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A review on green synthesis and recent applications of red nano Selenium

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A R T I C L E I N F O

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A B S T R A C T

As an essential micronutrient, the selenium nanoparticles (SeNPs) are gaining much importance in therapeutics, agriculture, nutrient supplements, and antimicrobial agents. Different techniques can synthesize these SeNPs. Nowadays, biological synthesis is favored because it is environmentally safe, least toxic, more stable, and requires less energy. The whole microbial cells or their extracts with added selenium oxyanions or selenium dioxides as the precursor synthesize SeNPs after incubation. The elemental selenium (Se^0) is significantly less toxic than its oxyanions, selenite, and selenate. Selenomethionine and selenocysteine are the primary organic forms of Se, involved in many proteins. Therefore, transforming these toxic materials into a biologically safe atomic structure becomes essential. Many microorganisms convert the environmentally available toxic selenium oxyanions such as selenate or selenite into less toxic elemental Se nanoparticles (Se^0). These microbes work as bio-nano-factories that utilize the Se anions into their metabolic pathways and detoxify them by forming the nanomaterial as the byproduct. In respiration, they may even use the selenite /selenate as a final electron acceptor. This review focuses on the biological methods for SeNPs synthesis, using various microbes reported and their applications in different fields.

Introduction

Selenium (Se), a metalloid discovered in 1818 by a Swedish Chemist, Jöns Jacob Berzelius, is named from the Greek word used for goddess moon, "Selene". It is a part of the Chalcogen family (Group 16) of the periodic table because of its metalloid properties. Naturally, Se is found immobilized within the rocks and released by environmental activities such as weathering rocks. Selenium undergoes its biogeochemical cycle in which its different oxyanions get transformed into the elemental Se within the anaerobic conditions by the naturally existing microbes and other sources in the environment [1,2]. Its commonly existing inorganic states in nature include selenide (Se^{2-}), selenite (SeO_3^{2-}), and selenate (SeO_4^{2-}), whereas its two major organic forms are selenocysteine (Sel-Cys) and selenometheonine (Sel-Met) that form several selenoproteins. This oxyanion of Se gets converted into an elemental state with a typical red color (Fig. 1) with significantly less toxicity in the reducing environment. The elemental form can be further reduced to selenide [3,4]. The major organic forms of Se, such as selenomethionine and selenocysteine, are found in the plants growing in Se enriched soil, which can transform the excess of Se into the volatile organic form, i.e., methyl selenocysteine is favored by some of the plant-associated microbes [5], and can be beneficial for the living organisms in its specific concentration as it forms amino acids such as selenocysteine in formate dehydrogenase [1] and other Se containing enzymes such as glutathione peroxidase, thioredoxin reductase, iodothyronine deiodinase. It also stimulates the immune responses [6,7], but on the other hand, excessive intake can elevate its accumulation into the tissues and show the toxic effect as its inorganic ions in the tissues can cause oxidative stress. The organic components such as Sel-Cys or Sel-Met may result in improper protein synthesis by getting miss incorporated while translation [8].

There is a very thin margin between Se deficiency and its toxicity. The ideal consumption of Se recommended by WHO in 2009 was found to be 50–55 μg per day in the human diet according to the individual body weight [9]. Whereas one of the investigation made by Zhang &

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Spallholz, 2011 mentioned the lower limit of dietary Se 55 μg and the highest limit of 400 μg per day for adults with crossing beyond 15000 μg leading to severe selenosis conditions as stated by some international bodies.

The research on Se grabbed attention in 1957 when one of the experiments on rats showed that adding Se in Brewer's yeast for feeding them helped prevent the fatal liver disease and muscular damages[7,9]. Selenium servers as a micronutrient in biological systems, and just like other micronutrients, its high concentration might result in a toxic effect [10,11]. Selenium nanoparticles (SeNPs) showed a low cytotoxic impact at one ppm concentration when cultured with human dermal fibroblast cells, human melanoma, and glioblastoma cells between 24 and 72 h [6]. Many microorganisms have been found with the potential to accumulate and transform the toxic forms of Se, such as selenate and selenite, to the less harmful form by the oxidation, reduction, and methylation reactions. This can be done by enhancing the bioavailability and volatilization of Se to continue the geological cycle of Se in the environment [12]. Microorganisms such as bacteria, fungi, archaea, and a few algae can reduce the Se-oxyanions to the elemental SeNPs. The nano Se is more promising because of increased absorptivity, improved bioavailability, and lower toxicity. These properties have increased the demand for SeNPs as nutritional supplements for livestock and human health. With its potential as antioxidants, antiviral, anticancer, antibacterial, anticancer drug carrier, and immune boosters, nano- Se has marked the boundaries of its applications in therapeutics [13]. However, over intake of Se than the dietary requirements can cause physiological damages within the organisms [14]. This review aims to highlight the studies performed on biogenic production of SeNPs to know their recognized significances, and to have a future perspective towards finding out the new methodologies and applications.

Selenium nanoparticles (SeNPs)

Nanotechnology has become a center of attraction for many scientific communities for many decades. The fascinating properties of the nanomaterials, such as their size (having at least one dimension as 100 nm), surface area to charge ratio, reactive surfaces, solubility, and thus increased bioavailability, have made them favorable in many fields such as agriculture, medicine, industry, biocontrol agents therapeutics, anticancer drugs, and targeted drug delivery as well [15]. Any of the three approaches can synthesize the nanomaterials; physical, chemical, or green synthesis (biological methods). The graph in Fig. 3 shows the comparative data of number of publications published in the recent years with respect to the above mentioned three different methods for SeNPs synthesis [16]. The physical and chemical processes require extensive energy-specific instrumentation, are thus highly expensive, and involve harsh toxic chemicals that produce environmentally hazardous compounds. Therefore, the products synthesized by the above methods face several application-based limitations in the pharmaceuticals and other consumables for the life forms. To solve these issues, green nanobiotechnology has been practiced using natural reducing agents to change the redox potentials of metals / metalloids oxyanions and convert them into their nano form. Fig. 2 outlines how the overall green synthesis of SeNPs is carried out. Microbes such as bacteria, fungi, and microalgae in optimal growth conditions with provided precursors utilize their reducing enzymes to transform the inorganic ions into the stable nanomaterial of that specific element [17,18].

SeNPs become drug nano-carriers in medicine as it exhibits high antioxidant and antibacterial activity [19].

