 ppm of SeNPs (Gharieb, Hassan, & Soliman, 2024). Inhibition of biofilm formation and hyphal transformation was observed at low concentrations (Rasha & Neihaya, 2025). Some nanocomposites achieved complete inhibition of fungal growth and reduced biomass at concentrations as low as 0.5 ppm (Desouky *et al.*, 2025).

Regarding in vivo applications, studies have shown promising results in improving health, disease resistance, and growth across various species. In Japanese quail, adding 0.4 g/kg of Che-SeNPs to the feed improved body weight and feed conversion ratio, increased antioxidant enzyme activity (SOD, GPX), reduced harmful lipids, enhanced immunity (IgG, IgM), and reduced the number of fungi and yeasts in the cecum (Alagawany *et al.*, 2021).

In plant applications, Foliar spraying of tobacco plants with nano-selenium was reported to provide 90.23% disease protection after 7 days and increased growth indices by 16.21–33.61% (Hashem *et al.*, 2021 & Yuan *et al.*, 2025). In bean plants, treatment with nano-selenium (0.0625 mM) resulted in the highest plant survival rate in that specific study (83.33%) and disease protection (72.27%) (Hashem *et al.*, 2021). Foliar spraying of wheat plants infected with *Septoria* leaf spot (at 30 mg/l) reduced disease severity, increased plant height by 30.7%, and significantly increased chlorophyll, phenol, and flavonoid content (Shahbaz *et al.*, 2023). In bean plants, treatment with nano-selenium (100 ppm) reduced the severity of *Alternaria alternata* infection to 20.5% and increased dry seed yield by approximately 30% (Taha *et al.*, 2023).

In mango trees infected with gummosis, spraying with nano-selenium (30 µg/ml) improved physiological indicators, including total chlorophyll (10.91 mg/g), proline content, soluble sugars, and SOD enzyme activity (Sadeghian *et al.*, 2012 & Rudrappa *et al.*, 2022). Treatment with nano-selenium (100 µg/ml) on wheat plants also reduced root and crown rot by 75% and increased yield (El-Saadony *et al.*, 2021).

Regarding fruits, Coating strawberries with the NCT/AmE/SeNPs compound was reported to provide complete protection against the onset of disease symptoms for 10 days (Tayel *et al.*, 2025). Similarly, coating oranges with the NCT/PPE/SeNPs compound provided 100% protection against green mold for the same duration (Nowruzi *et al.*, 2023). Foliar spraying with nano-selenium (0.5 mg/L) reduced the powdery mildew infection index on melons by 21–45% and increased the activity of catalase and peroxidase enzymes (Kang *et al.*, 2023). In a study on wheat,

treatment with nano-selenium (10 µM) stimulated growth and increased the contents of sugars and total proteins (Sonbol *et al.*, 2025).

These results reflect a diversity of synthetic and evaluation systems, with quantitative documentation of inhibition values, inhibitory and lethal concentrations, and indicators of protection and growth in plant and animal models. Although in vivo studies report promising disease protection (up to 90%), most were conducted under controlled greenhouse or laboratory conditions. Large-scale field trials are notably absent, and the environmental fate of SeNPs—including soil accumulation and effects on non-target organisms—remains largely unexplored. Furthermore, the variability in application methods (foliar spray, soil drench, seed treatment) complicates direct comparisons of efficacy across studies. Future research should prioritise field-scale validation and environmental risk assessment to bridge the gap between laboratory promise and practical agricultural application.

Biomedical Potential of Selenium Nanoparticles against Human Fungal Pathogens

Beyond their well-documented agricultural applications, selenium nanoparticles (SeNPs) have demonstrated considerable promise as antifungal agents against clinically relevant human pathogens. The opportunistic yeast *Candida albicans*, a leading cause of nosocomial fungal infections, exhibits marked susceptibility to biogenic SeNPs, with reported minimum inhibitory concentration (MIC) values ranging from 70 to 100 µg/mL (Alagawany *et al.*, 2021; Shakibaei *et al.*, 2015). Notably, SeNPs have also shown potent activity against non-*albicans Candida* species, including *C. glabrata*, *C. parapsilosis*, and *C. guilliermondii*, with MIC values of 90, 80, and 100 µg/mL, respectively (Alagawany *et al.*, 2021), underscoring their broad-spectrum anticandidal potential.

