



An Overview of Green Nanotechnology as a Contemporary Instrument for Sustainable Agriculture

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This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Green chemistry, which synthesises chemicals with less toxicity or reduces the toxicity of hazardous compounds, is an important tool for attaining sustainability. Green nanotechnology offers answers to a number of international problems. Green nanotechnology is seen as a crucial instrument for attaining sustainability in the food industry, animal feed, and agriculture. Green nanotechnology uses biological processes to synthesise nanoparticles (NMs), which find wide-ranging applications in the sustainable agricultural sector and related fields. Various plant tissues, including leaves, stems, barks, seeds, roots, fruits, and flowers, have been employed in the production of nanoparticles (NPs). Diverse natural resources are employed to maintain the agricultural industries. As this book chapter is not intended to cover the whole possibilities of this recently developed field of medicine and study, we will confine ourselves to a quick synopsis of green nanotechnology and the ongoing attempts to explore its potential uses in sustainable agriculture and related fields. Different engineered nanomaterials (ENMs) affect plants in different ways and through distinct mechanisms. In order to create systems for sustainable agriculture, this book chapter also thoroughly examines the absorption, translocation, interaction mechanisms, and phytotoxicity of numerous ENMs in plant species. This review emphasises the benefits and significance of using NMs, together with the primary technological challenges that may arise during the implementation of sustainable agriculture systems.

Keywords: Food security; nanoparticles; crops; yield; diseases.

1. INTRODUCTION

Global climate change, rapid population development, and an increase in anthropogenic activities have all contributed to a wide range of pollutants in various environmental matrices and stress-related degradation of soil quality, which has mostly resulted in a loss in crop yield worldwide [1]. One of the most concerning issues that is seriously jeopardising the stability of ecosystems at a rapid rate is abiotic stress [2]. Abiotic elements that most commonly impact agricultural productivity are heat, cold, salt, drought, and heavy metal toxicity. Diverse environmentally sustainable tactics are being utilised to mitigate the adverse effects of abiotic stressors, enhance plant resilience to stress, and address the worldwide concern of environmental contamination. A multidomain field encompassing chemistry, engineering, biotechnology, microbiology, and physics, nanotechnology is dedicated to the invention, improvement, and application of nanoscale (1–100 nm) structures [3]. Nanoparticles (NPs) are distinguished from bulk materials by their unique features, which include physical strength, stability, optical characteristics, reactivity, electrical conductivity, and magnetic properties. These attributes are a result of their minute size and high surface area to volume ratio [4]. As a result, NPs are used in a wide range of industries, including agriculture, pharmaceuticals, cosmetics, healthcare, biomedicine, textiles, food, optics, electronics, sensors, and optical devices. Through the use of biosensors,

nutraceuticals, genetically modified plants and animals, plant growth regulators and promoters, intelligent drug delivery systems, nanopesticides, nanoherbicides, and nanofertilizers, nanotechnology has the potential to completely transform the agricultural and biomedical industries [3].

NPs are created by a variety of chemical and physical processes, such as arc discharge, pyrolysis, sonication, laser ablation, and irradiation. Toxic byproducts are produced by the chemical procedures, which employ dangerous reducing chemicals as sodium borohydrate and hydrazine [5]. The employment of these technologies in clinical, biomedical, food, environmental, and agricultural applications is thus restricted by their combined energy and capital needs as well as the usage of toxic chemicals, nonpolar solvents, synthetic additives, and capping agents [2]. Thus, "green" chemistry and bioprocesses have become the centre of attention for researchers in their quest for a reliable, safe, nontoxic, and environmentally acceptable way to produce NPs [6]. Researchers have looked into using bacteria, fungus, actinomycetes, yeast, viruses, and plant components (seed, flower, leaf, bark, and peel) as well as enzymes to create homogeneous, stable NP synthesising agents at physiological pH, temperature, and pressure in an eco-friendly way. These species have the innate ability to biosynthesize NPs in either an extracellular or intracellular environment, making them potential biofactories for NP synthesis [7]. In biological