Selenium oxyanions reducing microorganisms can often be found in the soil contaminated with an excess of Se or in the agricultural land where Se containing fertilizer has been used. Some of them are capable to reduce SeO_4^{2-} to SeO_3^{2-} and then SeO_3^{2-} to Se^0, whereas many of them can reduce SeO_3^{2-} to Se^0 [20].

These reduction reactions may include oxyanion detoxification or microbial respiration by using selenate/selenite as the final electron acceptor or some enzymatic reactions. It is even said that the thioredoxin reductase, nitrite reductase [21] or other membrane reductases, the thiol group compounds such as Glutathione, Bacilithiol [22], and different respiratory enzymes could be the probable reasons behind SeNPs formation. Studies show that the microbes start synthesizing SeNPs in their exponential phase and reach maximum transformation in the stationary phase [23].

Food supplements including Se in their contents are advantageous to health as they increase the bioavailability and the controlled drug release kinetics in the body, increasing the efficiency of supplements. SeNPs-based food supplements have attracted great interest as a food additive for animals and humans [24]. SeNPs can be produced by physical, chemical, and biological synthesis. This review emphasizes the biosynthesis of SeNPs by various sources and their different applications that have been studied till now.

Sources for SeNPs synthesis:

Plants

The use of plants materials and their extracts is one of the proven methods that are quick, cost-effective, and at the same time environmentally compatible for synthesizing the NPs. The biomolecules and secondary metabolites from the different parts of plants significantly reduce the salts of metals/metalloids into their elemental nanomaterials [25]. Any part of the plant, such as fruits, leaves, stems, or nuts, is crushed. Their extract is being added into varying concentrations of selenium precursor solutions (salts/oxides of Se), optimizing the

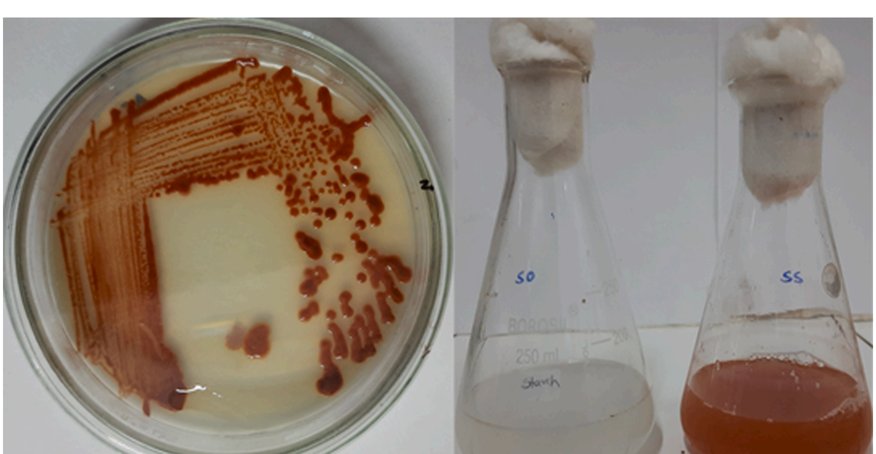

Fig. 1. Bacterial isolate from root nodules showing the transformation of sodium selenite into red nano selenium on sodium selenite containing agar plate (Left side); isolate in liquid medium with (red) and without (colorless) sodium selenite after the incubation of 24–48 hrs. (Right side) *(Images were taken after performing experiments in the Dr. Satish V Patil Laboratory of School of Life sciences, KBCNMU)*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

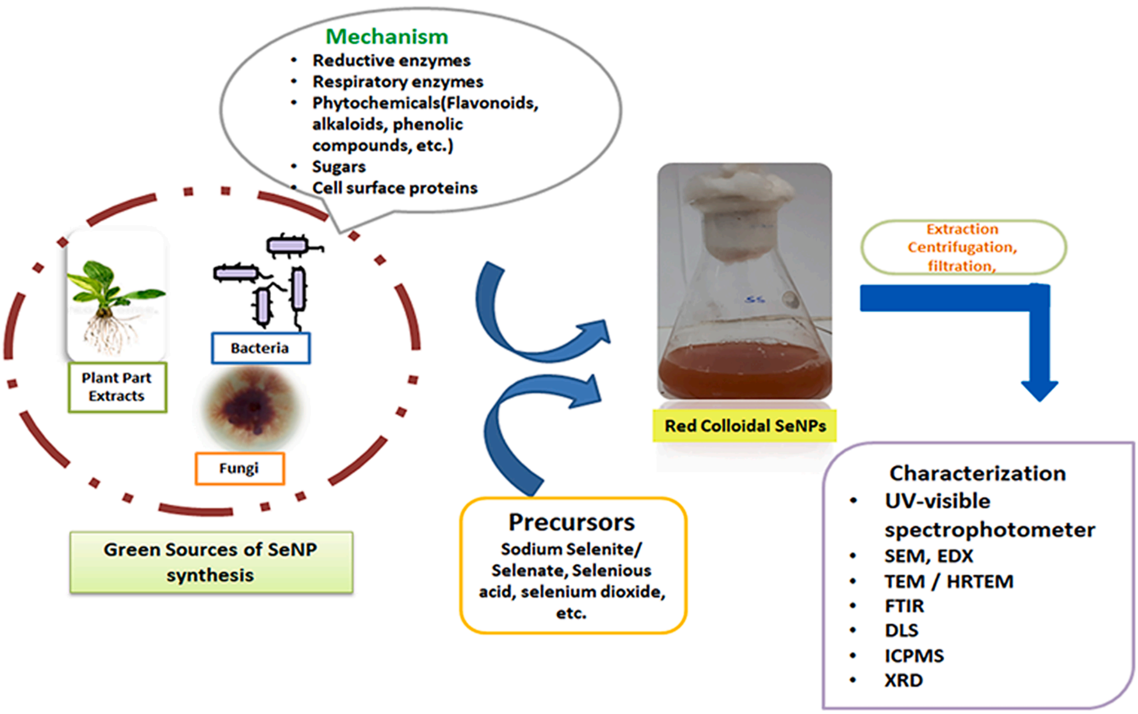

Fig. 2. Representation of overall process involved in biological synthesis of selenium nanoparticles (SeNPs).

