

Review

Agricultural sustainability and the challenges of selenium nanoparticles (SeNPs): Their role in supporting the environmental economy

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ABSTRACT

Agricultural sustainability faces significant challenges due to the overuse of synthetic fertilizers, which lead to environmental degradation and low nutrient use efficiency (NUE). Selenium nanoparticles (SeNPs) have emerged as a promising solution to these challenges due to their unique physicochemical properties, enhanced bioavailability, and potential to improve crop productivity while mitigating environmental contamination. This review explores the synthesis, characterization, and agricultural applications of SeNPs, comparing them with traditional ionic forms of selenium such as selenate and selenite. We discuss various methods for SeNP production, including biological, chemical, and physical techniques, and evaluate their advantages and disadvantages. Furthermore, we delve into the mechanisms of plant uptake, the role of SeNPs in improving plant growth, stress tolerance, and nutrient absorption, and their potential as biofertilizers, biostimulants, and remediation agents. The review highlights the potential of SeNPs to support sustainable crop production and environmental protection, offering a pathway to address global food security challenges.

Abbreviations

Nutrient utilization efficiency NUE
Nanoparticles NPs
Nanofertilizers NFs
Selenium nanoparticles SeNPs
Selenocysteine SeCys
Selenomethionine SeMet
Glutathione peroxidase GSHPx
Selenate SeO_4^{2-}
Selenite SeO_3^{2-}
Elemental selenium Se^0
Selenide Se^{2-}
Redox potential Eh
Selenomethionine selenoxide SeOMet
Methylselenocysteine MeSeCys
Phosphoselenate APSe

ATP sulfurylase APS
Adenosine phosphoselenate reductase APR
Glutathione GSH
Sulfite reductase SiR
O-acetylserine OAS
Selenomethyltransferase SMT
Cystathionine synthase CGS
Cystathionine-lyase CL
SeCys methyl synthase SMT
Dimethylselenide DMeSe
Dimethyldiselenide DMeDSe
Selenium tetrachloride SeCl_4
Selenous acid H_2SeO_3
Surface Plasmon Resonance SPR
Fourier transform infrared spectroscopy FTIR
Nuclear Magnetic Resonance NMR
X-ray Photoelectron Spectroscopy XPS

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Scanning electron microscopy SEM
 Transmission electron microscopy TEM
 Energy-Dispersive X-ray spectroscopy EDX
 Focused Ion Beam Scanning Electron Microscopy FIB-SEM
 scanning transmission electron microscopy STEM
 High-Resolution Transmission Electron Microscopy HRTEM
 Dynamic Light Scattering DLS
 Zero-valent iron nanoparticles ZVI NPs
 Cerium dioxide nanoparticles CeO₂ NPs
 Catalase CAT
 Superoxide dismutase SOD
 Ascorbate peroxidase APX
 Peroxidase POD
 Reactive oxygen species ROS
 Phenylalanine ammonia-lyase PAL
 1,3-glucanase GLU
 Volatile organic compounds VOCs
 Sodium dodecyl sulfate SDS
 Cetyl trimethyl ammonium bromide TAB
 Polyethylene glycol PEG

1. Introduction

Agriculture is under increasing pressure to meet the food demands of a rapidly growing global population, projected to reach 9.6 billion by 2050. This escalating demand is compounded by the impacts of climate change, urbanization, and the unsustainable exploitation of natural resources. In modern agriculture, the reliance on synthetic agrochemicals, including fertilizers and pesticides, has become indispensable. For instance, the global use of synthetic fertilizers reached 188.2 million tons in 2019 (FAO, 2022), and the use of pesticides amounts to approximately 4 million tons annually. However, the intensive use of these chemicals has raised significant concerns regarding environmental sustainability, soil health, and human health. These agrochemicals, while effective in promoting crop growth, have been linked to soil degradation, the depletion of beneficial soil microorganisms, air pollution, eutrophication of water bodies, and contamination of groundwater (Guo et al., 2018; Kazafy, 2015). Furthermore, the low nutrient use efficiency (NUE) of these chemicals exacerbates their environmental footprint, with nitrogen, phosphorus, and potassium NUE values often ranging from 30–35 %, 18–20 %, and 35–40 %, respectively (Buriak et al., 2022). This means that more than half of the applied fertilizers are lost through processes such as leaching, photolysis, hydrolysis, microbial immobilization, and degradation, which not only reduces their effectiveness but also increases costs for farmers and contributes to pollution (El-Saadony et al., 2021b). These challenges underscore the urgent need for innovative strategies to enhance nutrient uptake and reduce the ecological impact of agricultural practices.

One such strategy is the integration of selenium (Se), an essential trace element that plays a critical role in plant growth, stress tolerance, and human nutrition. Despite its importance, the bioavailability of selenium in soil is often low, limiting its effectiveness in enhancing crop yields and nutritional quality. Selenium deficiency in crops can lead to reduced plant health and lower concentrations of selenium in food, which can have adverse health implications for both humans and animals. Traditional methods of selenium fertilization, such as inorganic selenium salts, have shown limited efficacy due to poor absorption and the potential for toxicity at higher concentrations.

In recent years, the advent of nanotechnology has introduced innovative solutions to these longstanding challenges. Nanoparticles (NPs), particularly selenium nanoparticles (SeNPs), have garnered significant attention as a promising tool for improving nutrient uptake and crop productivity. SeNPs offer several advantages over traditional selenium forms, including enhanced bioavailability, targeted delivery, and reduced toxicity. Their small size and high surface area allow for more efficient absorption by plants, leading to improved plant growth,

increased yield, and enhanced resistance to environmental stresses. Furthermore, SeNPs can be tailored to release selenium in a controlled manner, thereby optimizing nutrient uptake and minimizing losses due to leaching or volatilization.

Recent studies have demonstrated the potential of SeNPs to enhance crop growth and nutritional quality. For example, (Wang et al., 2023) reported that foliar application of SeNPs at 20 mg/L increased maize fresh weight by 27 %, aligning with our findings that SeNPs enhance growth and yield. Similarly, in a study by Shang et al. (2019), the application of SeNPs to wheat significantly improved stress tolerance under saline conditions, supporting our results that SeNPs can mitigate the effects of environmental stress. In contrast, traditional selenium fertilizers have not shown such a substantial effect on stress tolerance (Zhang et al., 2019b). Additionally, (Zhang et al., 2019a) observed significant metabolic changes in spinach treated with CeO₂ nanoparticles, demonstrating the broader potential of nanomaterials to influence plant metabolic processes, a phenomenon also observed in our work with SeNPs. These findings reinforce the idea that SeNPs can enhance plant growth, stress tolerance, and nutrient uptake more effectively than conventional selenium fertilizers, providing a promising avenue for sustainable agricultural practices.

This review aims to explore the synthesis, characterization, and agricultural applications of SeNPs, highlighting their potential to revolutionize the use of selenium in agriculture. By comparing the efficacy of SeNPs with traditional selenium forms, we aim to demonstrate how nanotechnology can contribute to sustainable agricultural practices. The integration of SeNPs into farming systems has the potential to address critical challenges related to nutrient use efficiency, environmental sustainability, and food security, ultimately supporting the goals of sustainable agriculture in the face of a growing global population and environmental change.

2. Nanofertilizers as a promising technology

Nanofertilizers (NFs) are substances comprising macro and micro-nutrients at the nanoscale size, strategically administered to crops in a regulated manner. NFs have the potential to outperform even the most advanced polymer-coated traditional fertilizers, which have seen minimal progress in the past decade owing to their significant specific surface area (Shang et al., 2019)

2.1. Why NFs over synthetic fertilizers?

Conventional synthetic fertilizers exhibit low nutrient uptake efficiencies and are prone to high soil fixation rates (Shenoy and Kalagudi, 2005). Conversely, NFs have the potential to mitigate nutrient losses by facilitating direct crop internalization. NFs are coated or encapsulated with nanomaterials that regulate nutrient release according to plant requirements, thereby enhancing NUE (Qureshi et al., 2018). Notably, NFs exhibit extended nutrient release duration, typically spanning 40–50 days, in contrast to synthetic fertilizers, which typically last only 4–10 days. For example, synthetic urea fertilizers can rapidly lose more than 70 % of their nitrogen content shortly after being applied to the soil, primarily due to filtration and volatilization (Kahrl et al., 2010). Comparative studies have revealed that phosphorus-enriched hydroxyapatite NPs significantly enhanced plant height, shoot growth, and grain production, increasing soybean (*Glycine max* L.) yield by 18 % compared to synthetic phosphorus fertilizers (Liu and Lal, 2014). Furthermore, certain plant metabolic processes crucial for nutrient mobilization, such as phosphorus uptake, can be influenced by NPs (Han et al., 2022). Zinc NPs, for instance, facilitate the mobilization of synthesized phosphorus, enhancing absorption efficiency (Anjum et al., 2021). Moreover, NFs can integrate biosensors to regulate nutrient release and bioavailability according to the crop's growth stage, a capability absent in synthetic fertilizers (León-Silva et al., 2018). Lastly, NFs require minimal application due to their high efficiency, resulting in lower application rates

and costs compared to synthetic fertilizers.

2.2. NFs' characteristics

Due to their unique attributes, NFs offer environmental benefits compared to synthetic fertilizers (Pitambar et al., 2019). With particle sizes below 100 nm, NFs can infiltrate plants when applied as foliar or soil supplements, representing a significant feature (Seleiman et al., 2020). Their extensive surface areas confer high reactivity, enhancing nutrient accessibility and elevating plant NUE (Siddiqi and Husen, 2017). Additionally, NFs demonstrate high water solubility owing to their tiny size and strong adsorption to soil particles, contrasting with synthetic fertilizers' low solubility due to their larger particle size and limited water solubility. Encapsulation of fertilizers in NPs facilitates easier absorption and utilization by plants, thereby reducing the requirement for bulk fertilizers (Chhipa, 2017). For instance, the utilization of zeolite-based NFs promotes nutrient availability to plants throughout the growth cycle. Moreover, the controlled and delayed nutrient release from NFs (Basavegowda and Baek, 2021) curtails salt accumulation in soil, nitrogen losses via volatilization, leaching, fixation, and denitrification, while mitigating their toxicity to plants (Cataldo et al., 2021).

3. Selenium

3.1. Selenium as a vital trace element

Selenium stands as a crucial trace element essential for human health, known to enhance immunity, reduce the risk of certain cancers, and prevent various heart and cerebrovascular diseases (Zhang et al., 2019a). Naturally occurring in the Earth's crust, selenium was first isolated by chemist Jacob Berzelius in 1817, although its significance was recognized later in 1957. Schwartz and Foltz's discovery in 1957 that selenium could protect against muscular dystrophy and liver cirrhosis in rats marked a significant milestone in selenium research (Rayman, 2000). Another pivotal moment came with the identification of selenium in the enzyme glutathione peroxidase (GSHPx) (Weaver and Skouta, 2022), further underscoring selenium's importance in biological processes. Since then, the vital role of selenium in both animal and human nutrition has been recognized, highlighting its essential status in the human diet (Hartikainen, 2005). Selenium serves various purposes, with its anti-inflammatory and antioxidant effects being particularly noteworthy for human health (Bjørklund et al., 2022). The biological effects of dietary selenium primarily stem from compounds like selenocysteine (SeCys), selenomethionine (SeMet), and selenocysteine-containing amino acids (Hariharan and Dharmaraj, 2020). Numerous selenoproteins, including GSHPx, thioredoxin reductase, and iodothyronine-deiodinases, rely on selenium as a catalytic center, thereby playing crucial roles in scavenging free radicals, preventing oxidative stress, and enhancing immune function (Huang et al., 2012). Severe selenium deficiency in humans can lead to conditions such as Keshan disease or cancer (Zhao et al., 2013). Globally, around 500 million to 1 billion people suffer from selenium insufficiency, with 20 % of them residing in China (Ellis and Salt, 2003). Chinese adults consume only about 26.6 µg of selenium per day, as reported by the Chinese Nutrition Society's nutrition study, which falls short of the WHO recommendation of 50–55 µg/day for adults (WHO, 2009). The selenium nutritional status of China's population largely depends on the selenium content in rice (Chen et al., 2010). Studies indicate that cereals grown in naturally selenium-rich soils (with soil selenium content ranging from 0.5 to 1.0 mg/kg) can meet people's daily selenium requirements (60–80 µg/day) (Xie et al., 2021). Selenium is also essential for animals and poultry (Korzeniowska et al., 2019), with a diet containing 0.25 mg/kg of selenium being sufficient to enrich eggs (Attia et al., 2010). The health benefits for consumers could be enhanced by producing selenium-enriched animal products using selenium-fortified

feeds (Ullah et al., 2019). Individuals in selenium-deficient regions are believed to benefit from increasing their selenium intake through selenium-enriched foods (Ali et al., 2017). For instance, in Finland, a country historically deficient in selenium, the selenium levels in various foods and individuals significantly increased following the application of selenium fertilizer to the soil (Broadley et al., 2006).