In the context of invasive mould infections, SeNPs have exhibited significant inhibitory effects against *Aspergillus fumigatus*, the primary causative agent of invasive pulmonary aspergillosis in immunocompromised patients, with MIC values of 100 µg/mL (Shakibaei *et al.*, 2015). Similarly, *Aspergillus niger*, an opportunistic pathogen associated with otomycosis and pulmonary infections, was found to be highly susceptible to SeNPs, with MIC values as low as 6.25 µg/mL (Ghareeb *et al.*, 2025), indicating strain-specific variations in susceptibility that merit further investigation.

A particularly noteworthy finding is the potent antibiofilm activity of SeNPs against *C. albicans*. Biofilm formation is a critical virulence factor that confers resistance to conventional antifungals and is implicated in the pathogenesis of medical device-related candidiasis. SeNPs have been shown to inhibit *C. albicans* biofilm formation by up to 95% (Nile *et al.*, 2023; Rasha & Neihaya, 2025), suggesting their potential as an adjunctive or alternative therapy for biofilm-associated infections. Furthermore, the functionalisation of SeNPs with nystatin has been reported to enhance their antifungal efficacy against *C. albicans* biofilms, indicating a synergistic interaction that could reduce the effective dose of conventional antifungals and mitigate toxicity concerns (Nile *et al.*, 2023).

Crucially, the biocompatibility of biogenic SeNPs is supported by their low cytotoxicity towards mammalian cells. For instance, SeNPs exhibited an IC₅₀ value of 419.7 µg/mL against normal human lung fibroblast cells (WI-38), indicating a favourable therapeutic window (Ghareeb *et al.*, 2025). Additionally, SeNPs demonstrated a hemolysis rate of less than 2% at concentrations up to 1000 µg/mL, confirming their haemo-compatibility and safety for systemic administration (Ghareeb *et al.*, 2025).

Collectively, these findings position SeNPs as versatile antifungal agents with dual utility in agricultural and clinical settings. Their ability to combat both phytopathogenic and clinically significant fungi, coupled with their low toxicity and biofilm-disrupting properties, bridges a critical gap between plant protection and human healthcare. This dual functionality enhances the translational relevance of SeNP-based antifungals and supports their further development as broad-spectrum therapeutic agents.

Proposed mechanisms for the effect of chemical selenium nanoparticles (Che-SeNPs) on fungi

Selenium nanoparticles have been reported to exhibit antifungal activity that may surpass that of other selenium forms, such as sodium selenite (Khiralla & El-Deeb, 2015), selenium disulfide Wu *et al.* (2014) SeS₂, and selenite ions (Zhang, Li, *et al.*, 2021) Se (IV). Those prepared biochemically may outperform their chemical counterparts (Hartikainen *et al.*, 2000) and commercial antifungals (Tran & Webster, 2011).

This increased efficacy is thought to be attributed to their unique physicochemical properties, particularly their high surface-to-volume ratio and nanoscale size (Khiralla & El-Deeb, 2015), which enhance interactions with fungal cells and their ability to penetrate cells (Jha *et al.*, 2022), along with their relatively low toxicity (Desouky *et al.*, 2025).

First: Morphological and structural destruction of fungal cells

One of the most notable effects of nanoparticles on fungi is the severe morphological and structural destruction documented in electron microscopy images. Transmission electron microscopy (TEM) images have shown:

- Strong adhesion of nanoparticles to the fungal cell wall leads to a marked increase in the thickness of the cell wall and plasma membrane, a failed defense response, and the entry of nanoparticles into the cells, causing damage and aggregation of cellular organelles such as the nucleus, endoplasmic reticulum, and Golgi apparatus (Lazzano-Ramírez *et al.*, 2023). Severe morphological and structural destruction was observed, manifested by a significant change in the shape of fungal hyphae from their normal cylindrical form to a distorted structure, with separation and rupture of the cell membrane, loss of integrity of internal organelles, and the formation of pores in the cell, leading to cell death (Gunti *et al.*, 2019).