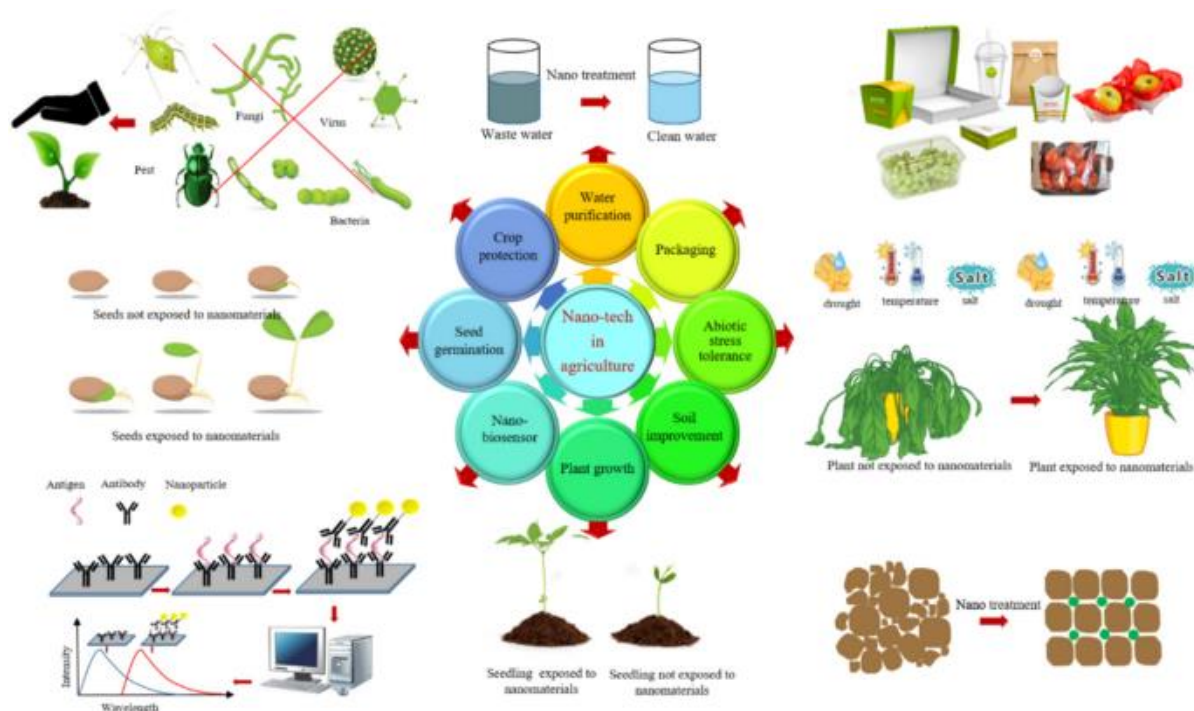


Fig. 1. Application of nanomaterials in agriculture

synthesis, amino acids, peptides, protein, polysaccharides, tannins, flavonoids, phenols, and vitamins play a crucial role as reducing and capping agents and are essential for regulating the size and form of nanoparticles. High dimensions, stability, and polydispersity characterise the biogenic NP (Fig. 1).

There are many reports on the use of biogenic NP in the biological and therapeutic domains, but there aren't many that discuss how it might be applied in the environmental and agricultural domains [8][9].

2. GREEN SYNTHESIS

The use of nanoparticles (NPs) or nanomaterials (NMs) is contingent upon the nature, size, shape, and biological corona of the NPs. The source of synthesis also affects these characteristics. It is possible to synthesise nanoparticles by using chemical, physical, and biological methods. Although monodisperse NPs can be produced through regulated chemical and physical synthesis, there are several drawbacks, including the creation of poisonous and hazardous byproducts and the attachment of excess substances to the NPs' surface. Due to these constraints, sustainable substitutes known as "green nanotechnology" have emerged. In this field, scientists primarily concentrate on using biological resources or environmentally friendly

production processes to produce nanoparticles. For over ten years, green techniques have been used to synthesise various metal nanoparticles, such as silver and gold [9,10]. Enhancing NPs' activity and lessening their negative effects on the environment and public health are the primary goals of green methods. Much study has been done to discover new environmentally friendly resources for the manufacture of nanoparticles and potential uses for them. The process of creating nanoparticles (NPs) by the help of various microorganisms, such as bacteria [11,12], fungi [13], yeast [14,15], and viruses [15,16], is known as "green synthesis." It is well known that green synthesis is quick, easy, and yields stable, biocompatible nanoparticles. In particular, it has been widely documented that medicinal plants can synthesise nanoparticles in a matter of seconds to hours, compared to the 24-48 hours that bacteria can typically synthesise. Numerous therapeutic plants, including Siberian ginseng, Panax ginseng, Rhodiola rosea, Cannabis sativa, Rowan berries, and others, have been documented to create metallic nanoparticles thus far. One intriguing method for producing large quantities of nanoparticles is plant-mediated synthesis. It does not require high temperatures or specific energy resources (such as ultrasonic waves), and it is economical, environmentally benign, and easily scalable. Enzymes, proteins, and secondary metabolites are the components that cause metal

