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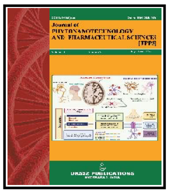

Review Article : Open Access

Green biosynthesis and characterization of silver nanoparticles using wheatgrass extract and its potential applications: A review

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Abstract

The biosynthesized or natural or green synthesis-derived drugs are in use for human existence in the management of any disease conditions. Nanotechnology refers to the fabrication, characterization, manipulation, and application of structures by controlling their shape and size at the nanoscale. The advancement of reliable and eco-friendly techniques for nanoparticle synthesis was a crucial development in the field of green nanotechnology. Traditional methods for synthesizing nanoparticles (NPs) are often costly, hazardous, and environmentally unfriendly. In response to these challenges, the use of natural sources such as bacteria, fungi, biopolymers, and plants, which act as capping and reducing agents, has emerged as a promising approach for synthesizing silver nanoparticles (AgNPs) using wheatgrass extract. The surface morphology and applications of AgNPs are significantly influenced by the experimental conditions under which they are synthesized. Existing scattered information on AgNP synthesis highlights the impact of various parameters and characterization techniques, spectroscopic techniques such as FT-IR, UV-Visible, other techniques such as DLS, sophisticated SEM and TEM studies, XRD and EDX on their potential properties and applications. This review emphasizes the recent use of natural sources for AgNP synthesis. The green synthesis of AgNPs using wheatgrass extract has proven effective in a wide range of applications, including anticandidal activity, antioxidant, antibacterial and antiangiogenic activities, biosensors, magnetic resonance imaging, cancer treatment, surface-enhanced raman spectroscopy (SERS), antimicrobial agents, drug delivery, gene therapy, DNA analysis, and more.

1. Introduction

Environmental engineering plays a crucial role in improving the quality of human life, and nanotechnology offers numerous applications within this field. In recent decades, eco-friendly, green strategies have gained significant attention for synthesizing various nanostructures. Methods such as the green synthesis of metal nanostructures, magnetic nanoparticles, nanocomposites, and metal oxide nanoparticles have emerged. Traditional nanomaterial synthesis methods face challenges due to the use of chemical agents as capping agents, surfactants, and reducing agents, which often introduce harmful contaminants into the environment and result in impurities in the final products. In contrast, green synthesis methods are favored due to their simplicity, eco-friendliness, cost-effectiveness, and accessibility. This approach utilizes natural agents like micro-organisms, plant extracts (Naikodi and Ansari, 2021; Chaithra *et al.*, 2021), fruit extracts, and biodegradable polymers, which influence the size, stability, and morphology of the nanostructures produced.

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Metal nanoparticles (MNPs) have garnered significant research interest due to their unique physicochemical properties and wide range of applications. These nanoparticles, typically ranging in size from 2 to 90 nanometers, play a critical role in determining their properties and potential uses. Additionally, their large surface area-to-volume ratio enhances their activity and performance across various applications (Kouhbanani *et al.*, 2019).

Nanotechnology is gaining prominence in science and technology due to its potential for creating new materials. Nanoparticles, with their small size, can effectively reach biological targets, making them highly valuable for biomedical applications (Figure 1). These applications extend beyond drug delivery to include innovative diagnostic and therapeutic techniques.

2. Types of methods for silver nanoparticlesynthesis

Several approaches have been explored for the synthesis of silver nanoparticles (AgNPs), including (i) physical methods, (ii) chemical methods, (iii) photochemical methods, and (iv) biological methods as illustrated in Figure 2. Each technique offers unique advantages and faces specific challenges, with common issues including cost, scalability, and particle size distribution, as summarized in Table 1. Among these methods, biological approaches are employed for AgNP synthesis, as they provide an eco-friendly process for nanoparticle synthesis whereas chemical approaches are frequently employed for AgNP fabrication, as they provide a straightforward process for nanoparticle synthesis (Tran *et al*., 2018; Singh *et al*., 2021; Philip, 2009).