3.2. Selenium in food supply chain: from soil to humans

Selenium is naturally present in sedimentary rocks from the Carboniferous to the Quaternary epoch (White, 2004). Globally, soils typically contain an average selenium concentration of around 0.4 mg/kg, although soils classified as seleniferous demonstrate higher selenium levels, ranging from > 2 to 5000 mg/kg (El-Ramady et al., 2014). Soil selenium content is influenced by several factors, including soil type, organic matter content, and precipitation levels (Sors et al., 2005). Regions characterized by mountainous terrain such as Finland, Sweden, and Scotland typically exhibit low soil selenium levels, whereas shale soils and arid regions tend to have higher selenium content. Many countries, including Brazil, France, the UK, Belgium, and others, are recognized to have areas deficient in selenium (Yin et al., 2012). Conversely, regions like the State of Para in the Brazilian Amazon, the Enshi district in Hubei province of China, and the North-East region of Punjab in India, among others, are reported to possess selenium-rich soils (Ma et al., 2024). Countries like Japan, Greenland, the USA, Venezuela, and Canada also contain selenium-rich regions (Yin et al., 2012). Peru, China, Chile, the United States, Canada, Zambia, the Philippines, Zaire, Australia, and New Guinea collectively hold nearly 80 % of the world's selenium deposits (Li et al., 2011).

3.3. Pathways of selenium uptake

Plant roots have the capacity to absorb various forms of selenium, including selenate (SeO_4^{2-}), selenite (SeO_3^{2-}), elemental selenium (Se^0), and organic selenium compounds like SeCys and SeMet, but they do not possess the capability to take up selenide (Se^{2-}) (White, 2018; Wu et al., 2015). The distribution of various selenium forms in soils is influenced by pH and redox potential (Eh) conditions. In well-aerated soils with pH and redox potential values exceeding 15, selenate typically prevails. Conversely, anaerobic soils with a neutral to acidic pH and pH and redox potential values ranging from 7.5 to 15 tend to harbor higher concentrations of selenite, hydrogen selenite (HSeO_3), and selenous acid (H_2SeO_3). The rhizosphere, the area immediately surrounding plant roots, acts as the main nutrient reservoir for plants. The unique characteristics of the rhizosphere significantly impact the accessibility of selenium to plants (Chen et al., 2010; Zhou et al., 2007). Through various mechanisms, plant roots selectively take up distinct selenium forms, leading to the production of diverse seleno-compounds due to mobility and metabolic activities within the plants.

3.3.1. Selenate uptake

Sulfur and selenium, both categorized as group-16 elements referred to as "chalcogens" on the periodic table, exhibit a spectrum of chemical properties, including similarities in their ionic and covalent radii. As documented by (Gupta and Gupta, 2017), it is well-established that plant roots can absorb selenate through sulfate transporters. According to (Schiavon et al., 2015), in *Arabidopsis thaliana*, selenate can cross the plasma membrane thanks to the existence of two high-affinity sulfate transporters, namely SULTR1;1 and SULTR1;2. Various plant species harbor diverse transporters for selenate uptake. While bioinformatics studies propose that selenium hyperaccumulators may possess distinct selenate transporters, the identification of a specialized selenate transporter in any organism remains inconclusive (Harris et al., 2014).

3.3.2. Selenite uptake

Selenite, characterized as a weak acid, can manifest in various forms,

sizes, and structures contingent upon specific pH and Eh condition (White, 2018; Zhou et al., 2018). Distinct pathways for selenite uptake appear to activate under varying pH circumstances. For instance, (Zhao et al., 2010) observed that in an acidic milieu, the rice silicon transporter OsNIP2;1 can uptake H_2SeO_3 . In partially acidic paddy soils, selenite primarily presents as HSeO_3^- , exhibiting structural resemblances to H_2PO_4^- . Consequently, investigations have demonstrated that the transport of H_2SeO_3 is facilitated by the phosphate transporter OsPT2 (Zhang et al., 2014). While it remains uncertain whether this relationship between the two chemicals is a common occurrence in plants, (Song et al., 2017) demonstrated in genetically manipulated tobacco lines that overexpression of OsPT8 enhances the uptake of phosphate and leads to increased selenium accumulation in shoots. It has become evident that multiple parallel pathways for selenite absorption exist in plants, with only a limited number connected to phosphate absorption. Furthermore, research into genes associated with selenium uptake in plants is still in its infancy. This highlights the need for more focused investigations into the molecular mechanisms governing selenium absorption, transport, and metabolism.

3.3.3. Organic selenium species uptake

Compared to inorganic selenium forms, there is notably less research dedicated to the investigation of plant uptake and transfer of organic selenium species. It was noted that the absorption rates of both SeCys and SeMet were approximately 20 times higher compared to the rates observed for selenate or selenite (Kikkert and Berkelaar, 2013). It is also suggested that amino acid transporters are likely to facilitate the entry of selenoamino acids into plant cells (Lima et al., 2018; Schiavon et al., 2020). Hence, it is logical to deduce that these amino acid transporters may also be involved in the uptake of SeCys and SeMet. Considering the array of amino acid transporter classes and the scarce research in this area, it is reasonable to speculate that additional amino acid transporters might also play a part in the assimilation of organic selenium forms.

3.3.4. Elemental selenium uptake

Multiple experiments have illustrated that red elemental selenium exhibits similar characteristics to a slow-release fertilizer, which, from an agronomic perspective, underscores the importance of ensuring a consistent supply of selenium to facilitate biomass production with nearly constant selenium levels. Recent studies have shown promising outcomes regarding the use of red elemental selenium in enhancing the selenium biofortification of crops and forages. Application of red elemental selenium, particularly at a concentration of 10 mg/L, demonstrated effects akin to those of slow-release fertilizers. This was evidenced by the consistent accumulation of total selenium in the stem and leaf of alfalfa across successive harvests, unlike selenium in its ionic forms, selenate and selenite (Kovács et al., 2021). The uptake of nanoparticles such as red elemental selenium is influenced by various factors including their physical and chemical properties, plant species, growth medium, and interactions within the plant-soil-microbe system (Kaszás et al., 2022). Certain nanoparticles have the ability to form complexes with organic compounds released by plant roots and soil microorganisms. Two pathways have been proposed for nanoparticle uptake, with the apoplastic route suggested for nanoparticles below 20 nm in size, as they can readily penetrate root cell membranes (Yu et al., 2024).

3.4. Selenium translocation from root to shoot

The xylem serves as the conduit for transporting selenium absorbed by plants, primarily in inorganic forms, from the roots to the shoot. The mode of external selenium supply influences this transport process. Selenate is readily taken up by plants and transported via the xylem. Subsequently, it is further distributed to reproductive organs through the phloem (Carey et al., 2012). In the xylem, selenate is the dominant selenium species; although trace quantities of SeMet and selenomethionine selenoxide (SeOMet) are also present (White, 2018). It is

believed that certain members of the ALMT transporter family, including AtALMT12, aid in the transfer of selenate into the xylem sap. The SULTR transporter facilitates the entry of selenate into mesophyll cells in most plants, where it accumulates within the vacuole. Although the transporter dedicated for conveying selenate across the tonoplast and into the vacuole remains unidentified, it has been proposed that homologs of AtSULTR4;1 and AtSULTR4;2 may function as efflux transporters in charge of the movement of selenate out of the vacuole (White, 2015). Within plant leaves, selenate undergoes conversion to selenite, which is subsequently transported to other tissues by becoming incorporated into organic selenium molecules. Selenate, SeMet, and methylselenocysteine (MeSeCys), all imparted through the xylem, are readily redistributed to sink organs via the phloem (Carey et al., 2012). In Arabidopsis, it appears that AtSULTR1;3 and AtSULTR2;2 play a role in facilitating the entry of selenate into the phloem, with the expression of genes encoding AtSULTR1;3 and AtSULTR2;2 being upregulated in response to selenium accumulation (Wang et al., 2018). Following uptake by the roots, selenite can swiftly transform into organic forms like SeMet and MeSeCys. Nonetheless, a potential challenge in enhancing the selenium content of crop plants lies in the tendency of these selenium-containing amino acids to predominantly accumulate in the root system, with minimal transport to the aerial parts of the plant. This distribution pattern largely arises from the fact that the majority of the edible portions of crop plants are situated above ground (Winkel et al., 2015). The transportation of ions or molecules to the aerial tissues of plants is influenced by both the transpiration rate and the rate of xylem loading (Renkema et al., 2012). (Kikkert and Berkelaar, 2013) demonstrated that in wheat and canola, various forms of selenium, including selenate, SeMet, and selenite/SeCys, are transported at significantly different rates. This inherent diversity in selenium uptake is governed by transporters located in the plasma membrane of root cells. Notably, OsNIP 2;1 in rice may serve as a potent positive regulator of selenium transport under conditions of low selenium concentrations in the supply medium (Liang et al., 2019).

3.5. Metabolism of selenium in plants

In plants, both selenium and sulfur follow the same metabolic pathway. Selenium is transported through the xylem from the roots to the chloroplasts in the leaves. The crucial step in selenium metabolism into organic components occurs when selenate is reduced to adenosine phosphoselenate (APSe), a process regulated by ATP sulfurylase (APS). Subsequently, selenate is further reduced by adenosine phosphoselenate reductase (APR), utilizing glutathione (GSH) as the electron donor. The enzyme sulfite reductase (SiR) then converts selenate into selenide. The subsequent reaction between selenide and O-acetylserine (OAS) can lead to the formation of SeCys (White, 2018). Alternatively, the enzyme selenomethyltransferase (SMT) can directly convert selenite into SeCys (Chauhan et al., 2019). SeCys can undergo further transformations into various chemical species through the actions of enzymes like cystathionine synthase (CGS), cystathionine-lyase (CL), and SeCys methyl synthase (SMT). These enzymes utilize the by-products of SeMet and MeSeCys, as well as MeSeMet, to facilitate these conversions. Additionally, further metabolic processes of SeMet and MeSeCys can lead to the production of selenoproteins and volatile selenium species like dimethylselenide (DMeSe) or dimethyldiselenide (DMeDSe) (Gupta and Gupta, 2017; White, 2018). Over the past few years, a multitude of genes linked to selenium absorption, transport, and metabolism in plants have been uncovered. To improve the accumulation, tissue-specific distribution, and chemical speciation of selenium in plants, it will be necessary to manipulate and harness the inherent variations in the expression of these genes. To maximize the biofortification potential of crops, which can ultimately improve animal and human nutrition; it is crucial to gain a comprehensive understanding of how these key genes interact.

4. Selenium nanoparticles

4.1. Synthesis of selenium nanoparticles

Currently, a significant area of focus in biotechnology revolves around the production of NPs, achieved by controlling their size and morphology through physical, chemical, or biological means (Table 1). Consequently, there has been a notable emphasis on NPs synthesis in recent years. This synthesis entails the reduction of metal ions to neutral metal atoms, typically accomplished by employing reducing agents derived from biological, physical, or chemical sources. As a result, nanomaterials undergo alterations in size, shape, structure, and atomic arrangement, rendering them highly bioavailable and less toxic compared to conventional forms (Prasad et al., 2017; Ram et al., 2014).

4.1.1. Physical synthesis

According to several studies, there are many physical ways to

produce SeNPs, including vapor deposition, hydrothermal, and solvothermal methods, pulsed laser ablation, and ultrasound-based synthesis (Khan et al., 2017). Using a fiber ytterbium laser with a wavelength between 1060 and 1070 nm and a repetition rate of 20 kHz (Kilohertz), selenium was laser-ablated in water to create SeNPs. As a result of extended laser fragmentation durations, the fundamental particle mass component decreased from an initial size of 800 nm to below 100 nm. The generated SeNPs were mass- and size-mono-dispersed. Numerous alternative methods have been utilized, such as physical evaporation techniques, hydrothermal processes, gamma-radiolytic reduction, and sonochemical procedures (Yu et al., 2024; Zhang et al., 2014).

4.1.2. Chemical synthesis

The chemical synthesis of SeNPs involves reducing a selenium salt using a reducing agent, typically in the presence of a stabilizing agent (Fig. 1). This prevents the aggregation of selenium atoms and allows for

Table 1
Pros and cons of different selenium nanoparticle (SeNPs) synthesis methods.