Scanning electron microscope (SEM) images also revealed:

- Severe destruction of fungal hyphae, with complete fragmentation, collapse of cell walls, and leakage of cellular contents (Zhang, Wang, & Xu, 2020). Shrinkage, decomposition, deformation, and fragmentation of hyphae compared with normal, intact hyphae (Wang *et al.*, 2015). A gradual transformation in treated hyphae from their normal shape to irregular deformities and swellings after 10 hours, clear degradation and leakage of cellular contents after 20 hours, and complete degradation and disintegration of hyphae after 30 hours of treatment (Tayel *et al.*, 2025). Clear deformation, loss of smoothness, severe deformities in hyphae, and complete inhibition of conidiation (Gouda *et al.*, 2025).

Second: Main mechanisms of action

1. Oxidative stress: Oxidative stress is widely considered to be a major mechanism underlying the antifungal effect. Evidence suggests that nanoparticles can stimulate the generation of Reactive Oxygen Species (ROS) within fungal cells (Menon & Shanmugam, 2020), causing oxidative stress that leads to a series of destructive effects, including Lipid peroxidation in cell membranes, as evidenced by the results of the study (Zommara *et al.*, 2025). DNA damage (Galić *et al.*, 2020), protein damage, and enzyme inactivation (Zhang, Wang, & Xu, 2020). They also cause leakage of cytoplasmic contents and damage to the cell wall (Gan *et al.*, 2020), inducing oxidative lesions and binding to iron ions (Mellersh *et al.*, 2002).

2. Disruption of cell membranes and walls: It has been proposed that nanoparticles may compromise the integrity of fungal cell membranes and walls in several ways:

A. Electrostatic interaction: The negative charge of the particles facilitates strong electrostatic interactions with positively charged components in fungal membranes, thereby disrupting membrane integrity (Serov *et al.*, 2023). In chitosan-based nanocomposites, positively charged chitosan molecules adhere to and interact with the negatively charged hyphal walls (Wang *et al.*, 2015). The electrostatic interaction between nanoparticles and the fungal cell wall or plasma membrane disrupts the wall, thereby allowing the passage of large molecules through the membrane (Anyasi *et al.*, 2017).

B. Binding to membrane components: Some studies indicate that the particles may bind to cell membrane components, including ergosterol (Zhang, Wang, & Xu, 2020).

C. Inactivation of thiol groups (-SH): Inactivation of thiol groups (-SH) in membrane proteins and enzymes affects membrane permeability. Furthermore, substituting selenium for sulfur in amino acids such as cysteine and methionine alters protein structure and disrupts enzyme function (Nie *et al.*, 2023).

3. Disruption of essential biological processes: It is hypothesized that nanoparticles can interfere with critical biological pathways in the fungal cell, as shown in:

Disruption of energy transfer, damage to mitochondrial components, disruption of the respiratory chain, inhibition of ATP production, and inhibition of vital enzymes such as cytochrome P450 (Nie *et al.*, 2023). Disruption of signaling pathways, including targeting of genetic material and disruption of the (RAS/cAMP/PKA) signaling pathways necessary for growth and the formation of biological membranes (B)Shahbaz *et al.*, 2023).

Thirdly: Effect on biofilms: Emerging evidence suggests that nanoparticles may play an important role in controlling the formation of fungal biofilms, which are the main source of resistance to antifungal drugs and food contamination (Fesharaki *et al.*, 2010). The particles have been shown to strongly inhibit biofilm formation and to cause biofilm degradation and severe damage to treated cells, including cell clumping, vesicles, membrane cracks, and cell lysis (Tran & Webster, 2011).

Fourth: Stimulation of host defenses: In addition to their direct effect on fungi, selenium nanoparticles stimulate the host's defenses, whether plant or animal, against fungal infection.

In animal models (fish):

This protective effect is attributed to improved antioxidant status—a significant increase in SOD and CAT activities and a decrease in MDA—and to improved

immune parameters, including increased lysozyme and phagocytic activity, and higher globulin levels (Alagawany *et al.*, 2021).

In plant models:

Foliar spraying with nano-selenium stimulated the plant's in vivo defenses through:

A. Enhancing the antioxidant status: increased activity of the antioxidant enzymes SOD, CAT, APX, POD, and PPO (Balois-Morales *et al.*, 2023); elevated levels of glutathione and proline; and reduced accumulation of H₂O₂ and MDA (Kang *et al.*, 2023).