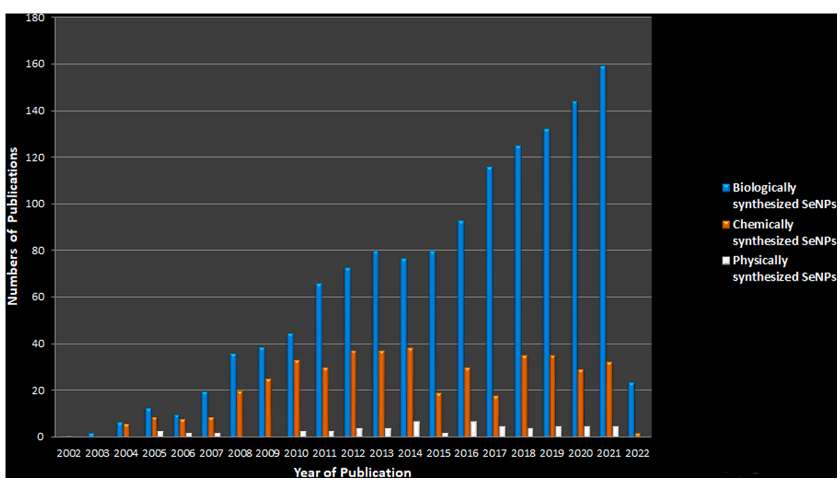

Fig. 3. Number of Publications on synthesis of SeNPs by different approaches since 2002 (https://pubmed.ncbi.nlm.nih.gov/?term=method+for+synthesis+of+selenium+nanoparticles on February 2022).

physical parameters to synthesize SeNPs of expected sizes within some hours. The chemicals present within the plants acts as stabilizing and capping agents, preventing the aggregation of synthesized material [26]. Sharma et al. (2014) used the concentrate of *Vitis vinifera*, which synthesized spherical SeNPs having average size of 3–18 nm and found to be capped by lignin compound present in the fruit. In one of the experiments, *Aloe vera* leaf extract was used with sodium selenite as a precursor to obtaining spherical SeNPs that showed antibacterial activity against *E. coli* and *S. aureus* and antifungal activity against food spoiling fungal species when fabricated in optimal physical parameters. The study found that amides and hydroxyl groups present in the *Aloe* extract were responsible for selenite reduction and stabilization of the NPs [27].

SeNPs constructed using *Emblica officinalis* (amla) extract have proved to be the better antimicrobials and antioxidants with the most negligible toxicity compared to sodium selenite salt. The phenols, flavonoids, and tannins present in the fruit are the reasons behind NPs fabrication [28]. *Withania somnifera* contains active constituents like alkaloids, flavonoids, phenolics, tannins, and terpenoids and behave as a reduction and capping agent for the producing SeNPs by reducing the selenious acid [19]. In one of the studies, SeNPs were synthesized using the waste orange peels. The resulted nanoparticles were of sizes 16 to 95 nm and were potentially able to inhibit the multi-drug resistant strain of *Klebsiella pneumoniae* [29].

Bacteria

Studies show that Microorganisms present within the environment can reduce selenium oxyanions (selenate/selenite) by assimilation and dissimilation reactions. The dissimilatory reduction helps the microbes in anaerobic respiration by conserving their metabolic energies. In aerobic conditions, these oxyanions may also be reduced to Se^0 through detoxification or redox physiological conditions in that organism. The assimilatory reduction involves various enzymatic reactions to reduce the selenite/ selenate resulting in Se^0 and might also take up this Se^0 to form organic selenium compounds [1,20].

Most extensively studied *Thaurea selenatis*, a β-proteobacterium isolated from the water bodies contaminated by Selenium, was reported to transform selenate into nano Se^0. The study mentions that this bacterium respires anaerobically using selenate as its terminal electron acceptor. The reduction of selenite to selenite takes place in the periplasmic space of the cell by the enzyme Selenate reductase (Ser ABC). Electrons required for this process are withdrawn from Cytochrome C_4 then transferred to the QCR complex. After entering the cytoplasm, selenite is reduced to elemental Se^0 nanostructures, probably by the nitrate reductase and arranged by Sef A protein. These SeNPs are transported outside the cell. On the other hand, *Rhodospirillum rubrum* reduced thiols such as Glutathione to transform selenite to SeNps [21,30].

The *Rhizobium* sp. isolated from a fermenter vessel formed SeNps from selenite by using Nitrite as the final e^- acceptor while respiration instead of using selenite. The study mentioned that the reduction was performed by an enzyme having molybdenum which utilized selenite as a substrate [31]. The endophytic selenobacteria, *Acinetobacter* sp. *E6.2* and *Bacillus* sp.*E5*, when supplemented with 5 mM Sodium selenite, produced SeNps of about 213 nm and 169 nm, respectively. The author intended to use these nanoparticles for Se biofortification in plants [32]. The bacterial species such as *Lactobacillus* sp., *Bifidobacterium sp*., and *Klebsiella pneumonia* were used for SeNp synthesis by the fermentation process. The nanostructures were homogeneous and sphere-shaped [33].

In 2018, Wang et al. reported for the first time that *Alcaligenes faecalis Se03* isolates from the gut of *Monochamus alternatus* were capable of transforming both 1 mM and 5 mM concentrations of sodium selenite into red amorphous Se^0, mainly extracellular. Here, they mention the thioredoxin reductase in the cytoplasm of bacteria responsible for reduction by using electrons from NADPH [34].

Other than the Bacteria, Cyanobacteria are also able for SeNps synthesis. In one of the studies, 20 cyanobacteria strains were screened for SeNPs fabrication. Among them, *Arthrospira indica SOSA-4* showed the best results forming orange-red SeNPs size 11.8 nm within two days. These nanoparticles showed the best antioxidant activities [35].

Fungi and yeast

Many Fungi have higher resistance to different metals, which makes them be prioritized while considering the synthesis of any metal or metalloid nanoparticles, mostly extracellular. The secondary metabolites of fungal proteins keep the nanoparticles stable in the liquid medium for a longer time [36].

The first report on mycosynthesis of SeNps was on the fungus *Alternaria alternata*. The inoculum supplemented with sodium selenite yielded nanoparticles of size 30 to 150 nm [37]. The fungal species reported for the extracellular selenium nanoparticles using SeO_2 as a precursor within an hour was *Aspergillus terreus*. Nanoparticles about 47 nm were characterized by UV– visible spectroscopy, DLS, and EDX [38]. The most prevalent plant symbiotic fungus, *Trichoderma sp*., combined with the SeNPs synthesized from their culture filtrate, has efficiently controlled the Downy Mildew conditions in pearl millet crops. The nanoparticles ranged from 49.5 to 312.5 nm and showed size-dependent activities against the zoospores of *Sclerospora graminicola* on chilly and tomato leaves [39]. The solid-state fermentation method using *Monascus purpureus ATCC16436* yielded SeNPs of the spherical particles approximately 46.58 nm. These particles effectively showed antioxidant activity of IC_{50} 85.92 μg mL^{-1} and anticancer activity when studied by MTT assay inhibited proliferation of human liver cancer cell line and breast cancer cell line [40].