reductions in microbes; in plants, these constituents include flavonoids, terpenoids, phenols, sugars, saponins, steroids, etc. The previously discussed biological elements aid in reduction and create a "capping layer" or "biological corona" that envelops the individual nanoparticles. The biological components released from the plant or microorganism in an extract or a culture media used for synthesis are contained in the biological corona that forms around the nanoparticles. In addition to preventing agglomeration and providing long-term stability for nanoparticles in aqueous solutions, this capping layer is crucial for the interaction of green nanoparticles with cells [20]. This facilitates the easy penetration of nanoparticles into plant, bacterial, fungal, and cell organelle cells [17]. As a result, the biological corona is crucially useful to the synthesis of nanoparticles and their various applications.

3. VARIABLES IMPACT ON THE GREEN SYNTHESIS

The temperature, pH, salt concentrations, time spent on synthesis, and synthesis source all affect the structure of the metallic nanoparticles during the process. The metallic nanoparticles develop and offer their activity based on these conditions (Fig. 2). The production of nanoparticles depends on reaction time, which determines the stability, size, and form of the particles [18]. Recent research by Singh et al. demonstrated that the entire agglomeration of nanoparticles at higher temperatures is caused by an increase in the reaction time during the synthesis of gold and silver nanoparticles. Green synthesis proceeds relatively quickly at high temperatures, between 70 and 90 °C [19]. The authors have observed that agglomeration with various plant extracts is brought on by longer reaction times and higher temperatures. The shape and size of nanoparticles are largely determined by pH. According to the literature, at



Fig. 2. Green synthesis of nanoparticles, method parameters and applications in sustainable agriculture

acidic pH levels, large nanoparticles generate less functional groups linked to the corona layer [21]. The impact of temperature on the shape of nanoparticles has also been extensively studied. Higher temperatures aid in rapid reduction and, if handled for an extended length of time, may result in agglomerations, according to the research. At higher temperatures, many biological components that would have been able to adhere to the nanoparticles under the corona become inactive as well. The instability of nanoparticles is caused by this. This is case-specific, though, because it doesn't include all green resources. Several studies have shown that even at higher temperatures, stable, tiny, monodisperse nanoparticles could form. For example, Singh et al. demonstrated the creation of 100% monodisperse silver nanoparticles at 90 °C from Rowan berries [19]. Another instance where spherical gold nanoparticles mostly generated at a lower temperature is given by Gericke et al. [22]. Nevertheless, a rise in temperature caused the nanoparticles' structure to alter, giving rise to rod- and plate-shaped nanoparticles [23]. The biomolecules that are available for reduction in the reaction also have an impact on the creation of nanoparticles. Huang et al. demonstrated how the concentration of sun-dried *Cinnamomum camphora* leaf extract caused the form of gold and silver nanoparticles to shift from triangular to spherical [24]. The morphologies of gold nanoparticles changed from spherical to triangular plates as a result of the concentration-dependent behaviour of *Aloe vera* leaf extract [25]. These illustrations show how important reaction components are to the creation of green nanoparticles.

4. FACTORS AFFECTING NANOPARTICLE'S TOXICITY A

Numerous parameters, such as the size and concentration of the nanoparticles (NPs) and the particular plant species involved, affect their toxicity in plants (Fig. 3). Comprehending these variables is crucial in evaluating the possible hazards linked to the application of NPs in agriculture.

4.1 Dimensions

One important factor influencing NPs' toxicity is their size. Because smaller NPs often have a bigger surface area, there is an increased chance of their interacting with cellular components and increased reactivity. This heightened reactivity may result in NPs being

absorbed and accumulating within plant tissues, which could have negative consequences.

4.2 Concentration

The amount of NPs sprayed on plants or in the environment can have an effect on how harmful they are. Elevated levels of nanoparticles have the potential to overpower the plant's defence mechanisms and cellular detoxification processes, resulting in stress reactions and cellular damage.