B. Stimulating defense pathways: stimulating polyamine and phenylpropanoid pathways, with increased levels of putrescine, spermine, salicylic acid, and indoleacetic acid (Kang *et al.*, 2023), and increased activity of the enzymes PAL, C4H, and 4CL, as well as increased levels of phenolic compounds, flavonoids, and lignin (Xu *et al.*, 2020 & Hashem *et al.*, 2021)

C. Improvement of cellular structure: Improvement of the cellular structure of plant leaves, reduction in fungal density, and the formation of defensive papillae at sites of fungal penetration (Xu *et al.*, 2020). Deposition of defensive callus (Moretti *et al.*, 2022).

Fifth: Factors influencing efficacy: The efficacy of antifungal nanoparticles depends on several factors

1. Concentration: Most studies have shown concentration-dependent efficacy, with efficacy increasing as concentration rises (Maringgal *et al.*, 2019).

2. Size: Smaller particles are more effective than larger ones; smaller particles (SeNPs-AGL, 8 nm in size) were more effective than larger particles (SeNPs-COF, 134 nm in size). This increased efficacy of smaller particles is attributed to their greater stability and larger surface-to-volume ratio, which allows them to interact more closely with microbial cell membranes and facilitate diffusion into cells (Jha *et al.*, 2022).

3. Nanocomposite Structure: Nanocomposites demonstrate greater efficacy than their individual components and than commercial fungicides:

- Nanocomposites prepared using Amla extract and nano-chitosan demonstrated antifungal efficacy superior to that of a commercial fungicide (Tayel *et al.*, 2025).
- The nanocomposite (NCS-Se NPs) exhibited complete (100%) inhibition of mycelial growth (Zhang, Wang, & Xu, 2007). The prepared nanocomposite exhibited antifungal activity superior to that of a commercial fungicide (Desouky *et al.*, 2025).

Mechanism of action of nanocomposites: Positively charged nanochitosan particles can carry and encapsulate AmE/SeNPs, transporting them toward fungal hyphae, where they adhere to and interact with the membranes. After penetrating the hyphae, the AmE/SeNPs are released from the nanochitosan to interact with cellular organelles and biological systems, thereby disrupting their functions (Tayel *et al.*, 2025).

The cytotoxicity of selenium nanoparticles and their effects on the growth of living organisms

The effect of selenium nanoparticles on normal cells depends critically on the dose used and the chemical form of the particles. Numerous studies have suggested that nanoparticles may exhibit high biocompatibility and safety at low and medium concentrations. In a study on laboratory animals, no adverse effects on white and red blood cells were observed at low and medium doses, whereas alanine aminotransferase and creatinine levels increased with the high dose (Alagawany *et al.*, 2021). Another study on normal cells (WI-38) confirmed that nanoparticles prepared using a biological method have a high IC₅₀ value (419.7 µg/ml), indicating low toxicity, as well as a very low hemolytic rate (less than 2%) on red blood cells, confirming their safety for cell membranes (Ghareeb *et al.*, 2025). This high biocompatibility may be attributed to organic molecules coating the nanoparticles, such as bacterial proteins or fungal extracts, which impart a high negative charge (e.g., -37.9 mV) that increases their stability and prevents harmful interactions with healthy cells (Ghareeb *et al.*, 2025; Nile *et al.*, 2023). An improvement in liver and kidney function was also observed in treated mice, manifested by a reduction in ALT, AST, urea, and creatinine levels, as well as the restoration of the histological structure of the liver and kidneys to their normal form under the microscope, confirming their biological safety at the specified concentrations (EL-Sayed *et al.*, 2024). However, some studies have reported moderate toxicity to normal cells such as Vero E6 (with a CC₅₀ value of 360.8.8 µg/ml) (Sonbol *et al.*, 2025). One study explained this phenomenon by noting that selenium nanoparticles are less toxic than ionic forms (selenates, selenites) due to their slow release and low solubility; but very small particles (45–100 nm) may be more effective at penetrating cells and producing reactive oxygen species (ROS), which may increase their toxicity if they accumulate at high concentrations (Zommara *et al.*, 2025). It is generally acknowledged that high doses may lead to significant oxidative stress, as evidenced by elevated liver enzymes and creatinine levels, which calls for caution and underscores the need to precisely define the safe range of use (Alagawany *et al.*, 2021).