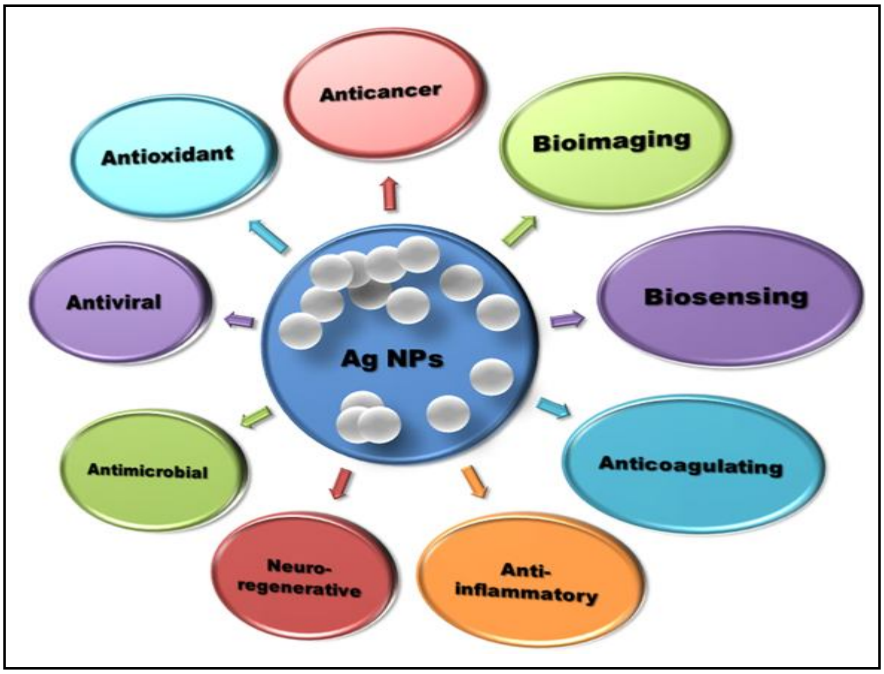

Figure 1: Silver nanoparticles and their biomedical applications.

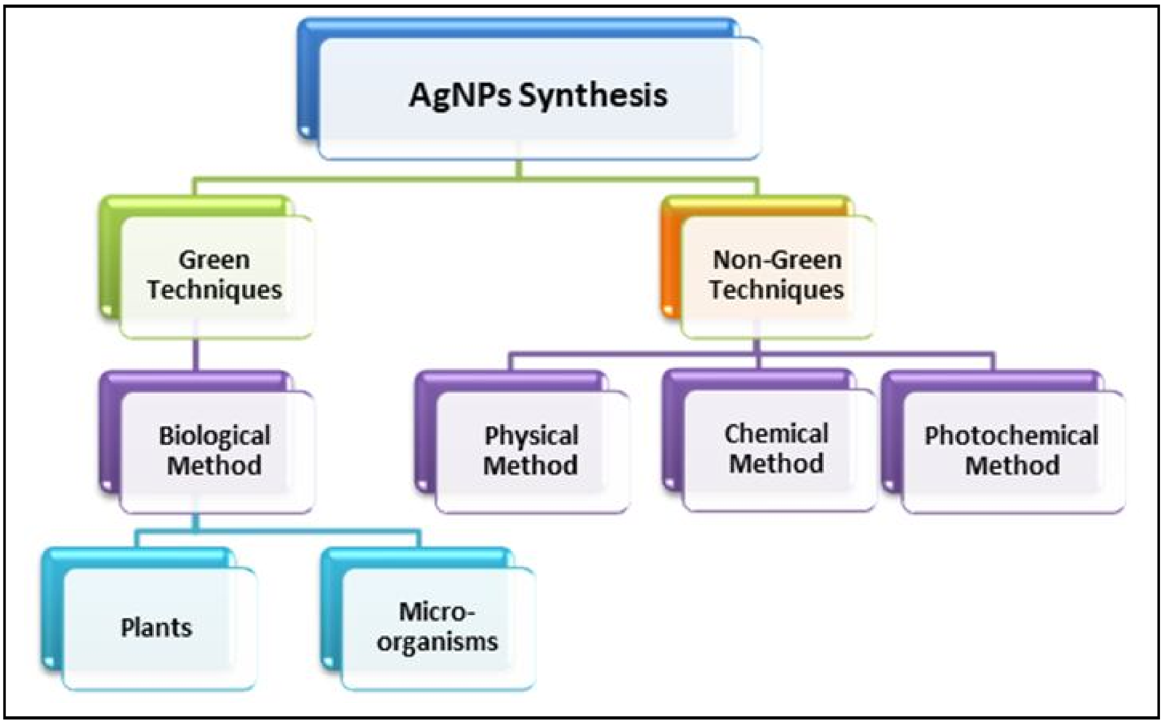

Figure 2: Methods of silver nanoparticles (AgNPs) synthesis.