4.3 Plant Species

The susceptibilities of various plant species to NPs differ. While certain plants may be more resistant to the negative effects of NPs, others may have systems in place that allow them to be tolerated or detoxified more successfully. Every plant species has unique physiological and biochemical traits that can affect how well it interacts with and reacts to NPs. It is significant to remember that NPs are not entirely harmful. When applied correctly, NPs can also benefit the growth and development of plants. NPs can be applied in a controlled manner at lower quantities, and their size and surface qualities can be optimised to increase nutrient uptake, stress tolerance, and overall plant health.

Research is now being conducted to better understand the toxicity of NPs in plants. This includes determining the underlying processes of NP-plant interactions, examining the effects of various NP characteristics, and assessing long-term consequences on food safety, ecosystem dynamics, and plant growth. Such research attempts to minimise possible dangers to plant health and the environment while ensuring the safe and sustainable implementation of nanotechnology in agriculture.

4.4 AgNPs

AgNPs are a type of commercialised nanomaterial that finds application in personal care and antibacterial agents in the medical arena [26,27]. AgNPs are an effective nanomaterial that may be used in agriculture to promote seed germination, plant growth, and development under stressful environmental conditions. Recently, plant biologists have become sufficiently interested in using AgNPs because of its environmentally benign qualities. AgNPs are primarily given to plants via foliar application, soil mixing techniques, or seed priming technology. Furthermore, the kind of

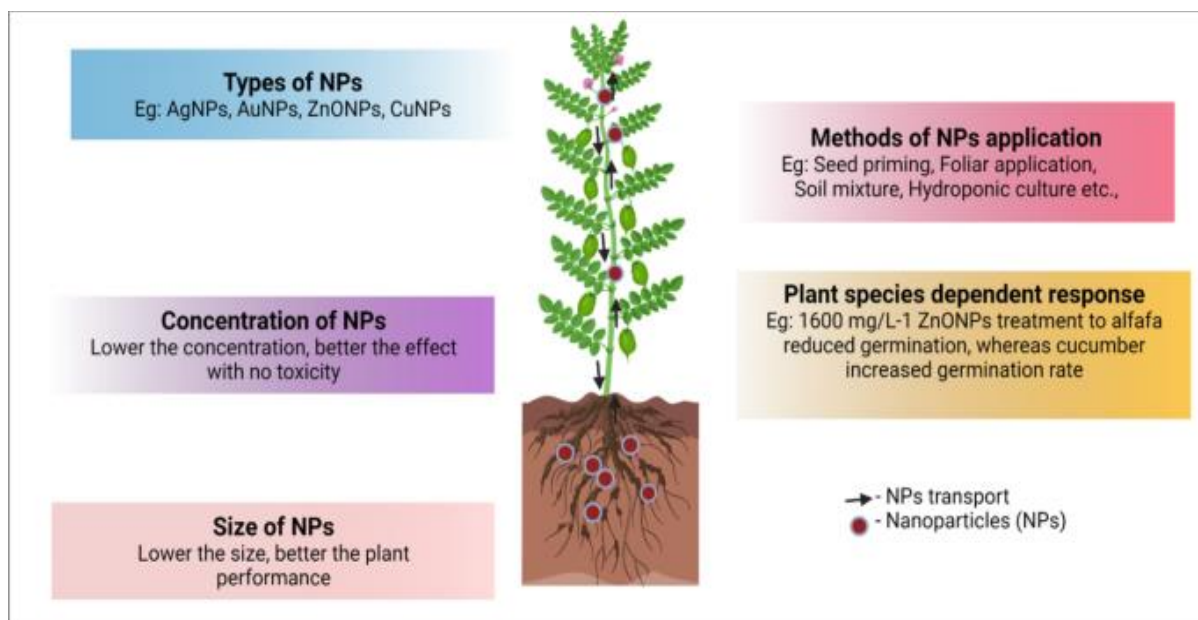


Fig. 3. Factors to be considered during the treatment of nanoparticle application to plants

application that was made had a significant influence on the improvement in plant performance. As a result, when various AgNPs treatment techniques are implemented, we will comprehend the main variations in plant development and performance under stress and under normal circumstances in this part. In order to assess if applying 5 and 10 mg/L of biocompatible AgNPs under standard conditions may enhance rice seed germination and starch metabolism, the extract from kaffir lime leaves was used in the synthesis of the AgNPs [28]. Compared to a treatment with silver nitrate (AgNO_3), AgNPs penetrating the seed coat enhanced water intake, increased ROS and H_2O_2 , and improved seed germination as well as starch metabolism. These findings supported the theory that seeds can be nanoprimed with AgNPs at low concentrations to loosen the cell walls of the seed coat and endosperms [28,29,30]. Furthermore, the low concentration of nanoparticles used in seed priming lowers production costs while also limiting the environmental dispersion of nanoparticles. Thus, seed priming appears as a sustainable method [10,11,12].