Biocompatibility data are encouraging; however, the absence of long-term toxicity studies, particularly concerning bioaccumulation in edible plant parts or animal tissues, represents a critical gap. Moreover, most toxicity assessments focus on mammalian cells, with limited data on environmental organisms such as

earthworms or beneficial soil microorganisms. Future research should prioritise ecotoxicological evaluations and long-term exposure studies to establish comprehensive safety profiles for SeNPs.

Improving plant growth, productivity and disease resistance

A series of studies suggests that nano-selenium may promote plant growth and enhances resistance to biotic stresses such as fungal diseases. For example, research on fava beans showed significant improvements in all growth indicators, with dry weight of vegetative mass increasing by 203%. This benefit has been attributed to nano-selenium's potential to boost starch content in chloroplasts, improve photosynthesis, and strengthen antioxidant defenses in plant cells (Hashem *et al.*, 2021). Similarly, wheat plants treated with 10 µM nanoselenium exhibited better growth and higher levels of soluble sugars and proteins. However, higher concentrations (100–25 µM) hindered growth, illustrating the dose-response relationship even in plants (Sonbol *et al.*, 2025). The protective role of nanocellin has been clearly demonstrated, especially in fungal resistance. In melon plants infected with powdery mildew, foliar spraying with 5 mg/L nanocellin reduced disease severity by up to 45%, increased antioxidant enzyme activities (SOD, CAT, APX), and doubled polyamine and hormone levels, including IAA and SA. It also stimulated the phenylpropanoid pathway, which produces defense compounds (Kang *et al.*, 2023). Similar results were observed in mango trees with leaf spot disease, in which levels of chlorophyll, proline, soluble sugars, and antioxidant enzymes increased, highlighting nanoselenium's role in boosting plant

immunity and reducing oxidative damage caused by fungal infections (B)Shahbaz *et al.*, 2023). Other crops, like wheat with stem spot disease (A)Shahbaz *et al.* (2023), and beans with leaf spot disease (Taha *et al.*, 2023), also showed notable improvements in growth parameters (length, weight, leaf area), biochemical contents (chlorophyll, phenols, flavonoids), and overall yield. These findings support nanoselenium's capacity to enhance photosynthesis and nutrient absorption. Additionally, post-harvest studies indicate that coating fruits with selenium-containing nanocomposites—such as mixtures of nanocellulose with amla extract or pomegranate peel—creates a protective barrier. This barrier inhibits fungi like *Botrytis cinerea* and *Penicillium digitatum* and maintains fruit freshness and quality over extended periods. The synergistic action of the components produces reactive oxygen species that damage fungal cell walls, thus preventing fungal growth (Tayel *et al.*, 2025&Salem *et al.*, 2022)

A critical review of studies on the antifungal activity of selenium nanoparticles (SeNPs)

The field of applications for selenium nanoparticles has experienced considerable growth, with a variety of sources of synthesis (chemical, bacterial, fungal, plant-based) and application models (animal, plant, laboratory) (Garza-Garcia *et al.*, 2022; Hernández-Díaz *et al.*, 2021).

To facilitate cross-study comparison and highlight the key features of the selected studies, the main synthesis parameters, antifungal activity data, and experimental models for the 12 analyzed studies are summarized in Table 3.

Table 3 Summary of synthesis parameters, antifungal activity, and experimental models of the 12 analyzed studies