Like filamentous fungi, the yeast sp. has also shown their remarkable properties in reducing the Se oxyanions into SeNPs. One yeast *Magnusiomyces ingens LH-F1* could use SeO_2 and synthesized SeNps of size 70–90 nm. The SDS-PAGE performed showed two protein bands identified on the surface of the nanoparticles could be the reason for particle stability. These SeNPs sowed antimicrobial activity against *Arthrobacter sp-W1* [41]. An easy and less time consuming method for SeNPs fabrication was established by using baker's yeast (*Saccharomyces cerevisiae*) extract. These nanoparticles had antimicrobial effects on some of the pathogens transmitted through foods including *E. coli. A. niger, A. fumigatus and S. aureus* [42]. Some more examples are introduced in the Table 1.

Characterization of SeNPs

Whether we use physical, chemical, or biological methods for synthesizing nanomaterials, what becomes more important is their identification and characterization. The combinations of some classical techniques with the upgraded latest technologies can give complete information such as its morphology, elemental compositions, stability, charges, surface chemistry, and adherence of biomolecules if any (in the case of biogenic nanoparticles) our nano-product.

Like gold, silver, copper, or other nanoparticles, selenium nanoparticles (SeNPs) exhibit their characteristic red color due to the Surface Plasmon Resonance (SPR) phenomenon after the reduction reaction. The intensity of this particular color can be recorded by studying the spectrum recorded by the UV–Visible spectrophotometric method. Also, the wavelength showing maximum absorption can be used for a rough estimation of the size of nanoparticles. Particles with smaller sizes tend to absorb lower wavelength light [43,44]. The SeNPs can be scanned in the wide range of wavelength from 200 to 800 nm, and it will then show the characteristic peak somewhere in between this range. Till now, different studies have reported various wavelengths for the maximum absorption peak, such as; SeNPs synthesized from the extract of *Emblica officinalis* fruit at 270 nm [28] extract of *Leucas lavandulifolia* at 293 nm [45], cell-free extract of B. pumilus sp.BAB-3706 at 300 nm [46], fungus *Fusarium oxysporum* at 217 nm [47], whereas the SeNPs formed by *Monascus purpureus* gave absorption maxima at 539 nm [40]. These variations in the absorption wavelengths are because of the SPR phenomena. It provides the primary confirmation of SeNPs formation from the precursor, i.e., sodium selenite, selenium oxide, or sodium selenate.

One of the advantages of biologically synthesized nanoparticles is their stability acquired due to the natural stabilizers, which can be either the metabolites or biomolecules of that particular source. FTIR (Fourier transform infrared spectroscopy) can identify this, which detects the corresponding functional groups with the nanoparticles. The FTIR results of SeNps synthesized from *Penicillium expansum* reveal the functional groups with the peaks like –OH, –NH (3247.5 cm^{-1}), and amide (1630.5 cm^{-1}) [48]. The FTIR results of SeNPs obtained from *B. amyloliquefaciens SRBO4* showed that amine, carboxyl, and aldehyde groups were responsible as the capping agents [49]. Other than FTIR, NMR (Nuclear Magnetic Resonance) can also be preferred to know the surface composition of the nanoparticles [50]. Another technique which is practiced for determining the surface chemistry by knowing the functional groups is X-ray Photoelectron spectroscopy (XPS). This creates an idea of how the nanomaterial is going to interact with other materials based on concept of binding energy of the nanoparticles [51]. The XPS analysis of SeNPs synthesized using Yeast, had a characteristic peak at 55.38 eV which represented the elemental state of Selenium (Se^0). The elements found in SeNPs product were C, O, N and Se3d. It also revealed the functional groups by having the peaks representing the functional group bonds of carbonyl, amide and aminoacid chains [52]. The starch stabilized selenium nanoparticles also had the XPS peaks at 55.6 and 56.6 eV which are specific for elemental selenium in its Se3d states [53]. Similar results were obtained in SeNPs stabilized using polysaccharide from *Polygonatu sibiricum*. The peak shifting at binding energies 55.4 and 56.2 eV indicated the formation of SeNPs and the

Table 1
Biological Sources for Synthesis if Nano Selenium.

Sr. No.	Name of the organism	Precursor used	Size (nm)	Applications	References
	Plants				
1.	*Aloe vera*	Sodium Selenite	50	Antibacterial, Antifungal	[27]
2.	*Leucas lavandulifolia*	Selenious acid	56–75	Antibacterial	[45]
3.	*Moringa oleifera*	Sodium selenite	18.85	_	[26]
4.	*Psidium guajava*	Sodium selenite	8–20	Bacteriacidal/ antibacterial	[105]
5.	*Vitis vinifera*	Selenious acid	3–18	_	[106]
6.	*Zingiber officinale*	Sodium selenite	100–150	Antibacterial, Antioxidant	[23]
7.	*Diospyros montana*	Selenious acid	4–16	Antibacterial, Antifungal, Anticancer: human breast- cancer cells	[60]
	Bacteria				
8.	*Desulfovibrio desulfuricans*	Sodium selenite	_	_	[107]
9.	*Lactobacillus acidophilus*	Sodium selenite	50–500	_	[33]
10	*L. casei*	Sodium selenite	50–500	_	[33]
11.	*Bifidiobacterium* sp.	Sodium selenite	400–500	_	[33]
12.	*Klebsiella pneumoniae*	Sodium selenite	100–500	_	[33]
13.	*Bacillus pumilus* sp. *BAB-3706*	Sodium selenite	10	Fabrication of H_2O_2 biosensors	[46]
14.	*Lactobacillus caseiATCC 393*	Sodium selenite	50–80	Anticancer Antibacterial	[108]
15.	*acillus* sp. *JASPK2*	Sodium selenite	21.9	Antimicrobial	[109]
16.	*Pseudomonas aeruginosa*	Sodium selenite	21	Molecular markers and biosensor for nanotoxicity	[58]
17.	*Agrobacterium* sp.	Sodium selenite	200–300	Anti dermatophyte	[110]
18.	*Azoarcus* sp.	Sodium selenite	123	Agriculture	[111]
19.	*Rhodopesudomonas palustris*	Sodium selenite	8–200	_	[112]
20.	*Streptomyces* sp. *(M10A65)*	Sodium selenite	20–150	Antibacterial, Larvicidal, and Antihelmenthic	[113]
21.	*Pantoea agglomerans*	Sodium selenite	<100	Antioxidant	[114]
22.	*Bacillus mycoide SE1TE01*	Sodium selenite	50–400	_	[115]
23.	*Azospirillum thiophilum (VKMB-2513)*	Sodium selenite	160–250	_	[116]
24.	*Azospirillum brasilense*	Sodium selenite	25–80	_	[117]
25.	*Stenotrophomonas maltophilia SeITE02*	Sodium selenite	160–250	_	[118]
	Fungi				
26.	*Aspergillus terreus*	Selenium dioxide	47	_	[38]
27.	*Lentinulaedodes*	Sodium selenite	180–190	_	[119]
28.	*Alternaria alternata*	Sodium Selenite	30–150	_	[37]
29.	*Gliocladium roseum*	Sodium Selenite	20–800	_	[67]
30.	*Monascus purpureus ATCC16436*	Sodium Selenite	46.58	Antioxidant Anticancer	[40]
	Yeast				
31.	*Magnusiomyces ingens LHF1*	Selenium dioxide	70–90	Antibacterial against	[67]
32.	*Saccharomyces cerevisiae*	Sodium Selenite	30–100	Antimicrobial against pathogens of nosocomial infections	[76]