The method of chemical synthesis of AgNPs in solution typically involves three key components: (i) metal precursors, usually silver nitrate ($AgNO_3$); (ii) reducing agents, such as ethylene glycol, which reduce metal ions to metal atoms; and (iii) stabilizing or capping agents, like polyvinylpyrrolidone (PVP), which control nanoparticle formation and prevent aggregation. However, the use of toxic chemicals in chemical synthesis raises significant environmental and health concerns. In response, there has been growing interest in developing greener routes for AgNP synthesis (Rafey *et al*., 2011).

Table1: Different methods of synthesis of silver nanoparticles and its benefits and drawbacks

S.No.	Synthesis methods	Synthesis principle	Benefits	Drawbacks
1	Physical method	The physical method for synthesizing silver nanoparticles (AgNPs) utilizes physical energy to produce nano-particles with a narrow size distribution.	Radiation acts as a reducing agent in this process, eliminating the need for hazardous chemicals and enabling the production of large quantities of silver nanoparticles in a single, short process. The resulting nanoparticles are free of contamination and do not require additional purification.	Costly, low yield, high energy consumption, solvent contamination, and inconsistent distribution.
2	Chemical method	Chemical reducing agents convert silver ions into silver atoms	Easy production, cost-effectiveness, and high yield.	Limited use of capping agents in AgNP synthesis can be toxic and hazardous. The resulting nanoparticles often require further purification due to surface contamination from chemical residues. Additionally, irregular particle sizes, the need for additional steps to prevent self-aggregation, and the release of harmful by products during synthesis are common challenges
3	Photochemical method	Photochemically generated intermediates facilitate the formation of metal cores and the reduction of metal ions.	A clean process that provides excellent spatial resolution and convenience.	High costs and specialized experimental conditions are required. (Natsuki *et al*., 2015)
4	Biological method	The reduction of silver ions to silver atoms using biomolecules was achieved through living organisms.	Cost-effective and abundant raw materials.	(Rauwel *et al*., 2015)

3. Wheat (*Triticum aestivum* L.)

In recent years, various eco-friendly methods have been developed for nanoparticle synthesis, particularly through biological processes. This includes the synthesis of silver nanoparticles using biological entities such as bacteria, yeast, fungi, and plants.Once among plant species known to be Wheat, a key cereal crop belonging to the Poeaceaefamily has played a vital role in ancient Indian culture for centuries and is renowned for its remarkable healing properties. As one of the primary staple foods for nearly 35% of the global population, wheatis widely cultivated in 102 countries covering 220.69 million hectares of area, which accounts for about 32% of the world's total cultivated land.Wheatgrass, derived from wheat, holds significant therapeutic potential due to its rich content of biomolecules such as chlorophyll, vitamins A, C, E, and B complex, bioflavonoids, minerals like calcium and magnesium, iron, and 17 amino acids, including 8 essential amino acids. Wheatgrass-extracted juice is known to boost energy by addressing nutritional deficiencies and detoxifying cells, blood, tissues, and organs. Wheatgrass extract is also effectively shown as an alternative to blood transfusions in terminally ill cancer patients. Recent research in India found that children with thalassemia who consumed 100 ml of wheatgrass juice daily experienced up to a 40% reduction in blood transfusion requirements, with no adverse effects.

Wheatgrass offers a wide range of health benefits, including reducing fatigue, improving sleep, boosting strength and naturally regulating blood pressure and blood sugar. It supports weight loss, aids digestion and elimination, and promotes the health of the heart, lungs, and reproductive organs. Additionally, it can help heal ulcers and skin sores, slow cellular ageing, enhance mental function, and alleviate conditions such as arthritis and muscle cramps. Wheatgrass has also been beneficial for various ailments, including anaemia, diabetes, cancer, eczema, constipation, kidney enlargement and the common cold. Incorporating wheatgrass into your regular diet can help you fully reap its advantages (Jadhav *et al*., 2023).