Method	Advantages	Disadvantages	Reference	
Chemical methods	Ascorbic acid	This method is relatively simple Cost-effective High stability of produced SeNPs Controlled small particle size Uniform size and shape of SeNPs. A convenient source of raw materials High activity High-temperature resistance	It may involve the use of toxic chemicals It may generate hazardous waste	(Zhang et al., 2023)
	Sol-Gel method	Provides control over particle size, shape, and surface chemistry Suitable for coating applications	Requires precise control of reaction conditions The process can be time-consuming	(Bokov et al., 2021)
	Electrochemical method	A relatively simple method High selectivity Allows for the synthesis of SeNPs with controlled size and shape	Electrochemical cells can be complex Electrode stability may be a concern	(Zhang et al., 2005)
Physical methods	Hydrothermal method (Microwave-assisted synthesis)	Low process temperature Low energy-efficient consumption Environmental friendliness Rapid synthesis Can produce well-dispersed SeNPs	Limited scalability for industrial applications Potential overheating issues	(Liu and Lal, 2014)
	Physical vapor deposition (PVD)	Allows precise control over size and shape Can produce high-purity SeNPs	Expensive equipment is required The process may not be suitable for large-scale production	(Rafique et al., 2020)
	Liquid phase pulsed laser ablation	Low equipment cost Easy to collect SeNPs The stable storage SeNPs in colloidal solution	Complex setup Costly equipment Safety concerns High energy consumption Limited scalability Limited material compatibility Contaminants, this can affect the purity and quality of the nanoparticles Limited control over surface properties The high-energy laser pulses used in LP-PLA can potentially induce damage or defects in the synthesized nanoparticles, affecting their properties and performance	(Van Overschelde et al., 2013)
	Photocatalysis	High preparation efficiency and reusable	Low stability of the produced SeNPs Ease transformation of the produced SeNPs into gray-black elemental selenium, which consequently enhances toxicity and activity loss. High energy consumption Creation of irritating compounds	(Triantis et al., 2009)
Biological method	Green synthesis (Plant extracts or biomolecules)	Environmentally friendly Often produces biocompatible SeNPs Is cost-effective	The process may not yield highly uniform nanoparticles Reaction parameters can be variable	(Cittrarasu et al., 2021) (Pyrzynska and Sentkowska, 2022)
	Biological synthesis (Biogenic) method or microbial reduction method	Simple process It is environmentally friendly Used reductants are easily accessible and biodegradable	Can be time-consuming Synthesis conditions may not be easily controlled	(Srivastava and Mukhopadhyay, 2015)

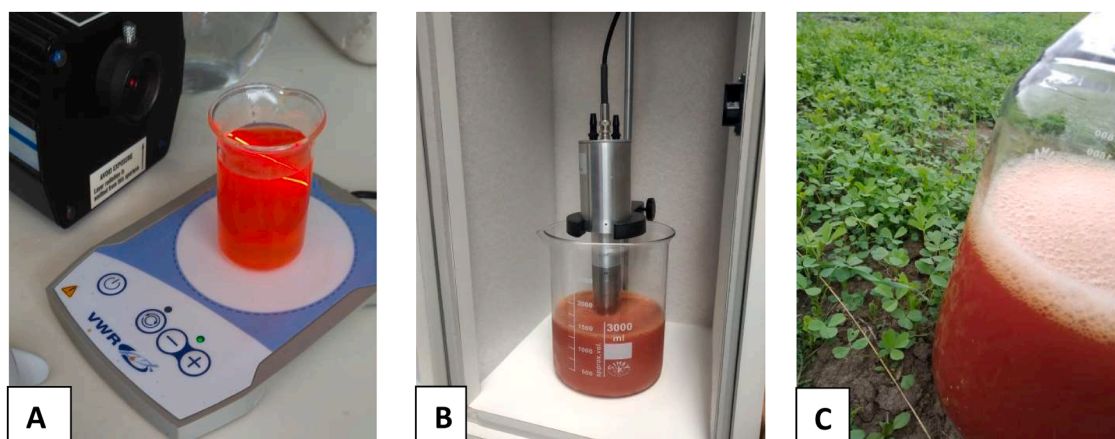


Fig. 1. A) chemical synthesis of selenium nanoparticles (SeNPs) using the ascorbic acid method, laser beam was applied to recognize the formation of nanoparticles; B) dispersion of the chemically-synthesized SeNPs using ultrasonic homogenizer; C) application of the chemically-synthesized SeNPs to alfalfa plantation in open field.

the production of stabilized NPs in a colloidal suspension. SeNPs are chemically synthesized from precursor compounds, which are inorganic forms of selenium. Various reducers have been employed in the production of SeNPs to prevent aggregation. These include glucose, ascorbic acid, fructose, cysteine, glutathione, sodium metabisulfite, and the ionic liquid 1-ethyl-3-methylimidazolium thiocyanate. Additionally, chemicals like SDS (sodium dodecyl sulfate), CTAB (cetyl trimethyl ammonium bromide), and polyethylene glycol (PEG 600) have been utilized for SeNP synthesis (Panahi-Kalamuei et al., 2014). Selenium tetrachloride (SeCl_4) served as the initial substance, which, upon dissolution in water, yields H_2SeO_3 . Subsequently, this selenous acid undergoes reduction using hydrazine hydrate (Panahi-Kalamuei et al., 2014).

4.1.3. Biological synthesis

Recently, the intersection of nanotechnology and biology has given rise to the emerging field of nanobiotechnology. This discipline harnesses various biological agents such as algae, bacteria, fungi, viruses, yeasts, and plants in diverse biochemical and biophysical processes. Utilizing biological synthesis through nanobiotechnologies holds immense promise for enhancing NPs production without relying on harsh, toxic, or costly chemicals. Moreover, the biosynthesis of SeNPs using different plant extracts or bacterial strains has revealed that each strain (or plant source) yields selenium spheres within distinct size ranges. Natural resources have emerged as a particularly attractive avenue for green synthesis, as this method avoids the use of hazardous chemicals while offering numerous advantages, including environmental sustainability, cost-effectiveness, and suitability for pharmaceutical and biomedical applications.

The biosynthesis of SeNPs often involves the reduction of selenate or selenite in the presence of bacterial or plant extracts, owing to their contents of phenols, flavonoids linked to amines, alcohols, proteins, and aldehydes (Husen and Siddiqi, 2014). The green chemistry concept prioritizes utilizing natural organisms such as microorganisms, microalgae, enzymes, plants, and plant extracts. This method provides a dependable, straightforward, non-toxic, cost-effective, stable, and environmentally friendly approach (Menon et al., 2017). The biogenic synthesis also allows for precise control of created NPs regarding their diameter and shape, where bio-based extracts serve as both stabilizing and reducing agents (Wadhvani et al., 2016). More than 16 distinct species of bacteria and viruses have been discovered to convert selenite into red elemental selenium, exhibiting varied shapes and sizes (Eszenyi et al., 2011; Lortie et al., 1992).

Synthesis of NPs entails the bioreduction of metallic ions into elemental forms, a process catalyzed by functional groups such as amines and alkanes, which are abundant in metabolites like flavonoids,

tannins, alkaloids, steroids, and terpenoids (Menon et al., 2019, 2017; Wadhvani et al., 2016). Whether NPs are synthesized extracellularly or intracellularly using plant systems, research has demonstrated that green synthesized NPs exhibit a significantly more potent inhibitory effect compared to chemically synthesized counterparts (El-Saadony et al., 2021a; Huang et al., 2016; Murali et al., 2017). Nevertheless, SeNPs exhibit instability and tend to aggregate in practical applications, underscoring the need to devise straightforward and effective techniques for achieving adequate dispersion and stabilization (Yu et al., 2024). Bioreduction methods employing certain plant extracts may present a viable solution for the rapid preparation of SeNPs (El-Saadony et al., 2021a).

4.2. Biological sources for SeNPs synthesis

4.2.1. Plants

One of the tested processes for producing NPs that is rapid, affordable, and ecologically friendly is the utilization of plant materials and their extracts (Table 2). The ions of metals and metalloids are considerably converted into their elemental NPs by biomolecules and secondary metabolites from various plant sections (Singh and Jha, 2016). Crushed plant parts, including nuts, leaves, fruits, and stems, are used as extracts mixed with varying concentrations of selenium precursor solutions. Physical conditions are adjusted to promptly generate SeNPs with the desired sizes. Compounds present in plant extracts act as capping and stabilizing agents, thwarting the aggregation of synthetic materials (Korde et al., 2020). (Sharma et al., 2014) produced spherical SeNPs with an average diameter of 3–18 nm by the extract of the *Vitis vinifera* and discovered that the fruit's lignin ingredient served as the cap on each particle. In a study, sodium selenite and *Aloe vera* leaf extract were utilized as precursors to produce spherical SeNPs, which exhibited antibacterial activity against *Escherichia coli* and *S. aureus*, as well as antifungal properties against food-spoiling fungi species (Fardsadegh and Jafarizadeh-Malmiri, 2019). The stability of the nanoparticles and the reduction of selenite were attributed to the presence of amides and hydroxyl groups in the *Aloe vera* extract (Fardsadegh and Jafarizadeh-Malmiri, 2019). Furthermore, compared to sodium selenite salt, SeNPs synthesized using *Emblica officinalis* (amla) extract demonstrated superior antimicrobial and antioxidant properties with minimal toxicity. The fruit's phenols, flavonoids, and tannins are what lead to the creation of NPs (Gunti et al., 2019). *Withania somnifera* includes active ingredients such as alkaloids, flavonoids, phenolics, tannins, and terpenoids. By decreasing selenous acid, these ingredients operate as a capping and reduction agent for the production of SeNPs (Alagesan and Venugopal, 2019). In one of the trials, leftover orange peels were used to

Table 2

Some plant extracts used for the biological synthesis of selenium nanoparticles (SeNPs) and their significant use.

Material used	Description	Significance	References
Clove (<i>Syzygium aromaticum</i>) and cumin (<i>Cuminum cyminum</i>) extracts	Ground clove and cumin tissues were suspended in distilled water then boiled at 70 °C for 10 min. The supernatants collected by filtration were used for the biosynthesis of SeNPs.	SeNPs displayed anti-inflammatory effect	(Jayavarsha et al., 2022)
Garlic (<i>Allium sativum</i>) extract	A mixture of garlic extract and 20 mM sodium selenite was subjected to heating at 70 °C for 15 min using a magnetic stirrer set to 150 rpm, maintaining an alkaline pH of 8.0. Subsequently, the solution was allowed to cool to room temperature and agitated for approximately 10 min until the color changed to brick red, indicating the successful synthesis of colloidal selenium nanoparticles.	Fast detection of salicylic acid in milk (milk adulteration)	(Aftab et al., 2023)
Clove (<i>Syzygium aromaticum</i>) extract	The cloves powder was immersed in distilled water for an hour at room temperature, followed by boiling at 90 °C for an additional hour, and then filtered to obtain the extract. The resulting supernatants were combined with 0.1 M sodium selenite at a ratio of 4:1.	SeNPs exhibited an inhibitory effect on the growth of A375 skin cancer cells	(Hemalatha et al., 2023)
<i>Elaeagnus indica</i> extract	<i>Elaeagnus indica</i> leaf powder was placed in a Soxhlet apparatus and extracted with distilled water at a ratio of 1:2. The resulting extract was then filtered and subsequently combined with selenous acid at a ratio of 4:1.	SeNPs demonstrated antimicrobial activity against various microorganisms, such as <i>Salmonella typhimurium</i> and <i>Fusarium oxysporum</i> . Additionally, SeNPs exhibited significant efficacy in degrading methylene blue dye.	(Indhira et al., 2023)
Lemon (<i>Citrus × limon</i> L.) plant extract	Fresh lemon leaves (5 g) were ground in 20 mL of Tris-Cl (pH 7.5) and subsequently centrifuged at 10,000 rpm for 5 min at 4 °C. The resulting supernatant was then combined with 10 mM sodium selenite at a ratio of	SeNPs reduced lymphocyte cell death and shielded against DNA damage when exposed to UVB radiation.	(Prasad et al., 2013)

Table 2 (continued)

Material used	Description	Significance	References
Almond (<i>Prunus amygdalus</i> Syn. <i>Prunus dulcis</i>) skin extract	1:2 under magnetic stirring. The mixture was shaken at 200 rpm for 24 h at 30 °C in absence of light. After being soaked in sterilized distilled water (SDW) overnight, almond nuts' skins were manually peeled, washed with SDW, air-dried, and ground. The almond skin powder was then suspended in SDW at a ratio of 1:25, boiled at 60 °C for 1 hour with continuous stirring in a water bath. Following this, supernatants were collected post-filtration using Whatman filter paper (#1) and centrifugation at 10,000 rpm for 20 min. These supernatants were subsequently incubated with selenium (IV) oxide, serving as a precursor of selenium, in the presence of ascorbic acid for 60 min at 60 °C.	SeNPs demonstrated a bactericidal effect against <i>B. subtilis</i> . Additionally, cotton fabric effectively coated with SeNPs exhibited significant antibacterial properties.	(Sadalage et al., 2020)
Holy basil (<i>Ocimum tenuiflorum</i>) leaf extract	A blend of <i>O. tenuiflorum</i> leaf extract at a concentration of 1 % and 10 mM sodium selenite was prepared at a ratio of 9:1, respectively, and stirred continuously at 130 rpm for 75 h at room temperature.	SeNPs demonstrated the ability to hinder the aggregation and expansion of calcium oxalate monohydrate crystals, suggesting promising potential in medical and pharmaceutical realms as a prospective inhibitor for calcium oxalate urinary stone formation.	(Liang et al., 2019)
<i>Moringa oleifera</i> leaf extract	Reduction of selenite ion into SeNPs using biomolecules such as quercetin and ascorbic acid in extract of <i>M. oleifera</i> leaf	SeNPs exhibit the capability to break down sunset yellow dye. Furthermore, SeNPs have demonstrated efficacy against three human cancer types: Caco-2 cells, HepG2 cells, and MCF-7 cells.	(Hassanien et al., 2019)
<i>Asteriscus graveolens</i> leaf extract	Following cleaning, <i>A. graveolens</i> leaves were finely powdered and then extracted with heated distilled water at a ratio of 1:10, respectively, while continuously stirred for 1 hour. The resulting suspension was	SeNPs exhibited a pronounced selective impact on HepG2 apoptosis by notably and swiftly elevating reactive oxygen species and lipid peroxidation levels. Concurrently, they reduced mitochondrial membrane potential	(Zeebaree et al., 2020)