Since NPs treated to seeds do not seep into the soil, seed priming can be recommended as a viable method for their commercial application. It also reduces the amount of NPs that disperse into ecosystems [31-36]. Due to their size- and concentration-dependent reactions, AgNPs should be used with caution when seed priming to enhance rice plant germination and growth.

Rice seedling germination and growth were inhibited when soaking in varying diameters (20, 30-60, 70-120 nm) and concentrations of AgNPs (100 and 1000 mg/L). In order to avoid AgNPs' phytotoxic effects on rice seedlings, it is imperative to take into account the ideal sizes and concentrations of AgNPs. AgNPs at a dose of 60 mg/L during seed priming enhanced the enzymatic activities, biochemical indices, and agro-morphological characteristics of sunflower plants. On the other hand, improved plant production, seed quality, and secondary metabolite contents of the sunflower plants were achieved through the combined approaches of seed priming and foliar treatment, suggesting that each application method can be used to enhance specific features of sunflower plants. However, the application of 150 mg/L AgNPs through soil resulted in an increase in toxicity in sunflower plants due to the accumulation of AgNPs in the root, leaf, and stem. This was evidenced by a decrease in total soluble proteins, lipid peroxidation, and chlorophyll and carotenoids contents [36]. When 200 mg/L of AgNPs were applied *in vitro* to cucumber and wheat plants, comparable phytotoxic effects were observed during the vegetative growth stage as opposed to germination [37]. Another study has demonstrated the beneficial effects of urea at low concentrations of AgNPs (10 and 15 mg/L) on reduced diseased condition in seeds, higher germination rate, increased chlorophyll contents, increased stomatal conductance, and higher seedling masses in oilseed rape and cucumber under thermal stress through foliar application

and seed priming [38]. AgNPs have been shown to boost growth metrics, photosynthetic pigments, proline, hydrogen peroxide (H₂O₂), and antioxidant activities in eggplant when sprayed topically on the plant during a drought [39]. This suggests that AgNPs can be used in place of toxic pesticides and highly concentrated mineral fertilisers. When biosynthesized AgNPs were pre-treated with *A. brassicicola*, the number of lesions was significantly lower than when plants were treated with *A. brassicicola* alone. These findings imply that the proper choice of AgNPs application can control plant development and performance in both stressed and unstressed environments.

4.5 AuNPs

AuNPs were extensively used in a wide range of industries, such as electronics, biology, chemistry, physics, medicine, and cosmetics [40]. Unfortunately, there aren't many studies on plant growth, development, and phytotoxicity that have been published. Both beneficial and detrimental impacts were generally shown by the plants exposed to AuNPs, and these effects mostly rely on concentration, particle size, shape, and species [41]. The manner in which nitrogen is applied to plants—that is, whether the uptake occurs through the leaves, roots, or seeds—is also very important [42]. An *in vitro* investigation conducted on *Arabidopsis* seedlings revealed that applying the tiniest AuNPs, measuring 10 nm, at the lowest concentration resulted in the formation of root hairs. However, the quantity and length of lateral roots exhibiting higher AuNP particle concentrations were reduced [43]. 25 nm-sized AuNPs at 500–1000 μ M concentrations applied to the soil for 40 days improved vegetative growth and seed germination in glory lillies [44].