T. aestivum also exhibits antimicrobial effects against pathogenic bacteria and fungi, primarily due to the presence of active peptides such as Puroindolines A and Puroindolines B (Ghobad *et al*., 2014). Flavonoids represent a diverse group of secondary metabolites in plants, crucial for regulating development and defending against environmental stress. Their biosynthesis involves the action of multiple enzymes, notably chalconeisomerase and flavanone 3-hydroxylase (Strygina and Khlestkina, 2022).

3.1 Silvernanoparticle synthesis using plant extracts

The use of plant extracts in the synthesis of non-toxic nanoparticles (NPs) has emerged as a promising and sustainable approach. Numerous studies have documented the successful biosynthesis of NPs using extracts from various plants. Plants are known for their natural ability to hyperaccumulate and biologically reduce metal ions. These properties make plant extracts an eco-friendly and efficient method for nanoparticle biosynthesis and detoxification.

Wheatgrassis a potential source for the various bio-active chemical compounds like alkaloids, steroids, tannins, glycosides, carbohydrates, rutin, chlorogenicacid, gallicacid and tocopherol obtained from the various extracts like hexane, chloroform, methanol and aqueous analysed by HPLC method (Anand *et al*., 2015). Further wheatgrass extract was treated with $AgNO_3$ solution for the preparation of silver nanoparticles by wheatgrass extract by green synthesis method.The synthesis of wheatgrass-AgNPstakes place when wheatgrass extract is treated with $AgNO_3$ solution, first the aqueous extract of wheatgrass yellow in colourand changes to cherry red after 1 h reaction with $AgNO_3$ solution, then the solution incubated at 35°C for 24 h leads to a change in color to brown colloidal solution indicates the formation of silver nanoparticles (Sangeeta *et al*., 2021).