(continued on next page)

Table 2 (continued)

Material used	Description	Significance	References
	filtered through Whatman filter paper (#1) and subsequently centrifuged for 30 min at 5×105 rpm. The supernatants containing <i>A. graveolens</i> leaf extract were combined with 0.01 M selenous acid at a ratio of 1:3 and left to incubate for a day at room temperature in darkness.	and glutathione levels, collectively influencing the fate of HepG2 cells.	

create SeNPs with a particle size ranging from 16 to 95 nm (Salem, 2022). SeNPs have been synthesized using various plants or their extracts, such as ginger extract (Menon et al., 2019), fenugreek seed extract (Ramamurthy et al., 2013), *Clausena dentata* leaf aqueous extract (Sowndarya et al., 2017), *Vitis vinifera* (Raisin) extract (Sharma et al., 2014), garlic cloves (*Allium sativum*) (Anu et al., 2017), *Capsicum annuum* extract (Li et al., 2011), *Terminalia arjuna* extracts (Prasad et al., 2015) lemon leaf extract (Prasad et al., 2013), extract of hawthorn fruit (Cui et al., 2018), *Aloe vera* leaf extract (Fardsadegh and Jafarizadeh-Malmiri, 2019), leaves extract of *Withania somnifera* (Alagesan and Venugopal, 2019), leaf and stem extract of *Leucas lavandulifolia* (Gunti et al., 2019), leaf extract of *Diospyros montana* (Kokila et al., 2017), and garlic extract (Aftab et al., 2023). Tarragon is readily available and easily collected, and its significant biological activities have led to its utilization as a medicinal agent in many countries. Additionally, its high levels of phenolic compounds, protein, and fiber content make it valuable for various applications (Aglarova et al., 2008; Gawlik-Dziki, 2012). Given these qualities, tarragon extract can also serve as a reducing agent for synthesizing SeNPs. Recent advancements in nanoparticle synthesis, as highlighted by (Kiani et al., 2024), have demonstrated the potential of green synthesis methods using plant extracts to produce SeNPs with enhanced stability and biocompatibility. These methods reduce the environmental impact and improve the efficiency of selenium uptake in plants

4.2.2. Bacteria

According to studies, environmental microorganisms can diminish selenium oxyanions (selenate/ selenite) by absorption and dissimilation processes. By preserving their metabolic resources, the dissimilatory reduction aids bacteria in anaerobic respiration. These oxyanions may also be converted to elemental selenium in aerobic circumstances by the organism's detoxification processes or redox physiological conditions. Assimilatory reduction uses a number of enzymatic processes to convert selenite/ selenate into elemental selenium and has the potential to take this elemental selenium up to create organic selenium compounds (Eswayah et al., 2016). *Thaurea selenatis*, isolated from selenium-contaminated water, has been observed to transform selenate into SeNPs. According to the study, this bacteria use selenate as its final electron acceptor during respiration under anaerobic conditions. Selenate reductase enzyme (Ser ABC) converts selenate to selenite in the periplasmic region of the cell. Upon entering the cytoplasm, selenite is converted into elemental SeNPs, likely facilitated by nitrate reductase and organized by the Sef A protein. These SeNPs are subsequently transported out of the cell. However, *Rhodospirillum rubrum* converted selenite to SeNPs by reducing thiols like glutathione (Butler et al., 2012). Rather than employing selenite as the final electron acceptor during respiration, *Rhizobium* sp., isolated from a fermenter vessel, produced SeNPs from selenite. According to this study, selenite served as the

substrate for a molybdenum-containing enzyme that carried out the reduction process (Hunter and Kuykendall, 2007). Upon the inclusion of 5 mM sodium selenite, the endophytic selenobacteria *Acinetobacter* sp. E6.2 and *Bacillus* sp. E5 synthesized SeNPs measuring approximately 213 nm and 169 nm, respectively (Durán et al., 2015). Spherical and homogenous SeNPs were produced by the fermentation process using bacteria from the genus *Lactobacillus*, *Bifidobacterium*, and *Klebsiella* (Wang et al., 2023). *Alcaligenes faecalis* isolated from the stomach of *Monochamus alternatus* was first shown to be capable of converting sodium selenite at doses of 1 mM and 5 mM into red, amorphous elemental selenium that was mostly extracellular using thioredoxin reductase and NADPH as an electron donor (Wang et al., 2018). Moreover, 20 cyanobacterial strains were examined for their ability to produce SeNPs. Among them, *Arthrospira indica* SOSA-4 demonstrated the highest performance, producing 11.8 nm orange-red SeNPs in just two days (Afzal et al., 2019).

4.2.3. Fungi and yeast

Since several fungi are more resistant to various metals, they should be given priority when synthesizing extracellular metal or metalloidal nanoparticles (Dusengemungu et al., 2021). The presence of fungal protein secondary metabolites contributes to the enhanced stability of the nanoparticles in the liquid medium over an extended duration (Shoebi and Mashreghi, 2017). The mycosynthesis of SeNPs was initially documented using the *Alternaria alternata* fungus, resulting in the production of SeNPs ranging in size from 30 to 150 nm when sodium selenite was introduced (Sarkar et al., 2011). *Aspergillus terreus* was the fungus identified as the progenitor of extracellular SeNPs with a particle size of 47 nm utilizing SeO_2 within an hour (Torres et al., 2012). The culture filtrate of *Trichoderma* sp., the most widespread plant symbiotic fungus, was applied for mycosynthesis of SeNPs, which successfully suppressed the Downy Mildew conditions in pearl millet fields (Nandini et al., 2017). Using *Monascus purpureus* ATCC16436, the solid-state fermentation technique produced SeNPs with spherical particles of around 46.58 nm (El-Sayed et al., 2020). The yeast has showcased remarkable proficiency in reducing selenium oxyanions into SeNPs. For instance, the yeast *Magnusiomyces ingens* LH-F1 exhibited the capability to utilize SeO_2 , yielding SeNPs ranging in size from 70 to 90 nm. Two protein bands that were found on the surface of the nanoparticles during the SDS-PAGE procedure might be the cause of the particle stability (Liang et al., 2019). The use of baker's yeast (*Saccharomyces cerevisiae*) extract was found to be a quick and simple way for making SeNPs (Salem, 2022).

The selection of plants (e.g., *Aloe vera*, *Emblica officinalis*, *Withania somnifera*) was based on their high content of bioactive compounds (phenols, flavonoids, terpenoids) that act as reducing and stabilizing agents for SeNPs (Fardsadegh and Jafarizadeh-Malmiri, 2019).

Characterizing SeNPs presents several challenges due to their unique physicochemical properties, such as their small size, surface charge, and high surface area, which complicate accurate analysis. One key limitation is the difficulty in determining the exact size distribution and morphology of SeNPs. Traditional techniques like dynamic light scattering (DLS) may not provide precise measurements at the nanoscale due to particle aggregation, which can skew results. Additionally, electron microscopy methods like transmission electron microscopy (TEM) are often used for high-resolution imaging but require sample preparation that can alter the native state of SeNPs, leading to potential inaccuracies (Cho et al., 2013). Surface charge and surface chemistry are critical for understanding the interaction of SeNPs with biological systems, but techniques like zeta potential measurements can be influenced by the dispersion medium, making it challenging to obtain consistent readings across different environments (Jain et al., 2014). Furthermore, quantifying the release of selenium ions from SeNPs, a key factor in their toxicity, requires highly sensitive analytical techniques, such as inductively coupled plasma mass spectrometry (ICP-MS), but this method can struggle with distinguishing between SeNPs and other forms of selenium

(Mlangeni et al., 2025). Thus, while there are advanced characterization techniques, their limitations in terms of sensitivity, sample preparation, and particle behavior under different conditions remain significant hurdles.

4.3. Techniques applied for characterization of senps

Regardless of whether nanomaterials are synthesized through physical, chemical, or biological methods, their identification and characterization are of greater importance. Combining traditional methodologies with cutting-edge technology allows us to gain comprehensive understanding of various aspects such as shape, elemental compositions, stability, charges, surface chemistry, and biomolecule attachments, particularly in the case of biogenic nanoparticles (Fig. 2). SeNPs, akin to gold, silver, copper, and other nanoparticles, exhibit a

characteristic red hue due to the Surface Plasmon Resonance (SPR) phenomenon triggered by the reduction reaction. Analysis of the spectrum obtained through UV-Vis spectrophotometric technique enables quantification of the intensity of this specific color. Additionally, an approximate assessment of the size of the nanoparticles may be made using the wavelength displaying the highest absorption. Smaller particles often absorb light of shorter wavelengths (Menon et al., 2020).

SeNPs can be scanned over a wide wavelength range, typically from 200 to 800 nm, revealing a unique peak within this spectrum. Various studies have reported different wavelengths for the maximum absorption peak, such as SeNPs synthesized from *Emblica officinalis* fruit extract exhibiting a peak at 270 nm (Gunti et al., 2019), extract of *Leucas lavandulifolia* at 293 nm (Pyrzynska and Sentkowska, 2022), *B. pumilus* sp. BAB-3706 cell-free extract at 300 nm (Prasad et al., 2015), and *Fusarium oxysporum* at 217 nm (Asghari-Paskiabi et al., 2018). However,

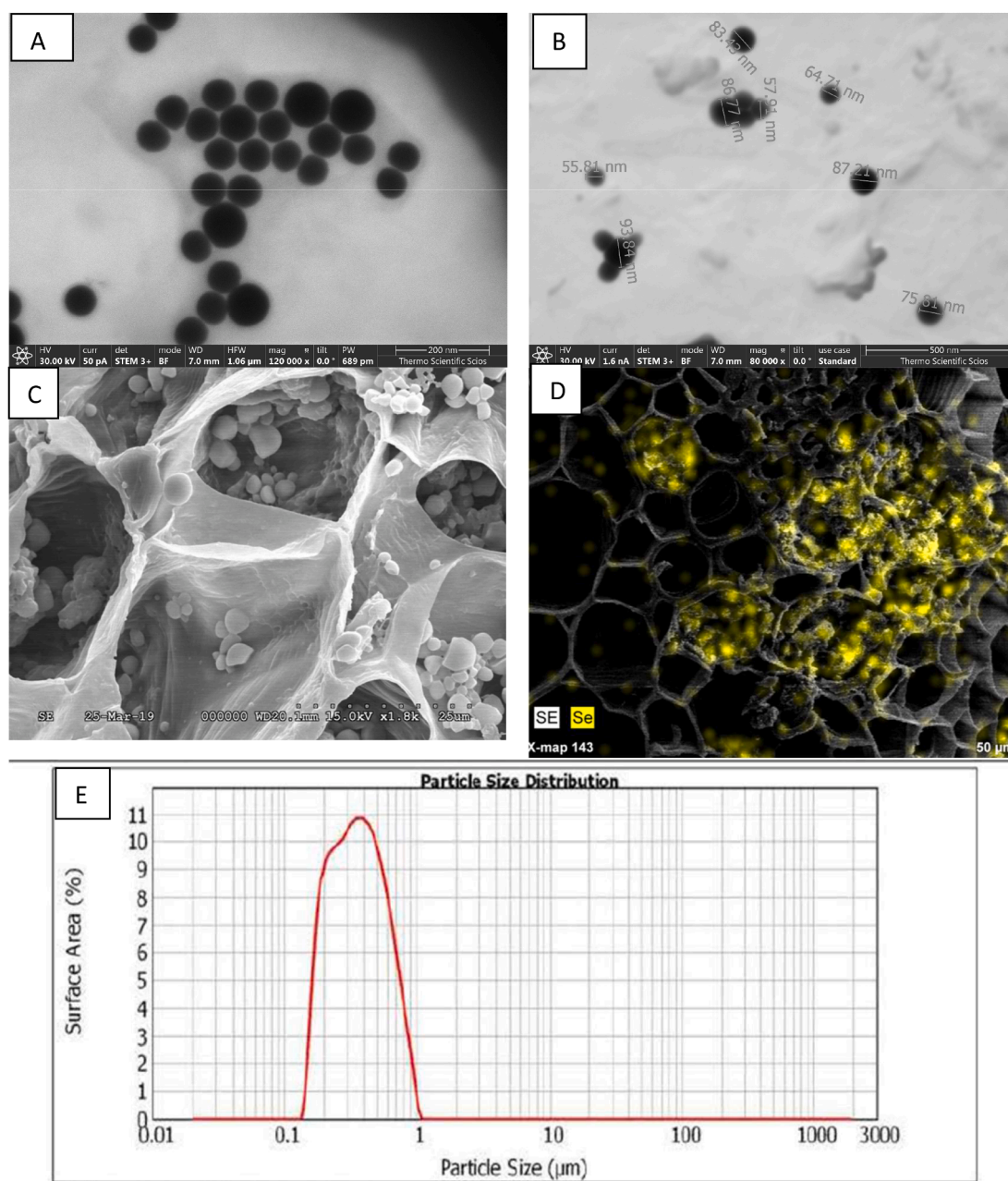


Fig. 2. A) Shape and homogeneity of chemically synthesized selenium nanoparticle (SeNPs; red elemental selenium) by ascorbic acid method under scanning electronic microscope (SEM); B) size of chemically synthesized SeNPs as shown by SEM; C and D) precipitation of SeNPs in stem tissues of Tulip plant under SEM; and E) particle size distribution graph for the chemically synthesized SeNPs.