(*continued on next page*)

Table 1 (*continued*)

Sr. No.	Name of the organism	Precursor used	Size (nm)	Applications	References
	Cyanobacteria				
33.	*Synechococcus leopoliensis*	Sodium selenite	220	_	[120]
34.	*Anabaena* Sp. *PCC7120*	Sodium Selenite	5–50	Antibacterial, antioxidant, antiproliferative against HeLa cell line, decolorization of methylene blue	[121]
35.	*Spirulina platensis (abdf2224)*	Sodium selenite	145 ± 6 and 171 ± 13 nm	Antioxidant	[122]
36.	**Archaebacteria** *Halococcus sulfodinae*	Sodium selenite	Rod shape 28 nm domain size	Inhibited proliferation in HeLa cell lines	[67]

polysaccharide complex [54]. Electron Microscopy, such as SEM (Scanning electron microscopy) and TEM (Transmission electron microscopy), are the most powerful techniques to illuminate the morphology of nanoparticles. SEM provides the surface features, and along with EDX mapping, it reveals the elemental composition in the sample [55]. Some other techniques, such as ICP- MS (Inductively coupled plasma mass spectrometry), also detect the constituent elements in the sample and give the concentration of the nano product formed. It is so sensitive that trace elements in the solution also get detected in the solution [43,50]. Besides showing the core diameter of the nanoparticles, TEM analysis also provides the dispersion with pattern and size distribution which is essential for knowing the functional significance of the nanoparticles. For example, the Nano- Se synthesized by the wet chemical method were finely dispersed, and their size ranged from 30 to 80 nm in the TEM images [56]. More advanced techniques such as HRTEM (High-resolution transmission electron microscopy), STEM (scanning transmission electron microscope), FIB-SEM (Focused Ion Beam Scanning Electron Microscopy) for imaging and studying the morphology of the nanoparticles are being practiced nowadays [43]. Like TEM, another technique named DLS (Dynamic Light Scattering) detects the size of nanoparticles based on the hydrodynamic diameter and finds the polydispersity index, which deals with the aggregates formed, if any [57]. Sometimes the size range of nanoparticles obtained by DLS may differ from the one obtained by TEM, which interprets that the particles might be aggregating in the colloidal suspension of DLS [58]. Zeta-potential measurement calculates the nanoparticles' overall charges, which results in their stability. More negative charges or more positive charge creates repulsion between the particles and lessens the chances of clumping of the particles. This can be well studied by combining DLS with Zeta potential analysis [50,59]. In one of the studies, the components of microbial extracellular polymer are shown to impart a negative charge on the selenium nanoparticles, which act as the capping agents and tend to provide some stability to the SeNPs [11]. X-ray diffraction elucidates the crystalline or amorphous nature and estimates the lattice structures of the nanoparticles. Its diffraction patterns also detect the SeNPs product's purity [44,60]. In combination with more advancement, all these analysis techniques can give complete knowledge about the Nanoparticles, which can enhance the information regarding their applications and risks.

Selenium nanoparticles in different domains

With its increasing research in nanotechnology, Selenium nanoparticles are rooting towards many different fields which are explained below and Fig. 4 focuses some of the major applications in several fields.

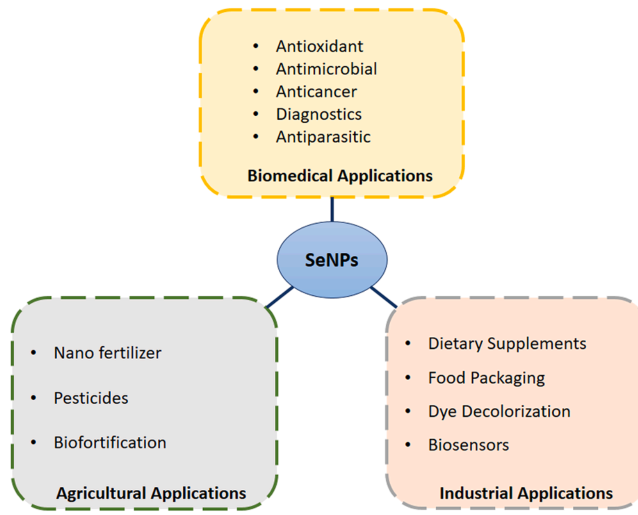

Fig. 4. Applications of Selenium Nanoparticles.