Study	Synthesis Route	Particle Size (nm)	Zeta Potential (mV)	Target Fungi	MIC (µg/mL)	MFC (µg/mL)	Experimental Model
Alagawany <i>et al.</i> , 2021	Chemical reduction	75.68	-23.26	<i>Candida spp.</i>	70–100	140–200	In vivo (quail)
Hashem <i>et al.</i> , 2021	Bacterial (<i>B. megaterium</i>)	29.72–74.36	Not reported	<i>Rhizoctonia solani</i>	0.0625 mM	1 mM	In vivo (bean plants)
Gharieb, Omara, & Soliman, 2025	Mycogenic (<i>P. citrinum</i>)	50–80	Not reported	<i>Fusarium spp.</i>	Not reported	Not reported	In vitro
Gharieb, Hassan, & Soliman, 2024	Mycogenic (<i>F. oxysporum</i>)	Not reported	Not reported	Mycotoxin-producing fungi	Not reported	Not reported	In vitro
El-Fallal <i>et al.</i> , 2024	Mycogenic (<i>F. fujikuroi</i>)	52–68	+32.3	<i>Fusarium spp.</i>	Not reported	Not reported	In vitro
Sonbol <i>et al.</i> , 2025	Mycogenic (<i>P. ostreatus</i>)	72–148	-10.5	<i>Candida albicans</i>	Not reported	Not reported	In vitro / In vivo (wheat)
Fesharaki <i>et al.</i> , 2010	Bacterial (<i>K. pneumoniae</i>)	100–550	Not reported	<i>Malassezia spp.</i>	10–50	80–190	In vitro
El-Saadony <i>et al.</i> , 2021	Bacterial (<i>L. acidophilus</i>)	46	-23.48	<i>Fusarium spp.</i>	20–40	40–60	In vivo (wheat)
Taha <i>et al.</i> , 2023	Bacterial (<i>B. cereus</i>)	41–102	Not reported	<i>Alternaria alternata</i>	100	Not reported	In vivo (bean plants)
Shakibaei <i>et al.</i> , 2015	Bacterial (<i>Bacillus sp.</i>)	80–220	Not reported	<i>Candida albicans</i>	70	Not reported	In vitro
Nile <i>et al.</i> , 2023	Bacterial (<i>P. terreus</i>)	220–240	-37.44	<i>Candida albicans</i>	Not reported	Not reported	In vitro
Salem <i>et al.</i> , 2022	Plant extract (pomegranate peel)	132.71–149.37	+31.7	<i>Penicillium digitatum</i>	22500	12500	In vivo (orange coating)

Note. MIC = Minimum Inhibitory Concentration; MFC = Minimum Fungicidal Concentration. "Not reported" indicates that the specific parameter was not explicitly reported in the original study

The studies analyzed (12 studies) have shown the promising potential of SeNPs in inhibiting pathogenic fungi, improving growth, and stimulating immunity (Alagawany *et al.*, 2021). However, a critical in-depth review reveals a recurring pattern of methodological strengths and weaknesses that warrant attention to improve the quality of future research; consequently, the field of antifungal selenium nanoparticle research is promising but remains methodologically in its infancy (Mondragon *et al.*, 2026). To advance it toward reliable, practical applications, standardized protocols are recommended that combine analytical rigor, experimental realism, and biosafety to enhance the credibility and reproducibility of the results (Percie du Sert *et al.*, 2020).

First: Common methodological strengths

1. Diversity of synthesis sources: Studies have presented multiple synthesis models (chemical, bacterial, fungal, plant-based), thereby expanding sustainable and environmentally friendly options.
2. Comprehensiveness of bioassays: Some studies were characterized by broad coverage of biological activities, such as assessing many different activities or combining laboratory bioassays with integrated animal studies, thereby adhering to advanced standards in bioassay methodology.
3. Accuracy in characterizing nanoparticles: Studies stood out for using a comprehensive suite of characterization techniques (TEM, XRD, DLS, Zeta, FTIR, EDX), enabling a precise understanding of particle properties and their correlation

with efficacy, consistent with the MIRIBEL guidelines for comprehensive characterization (Faria *et al.*, 2018).

4. Application to advanced models: Some studies went beyond the laboratory setting to explore promising applications in mice, fruit coating, and pot experiments with bean plants.
5. Use of advanced analytical techniques: One study stood out for using HPLC to assess mycotoxin inhibition, adding a qualitative dimension and complying with standard criteria for mycotoxin analysis.

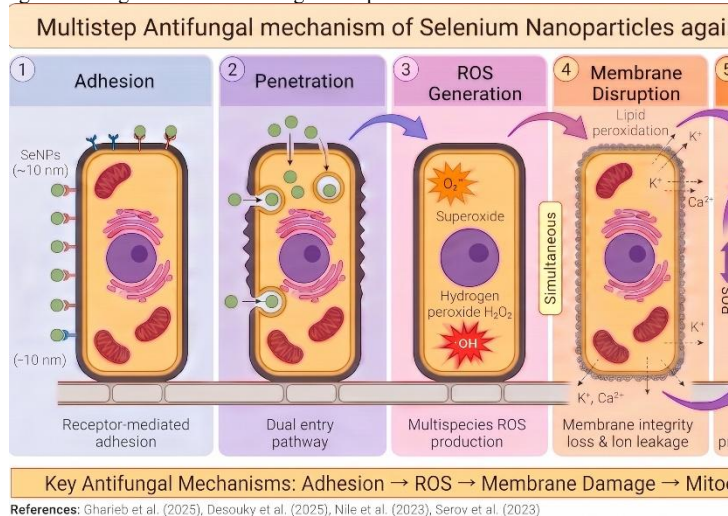
Secondly: Recurring methodological weaknesses