Monascus purpureus produced SeNPs that had absorption maxima at 539 nm (El-Sayed et al., 2020). The SPR phenomenon is what causes these fluctuations in the absorption wavelengths. It provides preliminary evidence indicating that selenite or selenate, serving as precursors, is the sources of SeNPs. The stability conferred by biologically produced NPs is a notable advantage, attributed to natural stabilizers derived from metabolites or biomolecules inherent in the source material. This stability can be elucidated through Fourier transform infrared spectroscopy (FTIR), which identifies pertinent functional groups in conjunction with the nanoparticles. In the FTIR analysis of SeNPs synthesized from *Penicillium expansum*, characteristic peaks such as -OH, -NH (3247.5 cm^{-1}), and amide (1630.5 cm^{-1}) were observed (Hashem et al., 2021). Similarly, (Ashengroph and Hosseini, 2021) reported that FTIR analysis of SeNPs produced from *B. amyloliquefaciens* SRBO4 revealed capping agents including amine, carboxyl, and aldehyde groups.

Beyond FTIR, Nuclear Magnetic Resonance (NMR) spectroscopy offers insights into nanoparticle surface composition (Mourdikoudis et al., 2018). Additionally, X-ray Photoelectron Spectroscopy (XPS) is employed to discern surface chemistry by identifying functional groups based on binding energies, offering insight into nanoparticle interactions with other materials (Korin et al., 2017). In XPS examination of SeNPs synthesized using yeast, a distinctive peak at 55.38 eV indicated elemental selenium, while peaks corresponding to carbonyl, amide, and amino acid chains revealed functional groups (Wu et al., 2021). Similarly, starch-stabilized SeNPs exhibited XPS peaks at 55.6 and 56.6 eV, characteristic of elemental selenium (Ahmed et al., 2021). Similar results were achieved with SeNPs stabilized using *Polygonatum sibiricum* polysaccharide. The synthesis of SeNPs and the polysaccharide complex was evidenced by peak shifts at binding energies of 55.4 and 56.2 eV (Chen and Murata, 2002). Electron microscopy techniques such as Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) are most effective for elucidating NPs morphology. SEM, in conjunction with Energy-Dispersive X-ray spectroscopy (EDX) mapping, provides surface characteristics and reveals the elemental composition of the sample (Cui et al., 2016). Additionally, Inductively Coupled Plasma Mass Spectrometry (ICP-MS), among other methods, can measure NPs concentration and constituent elements in the sample, offering high sensitivity to detect trace components in the solution (Mourdikoudis et al., 2018). TEM examination provides dispersion patterns, size distribution, and core diameter of the NPs, which are crucial for understanding their functional significance. For example, TEM images revealed SeNPs produced using the wet chemical technique varied in size from 30 to 80 nm (Korany et al., 2020). Modern techniques for imaging and studying NPs morphology have become more sophisticated, including Focused Ion Beam Scanning Electron Microscopy (FIB-SEM), scanning transmission electron microscopy (STEM), and High-Resolution Transmission Electron Microscopy (HRTEM) (Kizilyaprak et al., 2014). Dynamic Light Scattering (DLS), akin to TEM, measures the hydrodynamic diameter of NPs to determine their size and calculates their polydispersity index to assess the formation of aggregates (Souza et al., 2016). However, according to Dwivedi et al. (Dwivedi et al., 2013), nanoparticle size ranges obtained by DLS occasionally differ from those obtained by TEM, suggesting potential aggregation in the colloidal solution used in DLS measurements.

The stability of the nanoparticles is determined by Zeta-potential measurement of their total charges. More positive or negative charges cause repulsion between the particles, which reduces the likelihood of the particles clumping together. By combining DLS with Zeta-potential analysis, this may be thoroughly investigated (Mourdikoudis et al., 2018). It has been demonstrated in one study that the elements of microbial extracellular polymer provide SeNPs, which serve as capping agents and generally give the SeNPs some stability, a negative charge. The lattice structures of the nanoparticles are estimated and their crystalline or amorphous nature is clarified by X-ray diffraction. Its diffraction patterns can also gauge a product's purity, such as SeNPs (Menon et al., 2020). As these analysis techniques continue to evolve,

they offer comprehensive insights into nanoparticles, thereby advancing our understanding of their applications and potential risks.

4.4. Mode of application of senps

Due to the crucial function that selenium plays in activating plants' defensive mechanisms, SeNPs have priority over other nanoparticles. The usage of biogenic SeNPs is recognized as an ecologically biocompatible and environmentally acceptable method to improve crop productivity by reducing biotic and abiotic pressures in addition to the use of helpful microorganisms. Although, the agriculture industry did not pay much attention to greenly produced SeNPs, research was done to examine the potential of plant-based SeNPs for treating bacterial and fungal diseases in plants, as well as heat, heavy metal, and drought stressors.

Both soil and foliar application methods are commonly employed for producing selenium-biofortified crops, as selenium can be absorbed by plants through both root and foliar pathways (Dinh et al., 2018). Among these methods, foliar application has shown to be more efficient compared to soil application (Dinh et al., 2019; Wang et al., 2023). Current research has primarily focused on understanding the absorption and translocation processes of selenium, particularly selenate and selenite, through both root and foliar application techniques (Wang et al., 2023). Leveraging the unique properties of NPs, such as their small size, versatile surface chemistry, and enhanced stability, nanotechnology holds promise for improving the efficacy of agrochemical applications (Dinh et al., 2019; Lowry et al., 2019).

SeNPs demonstrate superior bioactivity, increased bioavailability, lower toxicity, enhanced dispersibility, and exhibit low-dose antibacterial activity in comparison to inorganic selenium (Cheng et al., 2022). However, the mechanisms and uptake pathways of elemental selenium in plants are not yet fully elucidated (Zhou et al., 2020). Generally, C4 cereals, such as corn and sorghum, exhibit higher light saturation points, chloroplast densities, and yields compared to C3 plants, including wheat, rice, and soybean (Naseem et al., 2021). Specifically, C4 plants can utilize the low CO_2 concentration in the leaf intercellular spaces for photosynthesis even under high temperature or drought conditions, resulting in increased photosynthetic rates and more efficient use of nitrate and water compared to C3 plants (Valeria and Santiago, 2011). Application of selenium at low concentrations promotes plant growth, while higher selenium levels can be toxic (Wang et al., 2022, 2023). Wheat roots may passively absorb SeNPs, whereas selenite uptake may involve an active transport mechanism (Hu et al., 2018). However, the comparison of selenium uptake between inorganic selenium and SeNPs in C3 crops or vegetables remains contentious (Cheng et al., 2022; Hu et al., 2018). Further research is needed to determine whether SeNPs are more effective than inorganic selenium in enhancing plant physiological responses, given their differing absorption and translocation pathways.

The application of advanced technology such as metabolomics enables the detection of significant yet imperceptible changes in plants exposed to exogenous SeNPs, zero-valent iron nanoparticles (ZVI NPs), and cerium dioxide nanoparticles (CeO_2 NPs) (Tian et al., 2018; Wang et al., 2022; Zhang et al., 2019a). For instance, in a study by (Wang et al., 2021), foliar application of ZVI NPs revealed a dose-dependent relationship between metabolites and mineral nutrition in maize leaves, with stress-related and antioxidant metabolites showing down-regulation at 50 mg/kg treatment and up-regulation at 500 mg/kg treatment. Similarly, (Zhang et al., 2019) observed significant down-regulation of several amino acids (such as threonine, tryptophan, l-cysteine, methionine, aspartic acid, asparagine, tyrosine, and glutamic acid) in spinach following foliar application of 3 mg/plant of CeO_2 NPs, indicating inherent phenotypic and metabolic alterations in spinach. Furthermore, a hydroponic experiment demonstrated that foliar application of SeNPs at 20 mg/L led to a significant increase in fresh weight by 27%. SeNPs could up-regulated 42 metabolites, including carbohydrates, amino acids, ketones, phenols, vitamins, and ten metabolic

pathways. These changes resulted in enhanced antioxidant capacity and improved physiological parameters, ultimately promoting maize growth (Wang et al., 2023).

4.5. Selenium nanoparticles uptake

To fully grasp the safety and potential toxicity of SeNPs in the context of their phyto-uptake and translocation, it is essential to have a thorough understanding of how different plants absorb them. To achieve this understanding, it is crucial to explore the process of SeNPs' uptake and movement within plants. While efforts have been made to investigate how SeNPs are absorbed into plant systems, this phenomenon remains incompletely understood (Hu et al., 2018). SeNPs navigate through the plant's cell wall and breach the plasma membrane, although effective penetration through the cell wall only occurs for nanoparticle aggregates with diameters smaller than the wall's pores (Wang et al., 2022). Acting as a protective barrier, the plant's cell wall hinders the entry of external substances, including SeNPs, into the cellular structure. The efficiency of this barrier relies on the pore size of the cell wall, which typically ranges from 5 to 20 nm (Carpita et al., 1979). Studies have revealed that SeNPs can adhere to plant roots, influencing the chemical and physical uptake processes in plants (Wang et al., 2023). The prevailing explanation for the translocation of engineered nanomaterials is that these materials can traverse both inside and outside plant tissues, eventually reaching the xylem (Rajae Behbahani et al., 2020). Once SeNPs have entered the plant's vascular system, engineered NPs possess the potential to be transported to the above-ground parts of the plant, utilizing the plant's transpiration and nutrient transport processes to facilitate their movement alongside nutrients. The attributes of NPs, including their physical characteristics such as shape and particle size, as well as their chemical properties like surface acid groups, surface adsorption, and metal and metal oxides solubility, typically exert significant influence on their behavior within the soil matrix (Altammar, 2023). Upon introduction into the soil environment, NPs undergo various environmental alterations, encompassing redox reactions, ion precipitation, sulfidation, or the formation of organic and inorganic complexes with high molecular weight substances (Husen and Siddiqi, 2014). Among these attributes, particle size emerges as a pivotal factor determining the physicochemical behavior of NPs in soil (Khan et al., 2017). Smaller NPs tend to be more easily immobilized by soil organic matter (Zhu et al., 2009), highlighting the importance of size-dependent morphology and its implications for NPs bioavailability. Chemoautotrophic bacteria have the capability to directly oxidize red elemental selenium (Se^0) into selenite. Microorganisms produce selenide, which can subsequently form complexes with metals or organic compounds, leading to the generation of metal or organic selenides. Ultimately, these compounds may undergo mineralization to yield bioaccessible forms of selenium (Luo et al., 2022). SeNPs tend to aggregate with higher quantities of particulate matter in soil, and this aggregation can influence the size-dependent characteristics of NPs, resulting in diminished bioavailability and biotoxicity (Yu et al., 2019). The smaller cerium (IV) oxide NPs exhibited greater susceptibility to transformation and released higher levels of cerium (III) ion compared to their larger counterparts. Conversely, in soil, larger cerium (IV) oxide NPs were found to contain higher concentrations of cerium in both roots and shoots, regardless of whether the soil conditions were flooded or aerobic (Mortazavi Milani et al., 2017).

4.6. Impact of SeNPs on crop management

4.6.1. As fertilizer

Fertilizers play a pivotal role in enhancing crop yields and agricultural productivity, while also ensuring food security in less developed regions (Kaur et al., 2014). The utilization of SeNPs as a selenium fertilizer source presents a viable alternative to conventional selenium fertilizers, leveraging innovative and emerging technologies. SeNPs are

employed to enhance the organic content of soil, thereby significantly impacting soil fertility (Gudkov et al., 2020). The concurrent application of humic material alongside SeNPs and other NPs further augments their stimulatory effect.