Biomedical applications

Antioxidant

Due to the low toxicity and biocompatibility, SeNPs have been known for their antioxidant activities for a long time. Various biological enzymes such as Glutathione peroxidase, Superoxide dismutase, thioredoxin reductase, and iodothyronine deiodinases function in dealing with the stress induced by oxidation and prevents cellular damages. By being a part of the commonly known seleno-proteins such as Glutathione peroxidase and thioredoxin reductase, the SeNps work efficiently in free radicles scavenging formed due to some oxidative stresses. The Glutathione peroxidase works in detoxification of various peroxides formed, while the thioredoxin reductase results in developing a redox system to follow detoxification [61]. The selenium nanoparticles perform radical scavenging based on their sizes; the smaller the size more compelling is the antioxidant. This property of nano-selenium makes it available for many medical applications, e.g., enhancing hair growth in fetus, recovering the reproductive issues caused by oxidative stress, and many others [13,62]. In one of the livestock reports, it was seen that the supplementation of Nano Se to the sheep and crucian carp fish has led to reducing lipid peroxidation and enhanced glutathione peroxidase activity, respectively [3]. The bimetallic nanomaterials, SeNPs conjugated with AgNPs (silver nanoparticles), further coated with quercetin and gallic acid, behaved efficiently as antioxidants with 59 to 62 % of the total activity [59]. Because of this well-known antioxidant property, the SeNPs have been potentially recommended to treat neurodegenerative diseases such as Huntington's disease, Parkinson's disease, and Alzheimer's disease. The study done on transgenic Huntington's disease model of *Caenorhabditis elegans (C. elegans)* states that when the worm was supplemented with lower doses of SeNps (less than 2 μM), it protected it from the oxidative damages, helped to balance the neurological malfunctioning and the behavioral function. It ultimately also reduced the accumulation of Huntington's protein in *C. elegans* [63].

Anticancer and drug targeting

Along with being recognized for their antioxidant property, selenium nanoparticles also can work as pro-oxidants depending on their concentration and size. This opens the door for SeNPs and their supplements in cancer therapies. They can be targeted with some drugs or alone on the specific malignant tissues. After being internalized, the pH alterations may result in free radicle generation within the cell, ultimately destroying the cell by affecting its mitochondria and endoplasmic reticulum. The overall reaction could also trigger several apoptotic pathways for districting the infected cell [61]. SeNPs have been reported to exhibit antitumor effects against glioma, lung cancer, and breast cancer. It has potent cytotoxicity against tumor cells with the least disturbance to the normal cells. This arrests cell cycle arrest and apoptosis enhancement [64]. The anti-proliferative activity of SeNPs worked well against A549 lung carcinoma cells and showed 50 % inhibition concentration (IC_{50}) as 0.25 µg/ml, which remarks the potential of SeNPs in their anticancer property [19].

In one of the studies, intracellular SeNps from *Bacillus lichiniformis* extracted using lysozymes effectively induced necrosis within the human prostate adenocarcinoma cells [65] The In vitro studies using SeNPs concentration of 2 µg/ml showed necroptosis in LNCaP-FGL cells. The oral supplementation of SeNps in C3H/HeJ mice had very low toxicity than that of L-Slelenometheonine. The Tumor necrosis factors (TNF) and Interferons (INF) were overexpressed on the application of SeNPs together with a decrease in expression of prostate-specific antigen [66].

Rod-shaped SeNps of dimensions 129 nm length and 100 nm diameter produced by the Halophilic organism, *Halococcus salifodinae BK18*, based on their dosage educed the cell proliferation in the HeLa cell line without affecting the HaCaT (normal) cells [67]. The surface decorated SeNps with polysaccharide and protein complex of edible mushroom worked against the MCF-7 human breast cancer cells by inducing apoptosis after 24hrs and inducing the oxidative stress in mitochondria [68].

The detailed studies of these applications would provide advancements in the implications of SeNPs for treating various types of cancer. The major concern in treating cancer with heavy drugs is their toxic effects on non-cancerous cells surrounding the tumor cells. Modern science focuses on the very well-known concept of targeted drug delivery, which protects the healthy cells to solve this issue. With the help of nanobiotechnology, the SeNPs can perform much better in targeting drugs to kill cancer tumors. Surface-coated selenium nanoparticles with 5-fluorouracil potentially destructed A375 human melanoma cells by inducing apoptosis which was the effect of caspase-9 activation with the IC50 in the range from 6.2 to 14.4 µM and without harming the healthy cells [69]. Other than 5-Fluorouracil, the use of Irinotecan with SeNps for ileocecal adenocarcinoma, GE11 conjugated with SeNPs to increase the dispersion and potency of Oridonin, SeNps modification with galactose for doxorubicin efficiently targeting the tumor cell and Anisomycin with SeNps to arrest the hepato-carcinogenic cells have also proved drug-carrying potential of SeNPs [15,70].

Anti-diabetic

Like cancer, diabetes, mainly defined as hyperglycemia, is another most prevailing concern globally. According to the latest data provided by IDF, i.e., International Diabetes Federation, 537 million adult populations are suffering from this disorder, which might hike to 643 million by 2030. In the year 2021, it was found that about 6.7 million of the human population died because of diabetes (*Facts and Figures. Retrieved from http://www.idf.org/aboutdiabetes/what-is-diabetes/facts-figures.html*)[71]. The scientific testimonies suggest that oxidative stress causing the destruction of pancreatic cells producing insulin is majorly responsible for diabetes. The same oxidation problem may also cause nephrological issues and kidney failure [72]. SeNPs are well known for their antioxidant effect and the crucial part of many reducing enzymes; their participation as the anti-diabetic component is much agreeable. SeNps can be used to reduce the complications in type −2-diabetes mellitus, such as beta cells dysfunction and insulin resistance. The effective mechanism is achieved by increasing the size of a protein named BAY 55–9837 by connecting the SeNPs. This prevents the protein from being flushed out of the renal passage. The SeNPs also keep away the β-cells of the pancreas from undergoing apoptosis and stimulate their insulin production [15,73]. The insulin concentration spiked in the diabetic rats induced by streptozotocin after treating them with Selenium nanoparticles. It was concluded that the SeNPs mimicked insulin in lowering hyperglycemia. Along with this, a decrease in cholesterol, fats, and LDL within the SeNPs provided animals were some more influential activities [74]. When SeNPs transported in liposomes were given to diabetic mice, it favored anti-diabetic mechanisms by maintaining Islets cell integrity and elevating the level of antioxidant enzymes such as Glutathione peroxidase and dismutase simultaneously rise in insulin and decrease in Glucose content of the cells [73].

Type 2 diabetes mellitus has adversely affected male reproductive functions. In one of the studies, rats were induced with heavy fat edibles and some amount of streptozotocin for making them diabetic. Further, they were given metformin dosage, SeNPs stabilized by Chitosan, and their combinations in different sets. The results mention that a combination of metformin and SeNPs decreases reproductive dysfunction and appropriately regulates the genes responsible for mitochondrial responses and steroidogenesis in the stimulated rats. This provides a better approach towards enhancing the therapeutics for diabetes and its complications [75].