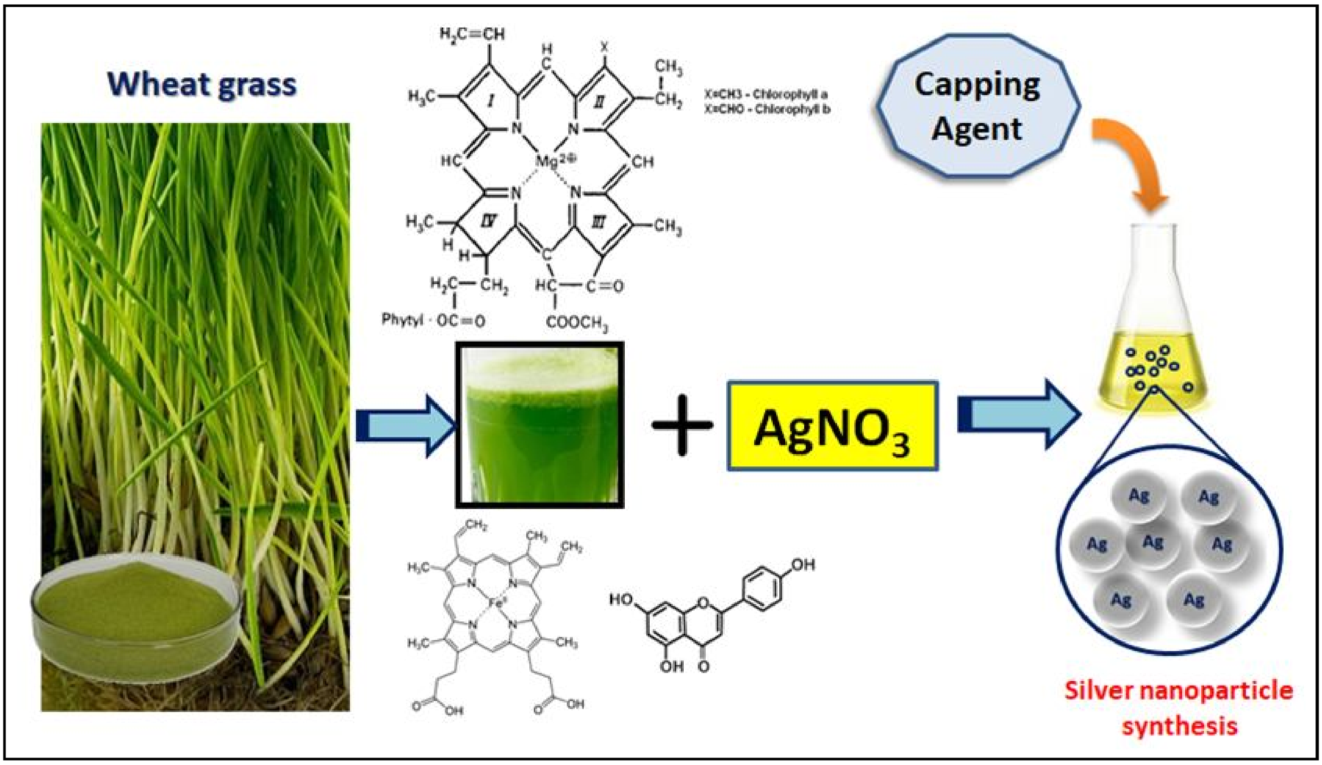

Figure 3: Typical representation of silver nanoparticles (AgNPs) synthesis.

In the structure of haemoglobin and chlorophyll, iron is the central metallic element in haemoglobin while Magnesium is the central element in chlorophyll. Wheatgrass rich as a source of chlorophyll and mainly responsible for inhibiting the metabolic activation of carcinogens showed anticancer properties (Figure 3). Silver nanoparticles offer a wide range of applications, largely influenced by their shape and size control, which plays a key role in enhancing their antimicrobial, physical, and chemical properties. The use of various capping agents not only helps to regulate particle size, prevent agglomeration, and shape the morphology of the nanomaterial but also contributes to the long-term stability of the nanostructures. Additionally, the nature of the interaction between the capping agent and the nanoparticle surface significantly affects the resulting nanostructure. The synthesis of spherical AgNP can be made by green synthesis because plant extracts contain secondary metabolites that are key factors in the morphology and stabilization of nanoparticles (Li *et al*., 2013). Wheatgrass boosts metabolism and restores alkalinity in the blood, and its high content of alkaline minerals helps reduce excessive acidity. Additionally, wheatgrass acts as a detoxifier and supports the restoration of healthy cells (Fahey *et al*., 2005). Wheatgrass has been used traditionally since ancient times to treat various diseases and disorders. Today, numerous wheatgrass suppliers in cities across India provide fresh wheatgrass daily through home delivery services, offering it for various health benefits and as a health tonic.

The formation of silver nanoparticles by wheatgrass extract was confirmed and characterized by various techniques like transmission electron microscopy (TEM), FT-IR analysis, powder x-ray diffraction (XRD) and UV-visible spectroscopy (Mohammad *et al*., 2019).

FT-IR offers wide scope as being a rapid, high throughput, non-destructive techniqueused for material evaluation,UV-visiblespectroscopy helps in primary characterization with the amount of conjugation and deals with short and long wavelength absorption, transmission of light and nuclear magnetic resonance (NMR). Spectroscopy is also one of the non-destructive sample techniques used in the molecular characterization to analyse the structural arrangement of atoms in the molecules and to describe the surface modification in the synthesized nanoparticles and requires a small amount of nature for analysis.The X-ray diffraction technique is one of the most widely used in nanoparticle characterization after its synthesis. Nanocrystals, nanoclustershaving surface ligands, and crystalline molecular structures can be studied through unique diffraction patterns produced by the scattering of X-rays from the sample. Scanning electron microscopy (SEM) is a high-resolution microscopic technique mostly used in the study of nanoparticle synthesis because of the imaging capability to describe the nature of particle size, size distributions, forms, surface morphology, *etc.,* and sometimes transmission electron microscopy (TEM) can be used as it is a more advanced and expensive technique for the characterization and nanoparticle surface morphological studies can be done more effective with higher resolution.