The decomposition of organic matter is controlled by microbial communities. Bacteria exist in certain surroundings as an organized and rigidly ordered polymicrobial community, where each member has a defined functional purpose. The soil functions as a bioreactor, accelerating a variety of biodegradation processes (Rajput et al., 2021). The effectiveness of selenium application in traditional fertilizer form is believed to be inferior to its application in SeNPs form concerning biological processes and productivity (Jain et al., 2015). Literature suggests that selenium exhibits specific stress-reducing properties in plants, including alleviation of high temperatures, drought, heavy metal accumulation, and salinity, by stimulating the production of secondary metabolites and enhancing the activity of antioxidant enzymes (Liu et al., 2022a). For example, in sandy soil, SeNPs enhanced the growth of groundnut cultivars by augmenting the plants' total soluble sugars, phenol content, photosynthetic pigments, lipid peroxidation, and antioxidant enzymes (ascorbic acid peroxidase, catalase, peroxidase), along with total flavonoids (Hussein et al., 2019). In another study involving barley (*Hordeum vulgare*) plants subjected to saline stress, treatment with SeNPs at a concentration of 100 mg/L led to a direct increase in selenium accumulation in leaves, enhancement in the levels of total phenolic compounds, and reduction in the levels of ROS-mediated cellular membrane damage markers such as malondialdehyde, which could affect metabolism and contribute to nutrient deficiencies (Schiavon et al., 2017). In a study on tobacco (*Nicotiana tabacum*) plants, SeNPs did not noticeably affect the shoot quantity; however, 50 mg/L of selenate completely inhibited shoot proliferation. The rate of root regeneration significantly accelerated with increasing SeNPs concentrations, resulting in larger and denser root systems at concentrations of 50–100 mg/L, leading to a considerable increase in fresh weight. Conversely, selenate entirely inhibited root development at concentrations ranging from 50 to 100 mg/L (El-Ramady et al., 2018a). Furthermore, the biological effects of SeNPs in plant tissue culture differed from those of the selenite ion. SeNPs concentrations between 50 and 100 mg/kg significantly promoted root system development (> 40 %) and organogenesis, whereas selenite did not exhibit such effects at any dose (El-Ramady et al., 2018a). Another notable aspect is that SeNPs have been shown to decrease the concentrations of heavy metals, which pose significant hazards to plants. The application of SeNPs has been found to notably reduce the levels of heavy metals in the environment, mitigating the adverse effects of these toxic substances on plant ecosystems. Most studies have indicated that selenium exhibits greater antagonistic effects against toxic elements such as lead (Pb) and cadmium (Cd). For instance, it was demonstrated that the concentrations of Cd and Pb in female spinach plants decreased by 66 % and 19 %, respectively, following SeNPs application. As the oxidation state of the plant decreases, female spinach plants become more resilient to Cd (Golubkina et al., 2017).

Several recent biochemical investigations have discovered that selenium increases the rates of photosynthetic reactions involving malic acid, succinic acid, and citric acid (Yin et al., 2019; Zahedi et al., 2019). Rice plants were shown to produce more chlorophyll when selenate and selenite were applied (Marques et al., 2020). Additionally, spraying SeNPs on sugarcane seedlings was said to improve the levels of chlorophyll a and chlorophyll b (Elsheery et al., 2020). Selenium was also shown to increase the antioxidant capacity and photosynthetic pigments in cowpea plants (El-Ramady et al., 2018b; Silva et al., 2018). Cowpea's chlorophyll a to chlorophyll b conversion was sparked by selenate foliar spraying, which also increased the plant's total chlorophyll content (Silva et al., 2020). Additionally, it was discovered that selenite increased the levels of chlorophyll a and chlorophyll b in wheat (Wu et al., 2020).

4.6.2. Biofortification

Researchers interested in integrating SeNPs into agroecosystems to mitigate potential losses associated with conventional fertilizers are intrigued by the prospect of gradual selenium release from SeNPs for plant biofortification purposes (Table 3). SeNPs offer a viable avenue for biofortification endeavors, aiming to augment the selenium levels in edible plant parts to prevent selenium deficiency in both humans and animals. Investigations involving tobacco and garlic (*Allium sativum*) have indicated that SeNPs pose lesser harm to plants compared to ionic selenium salts, namely selenate and selenite (Bano et al., 2021). SeNPs were reported to be assimilated into organic forms, such as SeMet, primarily accumulating in the root cell walls of plants, with uptake levels reported to be 1.7 times lower than that of selenate and selenite. Furthermore, SeNPs produced chemically were more readily absorbed than SeNPs produced biologically. For example, studies have shown that wheat roots exposed to chemically synthesized SeNPs with a diameter of 40 nm absorbed 1.8 and 2.2 times more selenium compared to wheat roots treated with SeNPs of 140 and 240 nm diameters, respectively. This underscores the importance of nanoparticle concentration and particle size in influencing absorption rates (Wang et al., 2020). The rising interest in using SeNPs for crop biofortification stems from their ability to improve the selenium content, nutritional attributes, and overall quality of edible plant parts. However, the effects on the body of consuming plant foods biofortified with SeNPs can vary significantly based on factors such as nanoparticle size, processing methods, and surface characteristics, as opposed to other conventional selenium sources (Wang et al., 2020). As a result, several *in vivo* studies must be conducted to ascertain whether consuming plant foods that have been biofortified with SeNPs has any negative impacts. Because of this, it is hard to generalize about the doses that are advised for consuming foods that have been biofortified using SeNPs (Verstege and Günther, 2023).

4.6.3. Effect of SeNPs on seed germination

SeNPs are useful in agriculture because they can affect the features of sowing and seed development. When subjected to the impacts of drought, disease, and pests, plants become more resistant to such conditions (Siddiqui et al., 2021). SeNPs have been shown to have a significant impact on seed germination and early ontogenesis. In barley (*Hordeum vulgare* L.) seeds, SeNPs increased both shoot and root length and accelerated germination rates (Ikram et al., 2021). These findings suggest that SeNPs could serve as a selenium source during seed development. Moreover, SeNPs were found to be less detrimental than ionic selenium forms, indicating their potential to support biochemical

Table 3

Amino acid content (g/100 g sample) in leaf protein isolated from fresh green biomass of alfalfa (*Medicago sativa* L.) treated by different selenium species (selenate, selenite, and selenium nanoparticles (SeNPs)) and concentrations.

	Control	10 Selenate	10 Selenite	50 Selenite	10 SeNPs	50 SeNPs
Lysine	4.55	2.65	3.20	2.79	3.07	3.07
Methionine	0.70	0.90	0.41	0.29	0.29	0.30
Phenylalanine	3.30	1.86	2.51	2.02	2.35	2.40
Threonine	1.40	1.33	1.55	1.37	1.52	1.61
Valine	2.50	1.54	2.09	1.64	1.91	1.89
Isoleucine	2.15	1.43	1.81	1.45	1.64	1.60
Leucine	4.35	2.54	3.50	2.82	3.24	3.23
Agrinine	2.80	1.38	2.17	1.57	1.79	1.86
Tyrosine	2.20	1.33	1.53	1.23	1.45	1.48
Proline	3.40	2.23	2.69	2.24	2.42	2.67
Histidine	1.25	0.80	0.90	0.71	0.85	0.90
Cysteine	0.80	0.74	0.34	0.13	0.24	0.28
Aspartic acid	3.05	2.97	3.15	2.82	3.14	3.42
Glutamic acid	3.90	2.86	3.92	3.44	3.74	3.71
Serine	1.30	1.27	1.47	1.31	1.42	1.46
Glycine	2.50	1.43	2.00	1.63	1.88	1.87
Alanine	2.70	1.54	2.08	1.70	2.03	2.01

processes and substitute selenium in seeds (Ikram et al., 2021). Notably, the highest germination percentage in barley seeds was observed with a SeNP application dose of 4.65 g/mL (Siddiqui et al., 2021). Further investigations into the effects of SeNPs on germination in other important crops like maize, rice, and soybeans are essential (Siddiqui et al., 2021). Additionally, SeNPs have been shown to promote organogenesis and root growth, with trace selenium levels benefiting the development of plants such as *Brassica oleracea*, potato, lettuce, and ryegrass (Bideshki et al., 2019).

4.6.4. Effect of SeNPs on pesticide usage

The utilization of pesticides is customary for managing pests and diseases; however, the escalation of pesticide residue levels and the emergence of pesticide resistance from prolonged use have heightened apprehensions among consumers regarding food safety and environmental contamination (Carvalho, 2017; Schiavon et al., 2020). Hence, there is a pressing need to develop innovative, environmentally sustainable approaches aimed at enhancing resistance, thereby reducing reliance on pesticides and effectively managing plant diseases and insect infestations. ROS are rapidly produced in plants during pathogen infections (Khursheed et al., 2022). Enhancing the activity of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and peroxidase (POD) in plants, reactive oxygen species (ROS) can be effectively neutralized (Mei et al., 2020). Studies have shown that sodium selenate can elevate APX activity in arsenic-stressed radishes, and selenium itself is recognized for its antioxidant properties (Joshi et al., 2021). Given the high bioavailability of SeNPs to plants, there is growing interest in the zero-valent oxidation state of selenium (Zhai et al., 2017). (Neysanian et al., 2020) reported that the bioaccumulated selenium content in plants treated with selenium is often substantially higher than the initial application content of selenate. Furthermore, the bioaugmentation effect of SeNPs surpasses that of its inorganic counterpart. Studies have demonstrated that foliar spraying of wheat with selenium leads to increased activities of SOD, POD, CAT, and APX, with SOD and CAT helping to mitigate the impact of harmful (Shah et al., 2020).

Moreover, observations indicate that tomato plants treated with SeNPs demonstrate heightened phenylalanine ammonialyase (PAL) and α -1,3-glucanase (GLU) activity, as well as elevated SOD activity and mRNA levels. To bolster the resistance of melon plants against powdery mildew, SeNPs treatments enhance antioxidant capacity, organic acids, and up-regulate genes related to Cuc-B synthesis (Kang et al., 2022). Also, *Sclerospora graminicola*, a plant disease, affects crops of maize and pearl millet. A prior work found that biosynthesized SeNPs suppresses *S. graminicola*'s proliferation, spore viability, and sporulation (Nandini et al., 2017).

4.7. SeNPs and abiotic stress resilience

NPs offer a means to mitigate the toxicity and aggregation of heavy metals and shield plants from environmental stressors such as salt or drought (Table 4). They can serve as a source of micronutrients, enhancing plant fitness and resilience under challenging conditions. In maize and strawberry plants, the application of plant-mediated SeNPs externally led to increased activity of APX, SOD, and CAT enzymes. This activation, in turn, induced the expression of genes related to antioxidant defense, thereby enhancing the plants' ability to withstand abiotic stressors (Huang et al., 2000). Additionally, strawberry plants subjected to salt stress responded positively to foliar spraying with SeNPs, which resulted in decreased levels of H₂O₂ and lipid peroxidation, along with increased activity of antioxidant enzymes such as POD and SOD (Zahedi et al., 2019). Moreover, (Rady et al., 2020) reported that tomatoes treated with 40 M of selenium exhibited enhanced drought tolerance, with several antioxidant enzymes including SOD, APX, and CAT contributing to this improvement. Specifically, SOD and CAT increased drought tolerance by 56 % and 44 %, respectively, while APX raised it by

Table 4

The supportive role of selenium nanoparticles (SeNPs) to plant growth and productivity under various biotic/ abiotic stresses.