Antimicrobial and antiviral

Nanomaterials are the best-proven alternatives against microbial pathogens rather than expensive antibiotics. Selenium nanoparticles have shown their antimicrobial and antibiofilm effects against many gram-positive and gram-negative bacteria like *Staphylococcus aureus, E. coli, Pseudomonas* sp., *Bacillus sp*., and many other species. The nano selenium particle can inhibit the attachment of the bacteria (Biofilm formation). SeNPs are coated on medical devices such as catheters, lenses, and heart valves to inhibit biofilms without using antibiotics. The selenium nanomaterials of size 30 to 100 nm by the yeast *Saccharomyces cerevisiae* are reported for their antimicrobial activities against the nosocomial pathogens. They exhibited higher zones of inhibition against *E. coli, S. typhiimurium, P. aeruginosa, and S. aureus* with the decreased size of the nanoparticles [76]. The SeNps from Bacillus *sp.* Msh-1 showed a potential reduction in biofilm formation by the pathogenic strains of *S. aureus, P. mirabilis*, and *P. aeruginosa* [77]. The extracellular SeNps acquired from *Streptomyces minutiscleroticus* M10A62 worked as good antibiofilm and antioxidant agents by reducing the 75 % of the growth of test organisms; *S. aureus* with being least toxic to the normal cells than that of the Selenium dioxide [78]. The formation of reduced oxygen species was formed while studying the antibacterial effect of SeNps created by *Providencia* sp. *DCX*.

The spherical nanoparticles of size 120 nm worked well against *S. aureus, B. cereus, B. subtilis, E. coli,* and *Vibrio parahemolyticus* within 12 h of incubation. This was also confirmed by the leakage test of proteins and polysaccharides on the test organisms [79]. The SeNPs function as antimicrobials by inducing ROS generations, rupturing the cells, inhibiting the metabolic pathways, or causing nucleic acid damages and producing malfunctioning proteins [80]. Most conventional antimicrobial agents remain ineffective against biofilm-forming microbes due to their restive mechanisms. Such resistance can be tackled by using alternative methods such as nanoparticles. SeNPs designed using *Bacillus licheniformis* substantially decreased foodborne pathogenic bacteria, including the *Salmonella* species, *Staphylococcus aureus*, *E. coli, B. cereus*, and *Enterococcus faecalis* as their biofilm production in the concentrations of 25 mg/mL and 20 mg/mL, respectively. This effective mechanism gives a direction for using biosynthesized SeNPs in food industries for packaging [81].

Selenium nanoparticles also have been observed to exhibit promising antiviral functions. SeNP conjugated with Oseltamvir improved the drug's action against the H1N1 human influenza virus by destroying its Hemagglutinin and neuraminidase on the virus surface. It also targeted ROS formation and activated p53 phosphorylation within the targeted cell [82]. Another study by the same author with SeNps and Amantadine complex also controlled the infection of influenza virus with fewer drug requirements and solved the problem of drug resistance [83]. The SeNPs from *Actinobcter* sp. at the concentration of 700 ppm maximally inhibited the type-1 Dengue virus [78].

Agricultural applications

The use of Nano fertilizers in recent years has extensively grown to increase crop yields. This limits hazardous chemicals and can work efficiently in very low amounts. SeNPs as fertilizers for plants seeks more attention as Se is one of the essential micronutrients. Unlike animals or microbes, Se is not essential for plants. Instead, the uptake of Selenium in plants may help their growth indirectly by reducing the oxidative stress [84]; resisting the drastic effects due to environmental changes [9], or protection against plant pathogens or pests [8] Plants tend to accumulate Se from the soil, which can be correlated with the bio-fortification of Se in those plants to fulfill the dietary requirement of the Se in humans and animals [5]. We know that excess salinity of the soil adversely affects the photosynthetic rate in crops, ultimately cutting down the produce. The same salt stress in *Fragaria ananassa* (strawberry) was diminished by spraying the solution of SeNps on its leaves. It surged photosynthesis and the antioxidant enzymes, the nutritional quality of the fruit, and the plant hormone levels together [85]. When concerned about the plant pathogens, especially the fungal attack, the study was done using SeNps synthesized from *Trichoderma harzianum JF309 (TSNP)* can be highlighted. These SeNPs showed control over the activities of mycotoxins of *Alternaria* species without showing any harmful effect on human cells. This could be more focused in the future for using the SeNps as biocontrol agents [86]. SeNps in concentrations of 265 to 532 μM has been seen to enhance the callus formation and rooting in tobacco tissues culturing without harming its chlorophyll content and not causing toxicity [36]. In contrast to chemically synthesized SeNPs, biosynthesized SeNPs using *Lactobacillus acidophilus ML14* were responsible for effectively reducing the crown and root rot disease caused by *Fusarium* species in wheat crops [87]. It has been reported that when the SeNPs were provided to the crops such as *Cyamopsis tetragonoloba* (cluster beans) and *Vigna Mungo* (black gram) in a pot assay, their chlorophylls and various amino acid content were increased [88,89].

Selenium nanoparticles have a multifold effect on agricultural produce, where they can increase crop productivity by inhibiting nematodes and several plant pathogens (especially fungi) depending on the dosage. Application of SeNPs to the plants have also shown an increase in their photosynthesis, reducing oxidative stress, increasing drought tolerance [90], affecting seed germination [91] as well as increasing Se content in crops help to fulfill the dietary intake of Se and reduce its deficiency [80].