1. Physicochemical characterization: Six studies relied on partial characterization (lack of ζ-potential or DLS), making it difficult to link properties to efficacy; this contradicts the minimum information required in nanobiotechnology research.
2. Absence of actual selenium concentration measurements (ICP-MS): None of the 12 studies used ICP-MS to measure actual concentrations in solutions or tissues, creating a gap in understanding the true dose and bioaccumulation.
3. Limitations of experimental models: Some studies relied on unnatural administration (injection) or were confined to in vitro settings, limiting the generalizability of the results to natural conditions.
4. Statistical issues: Some studies fell into the trap of pseudoreplication by analyzing individuals rather than replicates (Bazina *et al.*, 2025), and most lacked randomization and blinding.

5. Neglect of safety studies: In some studies, toxicity tests were not conducted on normal cells or non-target organisms, raising concerns about safe use.
 6. Weak molecular analysis: Most studies relied on conventional markers without examining the molecular mechanisms underlying growth inhibition or stimulation.
 7. Lack of comparison with standard fungicides: Most studies failed to include a positive control group with a commercial fungicide, making it difficult to assess relative efficacy.
 8. Lack of long-term stability studies: No study has assessed the stability of SeNPs during storage, which is essential for practical application.
- The wide variability in experimental design—from synthesis conditions to antifungal assays—limits the generalisability of conclusions. Without standardised MIC testing protocols (e.g., CLSI guidelines), direct comparisons of antifungal potency remain problematic

Based on the previous literature (Gharieb *et al.*, 2025; Desouky *et al.*, 2025; Nile *et al.*, 2023; Serov *et al.*, 2023) and the findings of the present study, a multistep antifungal mechanism of SeNPs against fungal cells can be illustrated, as shown in Figure 3.

Figure 3 Multistep Antifungal Mechanism of Selenium Nanoparticles (SeNPs) against a Single Filamentous Fungal Compartment



Third: Comprehensive recommendations for future research

1. Comprehensive nanoscale characterization: Adherence to guidelines for TEM, XRD, DLS, Zeta, and FTIR, and publication of data within the same study.
2. Measurement of actual concentration: Use ICP-MS or AAS to quantify Se in solutions, tissues, and food products.
3. Realistic experimental models: via environmental exposure (soil, water, food) rather than injection. Large-scale field trials. Application to real food surfaces.
4. Sound statistical design: Use of the mean of replicates as the unit of analysis. Application of Generalized Linear Mixed Models (GLMM) to hierarchical data. Documentation of randomization and blinding. Prior sample size calculation (power analysis).
5. Mandatory safety studies: Cytotoxicity tests (MTT) on normal cells. Assessment of the impact on non-target organisms (beneficial bacteria and earthworms). Determination of the maximum permissible dose.
6. Advanced molecular analysis: Use of qPCR to study the expression of resistance and virulence genes. Analysis of cellular signaling pathways (apoptosis, inflammation).

A notable gap is the lack of studies investigating the synergistic effects of SeNPs with conventional antifungal agents, which could offer combination therapies with reduced toxicity. Exploring such combinations may not only enhance antifungal efficacy but also reduce the required doses of conventional fungicides, thereby mitigating their environmental and health impacts

7. Comparison with reference standards: Inclusion of a positive control group using an approved commercial fungicide.
8. Stability and storage study: Recharacterization of the particles and testing of their efficacy after specified storage periods.

Translational Barriers and Future Perspectives for Selenium Nanoparticles as Antifungal Agents

Despite the promising antifungal activity of selenium nanoparticles (SeNPs) demonstrated in laboratory and greenhouse studies, several translational barriers must be addressed before their widespread agricultural and clinical application. Currently, no harmonised regulatory framework exists for SeNP-based antifungals. Agencies such as the EPA and FDA require comprehensive toxicological and environmental risk assessments before approval. The absence of standardised

protocols for SeNP characterisation, stability testing, and toxicity evaluation complicates regulatory submissions and delays commercialisation (Garza-Garcia *et al.*, 2022; Hernández-Díaz *et al.*, 2021). Recent critical reviews have concluded that SeNPs face significant regulatory hurdles due to insufficient long-term safety data and the lack of harmonised evaluation frameworks (Geng *et al.*, 2025; Li *et al.*, 2025).