Plant	Stress type	Mode of application	Growth conditions	Concentration	Findings	Reference
Dry bean (<i>Phaseolus vulgaris</i> L.)	Salinity (0.6, 1.6, 3.0, and 4.8 dS/m)	Foliar	Soil lysimeters under greenhouse conditions	5 and 20 ppm	The impact of SeNPs on enhancing tolerance to salinity stress in dry bean was found to be less significant than anticipated.	(Admasie et al., 2023)
Common Bean (<i>Phaseolus vulgaris</i> L.)	Salinity (7.55–7.61 dS/m)	Foliar	Field experiment	0.5, 1.0, or 1.5 mM (selenium dioxide nanoparticles)	- SeNPs improved growth and yield, photosynthetic pigments, proline, soluble sugars, selenium content, K^+/Na^+ ratio, cell membrane fluidity, and antioxidant enzymes activity. - SeNPs reduced Na accumulation in plant tissues, H_2O_2 content, electrolyte leakage, and malondialdehyde	(Rady et al., 2021)
Bitter-melon (<i>Momordica charantia</i>).	Salinity (50 and 100 mM NaCl)	Foliar	Growth medium (Coco peat:perlite at 2:1 ratio) under greenhouses conditions	10 and 20 mg/L (chitosan-SeNPs)	- Cs–Se NPs induced the activity of antioxidant enzymes, proline content, water retention, and K^+ content - Cs–Se NPs lessened malondialdehyde, H_2O_2 , and Na contents - Cs–Se NPs increased the yield and oil content	(Sheikhalipour et al., 2021)
Lemon balm (<i>Melissa officinalis</i>)	Salinity (50, 100, and 150 mM NaCl)	Foliar	Growth medium (perlite:coco peat:sand at 5:7:23 ratio) under greenhouse conditions	50 and 100 mg/L	- SeNPs enhanced plant growth - SeNPs reduced malondialdehyde content - SeNPs increased the activity of antioxidant enzymes, e.g., superoxide dismutase, catalase, and peroxidase - SeNPs up-regulated the expression of phenylalanine ammonia-lyase and rosmarinic acid (RA) synthase genes	(Ghasemian et al., 2021)
Strawberry (<i>Fragaria × ananassa</i> Duch.)	Salinity (25, 50, and 75 mM NaCl)	Foliar	Greenhouse conditions using a growth medium of perlite:coco peat:sand at 5:7:23 ratio	10 and 20 mg/L	- SeNPs lowered lipid peroxidation and H_2O_2 content - SeNPs induced the activities of superoxide dismutase and peroxidase - SeNPs increased the accumulation of indole-3-acetic acid and abscisic acid in plant tissues - SeNPs elevated the levels of several organic acids (such as citric, succinic, and malic acids) and sugars (such as sucrose, glucose, and fructose) in the fruits of strawberry plants	(Zahedi et al., 2019)
Tomato	Salinity (50 mM NaCl)	Foliar	Pot experiment under greenhouse conditions using a mixture of peat moss:perlite at 1:1 ratio	1, 5, 10, and 20 mg/L	SeNPs increased fruit yield SeNPs increased the contents of photosynthetic pigments SeNPs improved most of the antioxidant components in the tomato's fruits, namely lycopene, β -carotene, flavonoids, and phenols SeNPs amplified the activity of antioxidant enzymes in the tomato's leaves and fruits	(Morales-Espinoza et al., 2019)
Sweet Basil (<i>Ocimum basilicum</i> L.)	Salinity (50 and 100 mM NaCl)	Seed priming and root application	Petri dishes under laboratory conditions	100 μ M	- SeNPs improved the antioxidant enzymes activities - SeNPs decreased the contents of total polyphenols and malondialdehyde - SeNPs elevated the content of soluble protein	(Azizah et al., 2023)
Pomegranate (<i>Punica granatum</i> cv. Malase Saveh)	Drought (irrigation each 7, 14, or 21 days)	Foliar	Open field	20 mg/L SeNPs of 10 and 50 nm	- SeNPs declined lipid peroxidation and H_2O_2 content through enhancing antioxidant capacity. - SeNPs of 10 nm diameter showed better influence than that of 50 nm	(Zahedi et al., 2021)
Sorghum (<i>Sorghum bicolor</i> L. Moench)	Heat stress (38/28 °C)	Foliar	River sand under growth chamber conditions	10 mg/L	- SeNPs improved the antioxidant enzyme activities - SeNPs lowered the content of signature oxidants - SeNPs elevated the unsaturated phospholipids content - SeNPs induced productivity	(Djanaguiraman et al., 2018)
Carrot (<i>Daucus carota</i>)	Heavy metal (wastewater)	Foliar	Pot experiment under open field conditions using clay soil	10 mg/L SeNPs	- SeNPs diminished Ni, Cd, Pb, and Co accumulation in plant tissues - SeNPs increased the levels of proline and total phenols, activities of catalase, superoxide dismutase, peroxidase, and polyphenol oxidase. - SeNPs lowered the contents of malondialdehyde and H_2O_2	(El-Batal et al., 2023)

(continued on next page)

Table 4 (continued)

Plant	Stress type	Mode of application	Growth conditions	Concentration	Findings	Reference
Soybean (<i>Glycine max</i> L. Merrill).	Heavy metal (25 μ M/L arsenic)	Root application	Hydroponic experiment under greenhouse conditions	10 and 25 μ M/L	SeNPs boosted the translocation of arsenic in vacuole SeNPs diminished the electrolyte leakage and the generation of H ₂ O ₂ , oxidized glutathione (GSSG), and O ₂ ⁻ SeNPs enhanced the activities of glutathione S-transferase (GST) and monodehydroascorbate Reductase (MDHAR) SeNPs increased the contents of reduced glutathione (GSH) and phytochelatin (PC) SeNPs enhanced the activities of Na ⁺ /K ⁺ -ATPase and Ca ²⁺ /Mg ²⁺ -ATPase SeNPs induced expression of several transcription factors such as GmWRKY6, GmWRKY46, GmWRKY56, and GmWRKY106 SeNPs reduced the expression of GmPT1, GmPT2, GmPT3, GmPT4, and GmPT8	(Zeeshan et al., 2022)
Mung bean (<i>Vigna radiata</i>)	CeO ₂ -NPs stress (250, 500, and 1000 mg/L)	Foliar	Soil pot experiment under greenhouse conditions	25, 50 and 75 mg/L	- SeNPs lowered H ₂ O ₂ content, enhanced catalase and superoxide dismutase activities, and increased the contents of chlorophyll a, chlorophyll b, proline and dry matter content. - SeNPs increased Ce precipitation in the plant cell wall	(Kamali-Andani et al., 2023)
Bitter-melon (<i>Momordica charantia</i>)	Selenate	Root application	MS medium under laboratory conditions	1, 4, 10, 30, and 50 mg/L	- SeNPs below 10 mg/L improved biomass, whereas SeNPs above 10 mg/L lessened root meristem - SeNPs exhibited greater efficacy in promoting growth and organogenesis compared to selenate - Selenate exhibited higher toxicity to plants than SeNPs - High SeNPs concentration up-regulated the expression of WRKY1 transcription factor, phenylalanine ammonia-lyase (PAL), 4-Coumarate, and CoA-ligase (4CL) genes - Low SeNPs concentration induced the activity of the leaf nitrate reductase - High SeNPs concentration amplified leaf proline content - SeNPs induced the activity of peroxidase and catalase	(Rajae Behbahani et al., 2020)
Wheat (<i>Triticum aestivum</i> L. cv. Masr1)	Crown and root rot diseases (<i>Fusarium</i> spp.)	Seed priming	Growth medium (soil: sand at 1:1 ratio) under greenhouse conditions	50, 75, and 100 μ g/mL	- SeNPs suppressed crown root rot by 75 % - SeNPs improved plant development and yield by 5–40 % - SeNPs increased the content of photosynthetic pigments and elevated gas exchange	(El-Saadony et al., 2021a)
Groundnut (<i>Arachis hypogaea</i> L.)	Nutrient deficiency	Foliar	Pot experiment of sandy soil under greenhouse conditions	20 and 40 ppm	SeNPs enhanced the growth SeNPs enhanced levels of photosynthetic pigments, reduced lipid peroxidation, and increased the activity of antioxidant enzymes such as catalase, peroxidase, and ascorbic acid peroxidase SeNPs elevated the concentrations of total phenols, total flavonoids, and total soluble sugars	(Hussein et al., 2019)

44 %. Peanut plants subjected to foliar treatment with SeNPs for 45 days exhibited elevated levels of unsaturated fatty acids and antioxidant capacity, resulting in significantly accelerated growth (Hussein et al., 2019). Similarly, the exogenous application of SeNPs (10 nm) improved drought tolerance in pomegranate plants, leading to enhanced growth and productivity. SeNPs also bolstered the activity of photosynthetic pigments, improved the nutritional status, boosted antioxidant activity, and elevated total phenolic content levels (Zahedi et al., 2019). By mitigating oxidative stress, reducing metal absorption, and limiting metal translocation, SeNPs may offer beneficial effects at lower concentrations, safeguarding plants against metal toxicity (Sarraf et al., 2022). Additionally, selenium augmented the activity of SOD and CAT in

both the roots and leaves of Chinese cabbage exposed to Cd-stress conditions (Wu et al., 2017).

The intriguing qualities and unique bioactivities of SeNPs have garnered attention in agricultural applications (El-Ramady et al., 2018b), primarily demonstrating significant financial benefits in aiding plants to withstand heavy metal or other abiotic stress conditions (Hawrylak-Nowak et al., 2018). Selenium impedes the absorption and translocation of harmful metals from the roots to various plant parts, such as shoots, leaves, and grains (Gao et al., 2018). Se-NPs have been observed to enhance nitrate reductase activity, essential for proline synthesis, thereby increasing proline production. Selenium supplementation has also been shown to elevate the levels of pectin and

hemicellulose, as well as enhance cell wall thickness, thereby augmenting the cell wall's capacity to sequester toxic metals (Zhao et al., 2019). In assessing the toxicological, physiological, and biological impacts of heat stress on *Sorghum bicolor* L. Moench, SeNPs (10–40 nm) were employed (Djanaguiraman et al., 2018). Heat stress conditions resulted in elevated pollen germination rates and increased concentrations of unsaturated phospholipids, consequently enhancing seed yield. Following the application of SeNPs, there was an increase in seed yield percentage (14 %), seed set (19 %), and pollen germination (26 %) under heat stress conditions. Recent studies, such as (Zare-Bidaki et al., 2023), have shown that nanoparticles can significantly enhance crop yield and stress tolerance. SeNPs, in particular, have been found to improve antioxidant enzyme activity and nutrient uptake, leading to better plant growth under adverse conditions.

4.8. Impacts of SeNPs on plant gene expression and secondary metabolites

Selenium is taken up and moved within plants in a manner akin to the way sulfate and phosphate are absorbed and transported. At the cellular level, it has the capacity to substitute for sulfur in the synthesis of crucial macromolecules. This encompasses amino acids, specific structural and functional proteins, as well as potentially harmful, non-specific proteins, in addition to diverse selenium compounds (Sarwar et al., 2020). Utilizing SeNPs at different stages of plant cell and tissue culture affected processes such as cell division, tissue differentiation, epigenetic alterations, transcriptional profiling, and metabolic pathways in balsam pear seedlings (Rajae Behbahani et al., 2020). (Li et al., 2021a, 2021b) utilized transcriptomics and targeted metabolite analysis to discern variations in metabolites and genes linked to plant signal transduction and lignin production. Particularly, the cadmium-selenium treatment induced a significant upsurge in the number of genes associated with lignin synthesis (PAL, CAD, 4CL, and COMT) and the levels of specific metabolites (ersinol, phenylalanine, p-coumarin, cafestol, and coniferaldehyde). This resulted in the reinforcement of the structural integrity of root cell walls. Additionally, it enhanced signal transduction and the plant's responsiveness to hormones by stimulating the expression of genes (BZR1, LOX3, and NCDE1) and the accumulation of metabolites (brassinosteroids, abscisic acid, and jasmonic acid) in both roots and leaves. Table 5 shows the phytochemical composition of alfalfa after treating with different forms of selenium.

Table 5

Variations in concentrations of phytochemicals (µg/g) in leaf protein isolated from fresh green biomass of alfalfa (*Medicago sativa* L.) treated by different selenium species (selenate, selenite, and selenium nanoparticles (SeNPs)) and concentrations.

	Control	10 ppm Selenate	50 ppm Selenite	50 ppm SeNPs
Nicotinamide	5.1	5.9	4.8	5.4
Nicotinic acid	1.5	1.2	<1	1.2
Biotin	<1	<1	<1	<1
Riboflavin	36.6	23.6	27.2	33.1
Coumesterol	<1	<1	<1	<1
Formononetin	1.5	1.6	1.0	<1
Biochanin A	<1	<1	<1	<1
Genkwanin	<1	<1	<1	<1
Ferulic acid	18.8	22.7	19.2	18.7
Apigenin-7-O-glucuronide	58.3	68.1	57.0	43.1
Isoquercitrin	<1	<1	<1	<1
Liquiritigenin	<1	<1	<1	<1
Quercetin	<1	<1	<1	<1
Naringenin	<1	<1	<1	<1
Luteolin	1.5	2.9	3.0	1.6
Apigenin	11.6	11.1	9.9	8.0
Tricin	4.3	3.1	3.4	3.4
Medicagenic acid	<1	1.1	1.4	1.1

(Li et al., 2021a, 2021b) investigated the effects of Cd-contaminated soil stress and various concentrations of SeNPs (1–20 mg/L) on the metabolism of pepper plants, the nutritional quality of their fruits, and the composition of volatile organic compounds (VOCs). Notably, the application of SeNPs at a concentration of 5 mg/L upregulated the expression of genes associated with the phenylpropane branch-chain fatty acid pathway (BCAT, Fat, AT3, HCT, and Kas) and resulted in a significant increase in capsaicin (29.6 %), nordihydrocapsaicin (44.2 %), and dihydrocapsaicin (45.3 %) levels. Furthermore, the concentrations of VOCs, including amyl alcohol, linalool oxide, e-2-heptanal, 2-hexenal, ethyl crotonate, and 2-butanone, associated with crop resistance and quality, also exhibited a notable increase in response to SeNPs. Consequently, SeNPs played a beneficial role in enhancing the well-being of capsaicin crops by modulating the capsaicin metabolic pathway and influencing the amino acid and VOCs content.