Anti-parasitic

Since last few years, the therapeutic applications of SeNPs have increased their exploration for treating parasitic infection. A protozoan parasite, *Toxoplasma gonidii* is known to cause toxoplasmosis which show severe clinical issues including brain damage, weakened immune system and can also affect the embryo if transferred through placenta in pregnant woman. There is no vaccine available against this parasite and the treatment includes sulfadiazine and pyrimethamine which show disadvantageous complicacies in patients. When the effect of SeNPs synthesized by using *Bacillus sp*. MSh-1, was checked on toxoplasmosis infected mouse it gave the conclusions that the number of cysts in the tissues significantly decreased with increase in the expression of cytokines producing genes [92]. *Giardia deudenalis* causes intestinal problems, loss of weight, jaundice and vomiting like conditions after infection. Drugs such as Furazolidone, Metronidazole, and quinacrine are prescribed for its treatment. These drugs have shown some carcinogenic effects when studied in a laboratory. To solve this problem SeNPs were used to check their cytotoxic effect on *Giardia* cysts and they show same results with 0.3 mg/ml concentration, to that of metronidazole but with no adverse effects [93]. The treatment of Cystic echinococcosis or hydatid cyst which is caused because of the tapeworm known as *Echinococcus granulosus* includes heavy chemotherapeutic drugs suggested with surgery to remove the cysts. Bacterial SeNPs from *Bacillus sp*. MSh-1 in concentrations of 50 to 500 μg/ml and sizes 80–220 nm were treated on the protoscoleces for about 10 to 60 min exposure time. These SeNPs proved best at concentration of 500 μg/ml for 10 min and 250 μg/ml for 20 min and the viability of the cyst was checked by using eosin stain [94]. Leishmaniasis is another parasitic disease which is spread by the bite of sand fly. The biosynthesized SeNPs by *Bacillus* sp. MSh-1 gave the IC_{50} of 4.4 ± 0.6 μg ml^{-1} against the amastigote stage and 1.62 ± 0.6 μg ml^{-1} for promastigote stage of *Leishmania major* (MRHO/IR/75/ER), in addition to DNA destruction [95].

Miscellaneous

The use of Selenium nanoparticles has been expanding to all extents in recent years. Lateral flow immunoassays (LFIA) are used for rapid diagnosis in different applications. SeNPs synthesized by Ascorbic acid, SDS, and polyethylene glycol (PEG) for stabilization has been used to design an LFIA to detect clenbuterol has been developed [96]. The latest research on the global pandemic of SARS-CoV-2 created the essential production of test kits. The advancement in designing a rapid antigen test kit is based on identifying the IgG and IgM antibodies in the patient and using the stable Selenium nanoparticles as a probe to label the SARS-CoV-2 nucleoprotein on the detection kit strip [97]. SeNPs as a qualitative probe for lateral flow Immunoassays demonstrated more stability and sensitivity. The extract of *B. pumilus sp*. BAB-3706 is used to synthesize Selenium nanoparticles; these SeNPs are applied to fabricate H_2O_2 biosensors with 3 μM as the sensing limit [46]. The contamination of inflammatory drugs such as Diclofenac is a major concern for aquatic life and human health. This type of drug was found to be efficiently degraded (94.43 %) by SeNPs treatment combined with UV radiations and Hydrogen peroxide [98].

Dye decolorization of Basic Fuchsin from aqueous solution was achieved 100 % in light using 10 mg SeNPs synthesized from ascorbic acid [99]. The antimicrobial and antioxidant properties of SeNPs have opened up their way for being used in food packaging materials. The addition of SeNPs in limited concentrations into the polymers used for packaging can prevent the invasion of food spoiling microbes such as *S. aureus, E.coli*, etc. Also, these SeNPs have been used to design packing films for several fruits and dried fruits [80,100].

Toxicity of SeNPs:

It is clearly understood that Se is one of the vital elements in a whole biological system. According to the data, its essentiality is found beyond the concentration of 30 μg per day, and toxicity may induce if it exceeds 900 μg per day [7]. Also, the nature of Se species defines their toxicity. As mentioned earlier, its inorganic forms are more harmful than the elemental ones. Things become a bit complex when it comes to the nano-form (SeNPs). The method of synthesizing these nanoparticles also affects the safety levels of using them in several applications. The biosynthesized SeNPs from Bacillus sp. MSh-1 showed low toxicity in rats compared to the chemically synthesized SeNPs and Selenium dioxide [101]. The photochemical method of preparing SeNPs increases the cytotoxic effects even more than the organic or inorganic selenium compounds.

Like other Nanoparticles, SeNPs also have their toxicity and importance depending on the size and concentrations. Toxicity testing of

SeNPs on the male Sprague – Dawley rats for complete 14 days in different doses was performed, which clearly showed no side effects in the group provided with low SeNPs doses (0.2 to 0.4 mg/kg bw) but affected the higher dosage group (more than 2 mg/ kg bw) [102]. At the same time, both inorganic Selenium and Nano Se in higher concentrations induced physiological and metabolically abnormal conditions in the fish *Pangasius hypothalamus* [14]. SeNPs, when given in the concentration of 2 mg/ kg body weight to the mice for about 28 days, had positive effects like increased enzyme levels, decreased DNA damage, and less cell death in the bone marrow as compared to the organic or inorganic selenium components [103]. No severe toxic effects were observed in SeNPs given rats for checking the sub-lethal doses. There were slight changes in the glucose level and lower superoxide dismutase in the liver. Some dose-dependent lesions were seen in the liver and intestine. The report concluded that SeNPs could be given continuously but for a short period. For long-term cases, more pharmaceutical-based kinetics must be studied [104]. All these research revealed that SeNPs administration is completely dependent on its size and concentration simultaneously. Also, biologically synthesized SeNPs prove better in the application than chemically synthesized and the other forms of Selenium.

Conclusions

Compared to other nanomaterial synthesis methods, The Green or Biological synthesis has tremendously grown in recent times. Like the silver, gold, copper, zinc, and platinum nanoparticles, the use of selenium nanoparticles is also taking its way in Nanotechnology. Moreover, the microbial SeNps production helps explore different microbial strains to transform environmentally toxic selenite, selenate, or selenide into the desired shapes and sizes to enhance their activities. The physical parameters for the growth of these microorganisms can be manipulated to obtain effective nanoparticles. SeNps have proved to be better in all means for their antioxidant, antimicrobial, antiviral, and crop protection activities. SeNps are better biosensors and also show their remarkable therapeutically essential properties. They are the best antioxidants and pro-oxidants, which make them available as anticancer agents. Selenium has a very narrow window between its toxicity and its requirement. A thorough study needs to be conducted on the specific application used commercially. Also, the enhanced studies on molecular level could be more revealing in microbial synthesis of the SeNPs in controlled manner which will intensify their mode of application in future.

CRediT authorship contribution statement

Pradnya B. Nikam: Conceptualization, Writing - original draft. **Jitendra D. Salunkhe:** Writing - review & editing. **Tatiana Minkina:** Writing - review & editing. **Vishnu D. Rajput:** Writing - review & editing, Validation. **Beom Soo Kim:** Writing - review & editing. **Satish V. Patil:** Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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