The biosynthesized silver nanoparticles show considerable effect on germination rate and nodule formation where the nodule formation in plants directly affects the nitrogen fixation cycle. The seeds of groundnut treated with wheatgrass AgNPs increased the germination rate by 20% and nodule formation rate by 40%, respectively, compared to the non-treated seeds with wheatgrass-AgNPs (Nandedkar and Khandare, 2018).

Excellent antibacterial activity was reported in AgNPs that attracted global attention to develop more and more efficient nanomaterials (Sharma *et al*., 2022; Duraisamy *et al*., 2014). The bioactive chemical components present in the wheatgrass extract are responsible for the multifunctional activity like antibacterial, antifungal, anti-inflammatory, antioxidant, antimicrobial (Table 2) and few other reported as antileukemic, antiulcer, antidiabetic and antihypertensive.

Table 2: Wheatgrass leaf extract used for the green synthesis of AgNPs and their various activities

S.No.	Potential activity	Major bioactive chemical compounds/ Wheatgrass/Wheatgrass -AgNPs	Test microorganism	Reference
1	Antibacterial activity of hexane extract	Rutin, Chlorogenic acid	*V. cholera, Staphylococus aureus*	Anand *et al*., 2015
2	Antimicrobial activity	Wheatgrass-AgNPs	*Escherichia coli* (bacteria), *Staphylococcus aureus* (bacteria), *Aspergillusniger* (fungus)	Nandedkar and Khandare, 2018
3	Anticandidal activity	Wheatgrass-AgNPs	*C. albicans* MTCC3017*C. albicans* MTCC1637*C. albicans* MTCC183 *C. tropicalis* MTCC230 *C. glabrata* MTCC3814	Sangeeta *et al*., 2021
4	Antifungal activity	Wheatgrass extract	*Aspergillus niger, Aspergillus flavus, Trichormaviride*	Prasanna *et al*., 2016
5	Antioxidant activity	Flavonoids, Phenolics	–	Maria *et al*., 2017
6	Anti-inflammatory activity	Wheatgrass tablet	–	Sai *et al*., 2021

Candida was the most prevalent opportunistic fungal pathogen in humans, responsible for a range of infections from superficial mucosal issues to severe, life-threatening systemic conditions. According to the centers for disease control, it ranked as the third most common bloodstream pathogen found in hospitalized patients, with a mortality rate reaching up to 50% (Pfaller and Diekema, 2007; Ganguly and Mitchell, 2011; Richard and Cornelius, 2012; Wisplinghoff *et al*., 2004; Tournu and Dijck, 2012; Mathé and Dijck, 2013).

There is a pressing need for new and safer antifungal drugs to combat *Candida* infections. Silver ions have long been recognized for their potent inhibitory and bactericidal effects, along with their broad-spectrum antimicrobial properties. The target research is based on evaluating the effectiveness of silver nanoparticles (AgNPs) synthesized using aqueous wheatgrass extract against various *Candida* species.

3.1 Anticandidal activity of silver nanoparticles

Silver nanoparticles synthesized using aqueous wheatgrass extract were evaluated for their anticandidal activity against five different clinical isolates: *C. albicans* MTCC3017, *C. albicans* MTCC1637, *C. albicans* MTCC183, *C. tropicalis* MTCC230, and *C. glabrata* MTCC3814. The testing was conducted using the agar well diffusion method, with isolates obtained from the microbial type culture collection and gene bank in Chandigarh, India (Sangeeta *et al*., 2021).

3.2 Peroxide catalytic activity

Green and efficient method approaches for the synthesis of Ag nanoparticles using *T. aestivum or* wheatextract at room temperature without any harmful reducing agents such as sodium borohydride or dispersing agent. The green synthesized silver nanoparticles consisted of polydispersed, highly crystalline spherical particles with a size of less than 10 nm. The catalytic activity of silver nanoparticles in an aqueous solution was tested using hydrogen peroxide. Titration experiments were conducted with various concentrations, ranging from 100 µl to 1000 µl of a 30% hydrogen peroxide solution. These nanoparticles demonstrated catalytic stability with hydrogen peroxide up to a concentration of 200 µl. The silver nanoparticles remain stable up to a concentration of 200 µl and show high responsiveness at this level, though they oxidize at this concentration. UV-visible titration experiments demonstrated that the silver nanoparticles effectively facilitate the reduction of hydrogen peroxide, exhibiting excellent catalytic activity. Additionally, earthworms exhibited stability at concentrations of silver nanoparticles up to 1500 ppm (Waghmode *et al*., 2013).