Research indicates that SeNPs exhibit slow release in soil, enhancing beneficial microbial growth while exhibiting lower toxicity compared to ionic selenium forms (Liu *et al.*, 2021). However, a comprehensive review highlights that SeNPs can significantly modify soil physicochemical properties, alter microbial communities, and influence heavy metal migration, yet long-term ecological impact studies are still lacking (Tian *et al.*, 2025). Additionally, the behaviour of SeNPs in aquatic environments and their effects on non-target organisms, including earthworms and beneficial soil microorganisms, require further investigation (Serov *et al.*, 2023; Devi *et al.*, 2023).

Bioaccumulation poses a significant risk, particularly if SeNPs are used in edible crops or animal feed. Recent tracking studies have revealed that SeNPs absorbed through leaves are transformed into ionic ($Se(IV)$) and organic selenium species within plant tissues, and while all selenium forms can be transported downward, only $Se(IV)$ and SeNPs can be excreted via roots (Li *et al.*, 2025). Field studies on wheat have demonstrated that SeNPs effectively deliver selenium to grains, where it accumulates primarily in ionic form rather than as intact nanoparticles, thus reducing the risk of direct nanoparticle ingestion by humans (Wang *et al.*, 2025). In soybean, biogenic SeNPs at 5–10 mg/L enhanced the accumulation of organic selenium species (SeMet, SeCys, MeSeCys) in seeds, with significantly higher bioavailability compared to selenite treatment (45–56% vs. 19.6–34%) (Li *et al.*, 2025). Nevertheless, current data on the translocation of SeNPs from soil to plants and their distribution within plant organs remain scarce (Najjar & Abu-Khalaf, 2021; Lin *et al.*, 2021).

Long-term safety data are urgently needed. While acute toxicity studies suggest that SeNPs have relatively low cytotoxicity at moderate doses (Alagawany *et al.*, 2021; Ghareeb *et al.*, 2025), chronic exposure studies are virtually absent. A critical review of global regulations emphasises that SeNPs are considered promising delivery systems, yet their industrial application remains limited due to inadequate chronic safety data and the absence of unified assessment frameworks (Geng *et al.*, 2025). Comprehensive reviews on the clinical translation of SeNPs highlight the need for full pharmacokinetic modelling, long-term safety evaluation, and large-scale clinical validation (Li *et al.*, 2025). Similarly, recent work underscores the necessity of developing standardised toxicity assessment protocols, achieving scalable production, and ensuring regulatory compliance to drive clinical and commercial translation (Zhang *et al.*, 2025). The effects of low-dose, long-term SeNP exposure on cellular function, immune response, and reproductive health in animals and humans have not been systematically investigated, and the potential for SeNPs to induce oxidative stress or epigenetic changes over extended periods remains an open question (Zommara *et al.*, 2025; Sonbol *et al.*, 2025).

Addressing these translational barriers will require multidisciplinary collaboration among chemists, biologists, toxicologists, and regulatory scientists. Future research should prioritise the development of standardised characterisation and toxicity testing protocols, environmental fate studies under realistic agricultural conditions, long-term bioaccumulation and safety studies in relevant animal models, and engagement with regulatory bodies to establish clear guidelines for SeNP-based product approval.

CONCLUSIONS

Biosynthesized selenium nanoparticles (SeNPs) have been shown to exhibit promising antifungal activity against a broad range of phytopathogenic and clinically relevant fungi. Their efficacy is primarily governed by size, surface charge, and coating material. Antifungal mechanisms reported in the literature include inducing oxidative stress, disrupting membranes, and stimulating host defenses. In vivo applications demonstrate significant disease reduction (up to 90%) in plants and improved health parameters in animal models. However, critical methodological flaws persist across studies: incomplete physicochemical characterization, lack of accurate selenium quantification (ICP-MS), limited safety assessments, and absence of long-term stability studies. Future research should consider adopting standardized protocols to translate SeNPs into reliable antifungal agents.

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