4.9. Effects of SeNPs on humans and animals

SeNPs have attracted considerable attention for their potential uses as food supplements and therapeutic treatments. Their biomedical and pharmaceutical applications are promising due to their antioxidative, antimicrobial, antidiabetic, and anticancer characteristics. Moreover, SeNPs have demonstrated effectiveness in alleviating the harmful impacts of chemicals and heavy metals. Their efficient adsorption capabilities also make them valuable for remediation efforts in both water and soil contaminated with various metals and heavy metals (El-Ramady et al., 2018a).

Selenium plays a crucial role as a micronutrient essential for humans, plants, and animals (Rayman, 2000; Xu et al., 2020). It is integral to the synthesis of selenoproteins, which include various well-defined selenoenzymes, many of which function as oxidoreductases (Labunskyy et al., 2014; Rayman, 2000). Selenium is a fundamental component of the amino acid selenocysteine (Yang et al., 2019), which is the building block for selenoproteins and selenoenzymes, such as peroxidases and reductases. This essential trace element is indispensable for the maintenance and growth of both animals and plants (Qiu et al., 2018).

SeNPs are of great significance in research owing to their superior absorption capacity and reduced toxicity compared to both organic and inorganic counterparts (Bhattacharjee et al., 2019). They are highly esteemed as a promising material for various applications due to their unique characteristics, including heightened biological activity, improved bioavailability, minimal toxicity, excellent particle dispersion, and significant surface area (Torres et al., 2012).

SeNPs are central to various physiological processes crucial for human well-being, such as growth, reproduction, and immune regulation (Hosnedlova et al., 2018). In modern times, SeNPs are widely utilized in medical diagnostics and drug delivery systems, addressing conditions like cancer, muscular dystrophy, diabetes, liver fibrosis, bacterial and fungal infections, among others (Khurana et al., 2019). Moreover, SeNPs are utilized in addressing heavy metal and chemical toxicity owing to their capability to offset the impacts of these substances. Biologically synthesized SeNPs have shown efficacy in shielding against DNA damage and cell demise induced by As (III) (Prasad and Selvaraj, 2014). Likewise, SeNPs function as antagonists in alleviating neurotoxicity and nephrotoxicity induced by cadmium chloride in rats (Sadek et al., 2017). In a particular study, SeNPs demonstrated their antagonistic properties against hexavalent chromium (K₂Cr₂O₇) thyrotoxicity by mitigating oxidative stress and cellular damage in the thyroid gland of rats (Hashem et al., 2013). Similarly, SeNPs have exhibited a preventive impact on abnormal liver metabolism induced by hexavalent chromium in chickens (Luo et al., 2019). Cisplatin, an anticancer medication, has limited utility due to its associated adverse effects. However, SeNPs offer protection against Cisplatin-induced nephrotoxicity (Liu et al., 2022a) and gonadotoxicity (Rezvanfar et al., 2013) by utilizing their antioxidant properties to regulate the production of ROS. The inhibitory effect of SeNPs on 4T1 breast cancer cells was examined,

revealing their immunomodulatory effects on the immune response targeting cancer cells (Beheshti et al., 2013). Additionally, in another investigation, SeNPs conjugated with folate (FA-SeNPs) exhibited antagonistic effects against multidrug-resistant liver cancer cells (Liu et al., 2015).

4.10. Selenium and SeNPs in ecosystems

The release of selenium into the environment is primarily attributed to industrial and agricultural activities, thereby becoming accessible to aquatic and terrestrial ecosystems' fish and wildlife (Hamilton, 2004). Various sources contribute to selenium contamination, including agricultural runoff, sewage water, fly ash emissions from coal-fired thermal power plants, effluents from oil refineries, and inadequately managed mining operations dealing with phosphates and metal ores (Staicu et al., 2017). Wastewater containing selenite and selenate can be efficiently treated in activated sludge systems, where SeNPs are formed and subsequently captured within the biomass, leading to the removal of total selenium from the wastewater. A significant portion, over 94 %, of the captured selenium within activated sludge flocs exists in the elemental selenium form, comprising both amorphous/monoclinic selenium nanospheres and trigonal selenium nanorods (Jain et al., 2015). The biological conversion of soluble selenate into insoluble elemental selenium provides a means to extract and recover selenium from aqueous streams. The cost-effective efficiency of biological selenium recovery depends on specific properties of SeNPs, including their size, density, stability, hydrophilic nature, and affinity for binding to biomass (Hageman et al., 2017).

SeNPs offer a valuable solution for addressing water and soil contamination caused by inorganic pollutants due to their exceptional adsorption capabilities. For instance, SeNPs have demonstrated effective adsorption of Cu, Cd, and Zn, suggesting their potential as a superior option for removing these metals from both water and soil (Jain et al., 2016). Another study reported the adsorption of Cd (II) from aqueous solutions by biogenic SeNPs (Yuan et al., 2016). Currently, the utilization of nanomaterials for removing heavy metals from wastewater is gaining significant attention. There is an increasing demand to develop new technologies that harness nanomaterials for treating water and soil contaminated with heavy metals (Yang et al., 2019). Similarly, the recovery and recycling of metals can also be achieved using biogenic SeNPs owing to their ability to adsorb various metal ions. The use of nanoparticles for environmental remediation has gained significant attention, as demonstrated by (Nejati et al., 2023). SeNPs, in particular, have shown exceptional adsorption capabilities for heavy metals such as Cu, Cd, and Zn, making them a promising solution for water and soil contamination.

5. Potential risks and environmental impacts

SeNPs represent a double-edged sword in nanotechnology applications - while their unique properties enable groundbreaking advances in medicine, agriculture, and environmental remediation, their widespread use necessitates careful consideration of potential ecological consequences. The environmental fate and biological effects of SeNPs differ significantly from bulk selenium due to their nanoscale-specific characteristics, including enhanced reactivity, greater bioavailability, and ability to cross biological barriers. In aquatic systems, SeNPs have demonstrated concerning bioaccumulation potential, with studies showing uptake in fish leading to gill damage, hepatic stress, and reproductive impairment at concentrations as low as 0.1 mg/L (Oancea, 2022). These effects may cascade through food webs, as demonstrated by elevated selenium levels in piscivorous birds consuming contaminated fish, potentially leading to teratogenic effects in developing embryos.

The terrestrial impacts are equally concerning, with soil-dwelling organisms showing varied sensitivity to SeNP exposure. Earthworms

exhibit reduced growth and reproduction at environmentally relevant concentrations (5–10 mg/kg soil), while certain microbial communities experience shifts in diversity and metabolic function that could disrupt critical nutrient cycling processes (Ren et al., 2022). Plant studies reveal species-dependent responses, with some crops accumulating selenium to potentially toxic levels while others show stunted root development and chlorophyll degradation. Of particular concern is the potential for trophic transfer, as demonstrated by studies showing SeNP accumulation in leafy vegetables followed by detection in herbivorous insects and their predators (Li et al., 2021a, 2021b).

At the cellular level, SeNPs induce oxidative stress through multiple pathways, including direct ROS generation, depletion of antioxidant enzymes, and mitochondrial dysfunction. The particles' high surface area facilitates interactions with cellular membranes and organelles, while their dissolution releases selenium ions that interfere with sulfur metabolic pathways (Bano et al., 2022). These mechanisms may explain observed genotoxic effects in multiple model organisms, raising concerns about chronic low-dose exposure scenarios.

Current risk assessment frameworks struggle to address three key challenges: (1) the dynamic transformation of SeNPs in environmental matrices, (2) the lack of standardized testing protocols for nanoscale-specific effects, and (3) the potential for synergistic interactions with other environmental contaminants (Walters et al., 2016). For instance, SeNPs may alter the bioavailability of coexisting heavy metals or interact with natural organic matter to form hybrid structures with unknown toxicity profiles.

Emerging mitigation strategies focus on three fronts: (1) intelligent design of coated or stabilized SeNPs to control dissolution rates and reduce bioavailability, (2) development of advanced wastewater treatment systems specifically targeting nanoparticle removal, and (3) implementation of tiered monitoring programs in areas of heavy SeNP use (Devi et al., 2023). Recent advances in biodegradable SeNP formulations show particular promise, with some chitosan-encapsulated versions demonstrating reduced environmental persistence while maintaining functional efficacy.

6. Regulatory and economic aspects of SeNPs in agricultural settings

The integration of SeNPs into agricultural systems presents a complex interplay of regulatory uncertainties, economic constraints, and scalability challenges that must be systematically addressed to realize their full potential. Globally, regulatory frameworks for nanomaterials in agriculture remain fragmented and underdeveloped, creating significant barriers to commercialization. While the European Union has established preliminary guidelines through EFSA requiring comprehensive safety assessments for nanomaterials in food production, these regulations lack specificity for SeNPs, particularly regarding their long-term environmental fate and trophic transfer potential (Rauscher et al., 2017). In the United States, the absence of clear EPA or FDA guidelines for agricultural nanotechnology has resulted in a regulatory vacuum, leaving manufacturers and farmers uncertain about approval pathways and liability concerns (Wiesner et al., 2006). This regulatory ambiguity is compounded by the unique properties of SeNPs—their small size, high reactivity, and potential for bioaccumulation—which complicate traditional risk assessment models and necessitate the development of nano-specific testing protocols. Economically, the production costs of SeNPs remain a critical bottleneck, with current synthesis methods being 3–5 times more expensive than conventional selenium fertilizers due to the need for precise control over particle size, shape, and surface functionalization (Gudkov et al., 2020). Although biological synthesis using plant extracts or microorganisms offers a promising route to reduce costs and improve sustainability (Meenambigai et al., 2022), scaling these methods to industrial levels while maintaining product consistency remains a significant challenge, as batch-to-batch variability can compromise nanoparticle efficacy and safety. The economic

viability of SeNPs is further influenced by regional factors: in selenium-deficient regions like parts of Sub-Saharan Africa and North-east China, SeNP-based biofortification programs may justify higher costs due to their potential to address critical public health needs (Mahra et al., 2025), whereas in regions with selenium-sufficient soils, the adoption of SeNPs will likely be limited to high-value crops where their demonstrated benefits—such as 15–25 % yield increases and 30–40 % reductions in pesticide use (Ahmed, 2022)—can offset production expenses. However, achieving widespread adoption will require not only technological advancements, such as continuous-flow synthesis systems that could reduce production costs by ~40 %, but also policy interventions like subsidies for sustainable nanotechnology adoption or tax incentives for farmers transitioning from conventional inputs. Additionally, the development of precision delivery systems to optimize dosage and minimize environmental release will be crucial for both economic and ecological sustainability. Current economic models suggest that SeNPs are unlikely to achieve broad-scale use in staple crops until production costs decrease by at least 50 %, highlighting the need for both public and private sector investment in R&D (Qin et al., 2025). Ultimately, the successful integration of SeNPs into agriculture will depend on a coordinated effort among regulators, researchers, and industry stakeholders to address these multifaceted challenges while maximizing the technology's potential to enhance food security and sustainability.

7. Future research directions, emerging challenges, and opportunities for SeNPs in agriculture

While SeNPs show immense promise for sustainable agriculture, significant challenges in characterization, environmental risk assessment, and practical implementation must be addressed to enable their responsible adoption. A critical limitation lies in characterization techniques—current methods struggle to accurately quantify SeNP transformations in complex biological matrices, distinguish between ionic and nanoparticulate selenium, and track long-term environmental fate due to their dynamic surface chemistry and aggregation tendencies (Cheng et al., 2022). These analytical challenges complicate both safety evaluations and quality control during scaled-up production, where maintaining consistent particle properties (size, shape, surface charge) remains problematic (Gudkov et al., 2020). Future research should prioritize developing advanced characterization platforms combining synchrotron-based X-ray spectroscopy, single-particle ICP-MS, and machine learning-assisted imaging to overcome these limitations. Simultaneously, comprehensive environmental risk assessments must evaluate SeNP bioaccumulation potential across trophic levels and their impacts on soil microbiome functionality using standardized ecotoxicological protocols (El-Saadony et al., 2021b; Jain et al., 2015). On the technological front, emerging opportunities include stimuli-responsive delivery systems (e.g., pH- or enzyme-triggered SeNP release) and CRISPR-engineered crops with enhanced selenium metabolism, which could dramatically improve efficiency while reducing environmental dispersion (Liu et al., 2022b). Regulatory harmonization represents another urgent need, requiring collaborative efforts to establish nano-specific safety guidelines that balance innovation with precautionary principles. For practical implementation, cost-reduction strategies like continuous-flow bioreactor synthesis and farmer-centric demonstration trials will be essential, particularly for smallholder adoption (Khan et al., 2017). The most promising yet underexplored research frontiers include SeNP applications in climate resilience—such as drought-stress mitigation and heavy metal phytoremediation—and their integration with precision agriculture technologies for targeted delivery. Addressing these multidimensional challenges through interdisciplinary collaboration will be crucial to unlock SeNPs' full potential while ensuring environmental and food safety.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to improve the readability and language. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Data availability

Data will be made available on request.

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