3.3 Effect on germination, early seedling development, and metabolome of wheat

The effects of bio-synthesized silver nanoparticles (Bio-AgNPs) on grain germination, quick development of seedlings, and roots metabolic profiles, coleoptile, and endosperm of wheat were studied. The detrimental effects of Ag nanoparticles on roots were evident through root shortening, thickening, browning of root tips, epidermal cell death and progression of damage from the apical meristem to the root hair zone, along with inhibition of root hair development. AgNPs also induced reactive oxygen species production in roots and altered the metabolic profiles across all tissues. Notably roots accumulated sucrose, maltose, 1-kestose, phosphoric acid and various amino acids (Lahuta *et al*., 2022).

Application of biologically synthesized silver nanoparticles (AgNPs) at concentrations of 40 mg/l inhibited the growth of wheat seedlings leading to the development of a brown coloration in the root caps and an increase in fluorescence, which indicates the production of reactive oxygen species (Lahuta *et al*., 2022).

3.4 Physiological effects of silver nanoparticles

Silver nanoparticles and silver nitrate significantly reduced the levels of chlorophyll 'a' and 'b', carotenoids and total protein content in the leaves. Additionally, treatments with AgNPs and $AgNO_3$ led to increased proline accumulation, lipid peroxidation, and catalase activity in the wheat seedling tissues (Karimi and Mohsenzadeh, 2017).

3.5 Effects of environmental contamination by Ag NPs on the metabolism and growth

Accumulation of silver (Ag) was observed in the shoots, suggesting that the metal was taken up and transported from the silver nanoparticles (Ag NPs) present in the sand. Transmission electron microscopy confirmed the presence of Ag NPs in the shoots of plants whose roots were exposed to Ag NPs or high levels of Ag ions. This exposure led to oxidative stress in the roots, as evidenced by increased levels of oxidized glutathione and the upregulation of a gene encoding metallothionein, which is mainly involved in metal ion sequestration and detoxification (Dimkpa *et al*., 2012).

3.6 Photosynthetic activity

The treatment with AgNPs significantly affected growth parameters including root and shoot length of wheat seedlings. Additionally, biochemical parameters such as catalase activity, glutathione levels, flavonoid content and chlorophyll concentrations were also impacted. At a 5 mM concentration, AgNPs notably suppressed photosynthetic activity causing severe damage to photosystems. This suggests improper regulation of PSI electron transport, leading to structural damage to the chloroplasts, including the photosystems themselves (Rastogi *et al*., 2019).

3.7 Catalase activity

Numerous studies have shown that the phytotoxic effect on plants was mediated by oxidative bursts, which may trigger a protective mechanism by boosting antioxidant production. Catalase, an enzyme that decomposes hydrogen peroxide into water and oxygen, was a key player in this process. Enhanced catalase activity suggests increased hydrogen peroxide levels, while elevated GSH levels in AgNPs-treated samples indicate a greater production of glutathione (Hossain *et al*., 2015; Rastogi *et al*., 2017).

4. Conclusion

The biological methods employing green silver nanoparticle synthesis are considered the best tools due to their nontoxic nature and also biocompatible applications. Wheatgrass has been traditionally used since ancient times to treat a variety of ailments and many firms' supplies across the country offer fresh wheatgrass daily, promoting it for its numerous health benefits and as a natural health tonic. Green synthesis of Ag nanoparticles using wheatgrass extract without any harmful reducing agents or dispersing agents demonstrated catalytic stability, effects of biosynthesized silver nanoparticles detrimental effects of Ag nanoparticles on roots. It also induces reactive oxygen species production in roots and can alter the metabolic profiles, catalase activity, and increased levels of oxidized glutathione by silver nanoparticles were reported successfully. The green synthesized nanoparticles can be obtained by the various eco-friendly biological methods which include various plant parts, microorganisms and algae. The formation of the wheatgrass AgNPs can be confirmed and characterized by different sophisticated analytical techniques.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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