4.6 ZnONPs

Because plants without zinc (Zn) have much less protein, glucose, and chlorophyll creation, zinc is an essential mineral for plant growth and development. Because zinc oxide (ZnO), also known as ZnSO₄.7H₂O, is poorly soluble in soil and has a low zinc bioavailability for plants, its usage as fertilisers is restricted [45]. ZnONPs have so received particular interest in the sphere of agriculture. It has been demonstrated that zinc nanoparticles can pierce the seed coat, increasing the expression of aquaporin genes related to water uptake, seed vigour, bioavailability, soil solubility, and slow and progressive release. Compared to bulk ZnSO₄

treatment, the ZnONPs primed seeds had a stronger impact on growth and physiological status. This could be because of the nano size's increased ability to be absorbed and assimilated. Because zinc is involved in the early phases of coleoptile and radicle development, adding zinc to the primed solution in a study increased the budding and seedling growth of wheat seedlings [42]. ZnONP-treated seeds with elevated α -amylase levels have the potential to enhance the availability of soluble carbohydrates, leading to improved overall metabolic activity through increased germination rate, seedling length, and seed water intake [46,47]. The amount of ZnONPs employed in foliar spraying is more than in seed priming, but it is still relatively low when compared to the soil mixture method in wheat plants. For example, ZnONPs applied to wheat tissues via soil utilised a total of 500 mg for four plant repetitions [43], whereas ZnONPs applied by foliar spray required only 200 mg for four replicates, indicating the effective use of ZnONPs for plant bio-fortification. Additionally, in Cd-stressed water-deficient conditions, this study demonstrated the efficacy of ZnONPs in enhancing growth, chlorophyll contents, Zn contents, and by lowering oxidative stress and cadmium (Cd) contents. Additionally, we discovered that the selection of plant species and NP concentration generally influence the ZnONP seed priming on germination [44]. Higher concentrations of ZnONPs have been shown to impact the quality of germination. For example, with 1600 mg/L ZnONPs treatment, the germination rate of alfalfa was lowered to 40%, and of tomato seeds by 20%, but the germination rate of cucumber seeds rose compared to the control. When compared to foliar spray and soil combination, the germination rate of egg plant seeds treated with 100 mg/L ZnONPs was improved by decreasing seed dormancy [41,45, 46,47]. Similarly, foliar treatment of habanero pepper plants resulted in concentration-based physiological responses. The foliar spray exhibited varying functions depending on the ZnONP content.

5. AGRICULTURE'S EXPOSURE TO METAL NANOPARTICLE COATINGS

In order to achieve sustainable agriculture, supply nutrients or pesticides to crops, increase crop production and agricultural performance, reduce waste, and treat infectious plant diseases, NPs are constantly being researched for their potential use in agriculture. More internalisation from plant parts (leaf, root, etc.), strong reactivity due to the increased surface

area to volume ratio, and enhanced bioavailability in plants are just a few benefits of nanoparticles (NPs) smaller than 100 nm. However, aggregation, instability, dissolution in aqueous suspension, and soil adsorption are problems that these NPs face. These problems may result in a decrease in their activity, inadequate transport inside the plant, phytotoxicity, and toxicity to the plant's surrounding ecosystem. These NPs can be coated with different chemical moieties, which aid in decreasing agglomeration and controlling dissolution, to increase their efficacy. Natural organic matter (NOM), polymers, zwitter ionic surfactants, proteins, etc. are examples of these chemical moieties. Moreover, these coatings actively engage in nano-bio interactions as soon as they enter the cell and aid in the translocation of NPs inside the plants. Numerous techniques, including physical adsorption, chemical adsorption, covalent coupling, association through hydrogen bonding, electrostatic interactions, or hydrophobic interactions, have been employed to apply these coatings on NPs [6]. NPs intended for agricultural applications are often coated with humic compounds, also known as humic acids, which make up a significant portion of the organic part of soil [35,36].

6. LIMITATIONS AND POTENTIAL FUTURES

The nanotechnological tools meant for use in the agricultural sector have certain benefits (Fig. 4) and some disadvantages, much like any other technology. Only two nations in the world—the European Union and Switzerland—have included measures unique to nanoparticles in their agricultural laws. Non-EU nations, however, continue to rely implicitly on industry recommendation because their current laws do not contain any provisions specifically pertaining to nanoparticle. In light of the rapidly expanding field of nanotechnology and its applications therein, uniform policies and regulations pertaining to the synthesis, use, and removal or elimination of nanomaterials from the environment must be developed and updated on a regular basis in accordance with the most recent advancements in nanotechnology. The application of NPs in agriculture is still in its early phases, and it will take time for their full potential to be realised when theory and field knowledge catch up. The typical obstacles that industries and researchers must overcome for this include high processing costs, standardising research techniques, and worries about environmental and public health [48,49]. Temporarily solving this

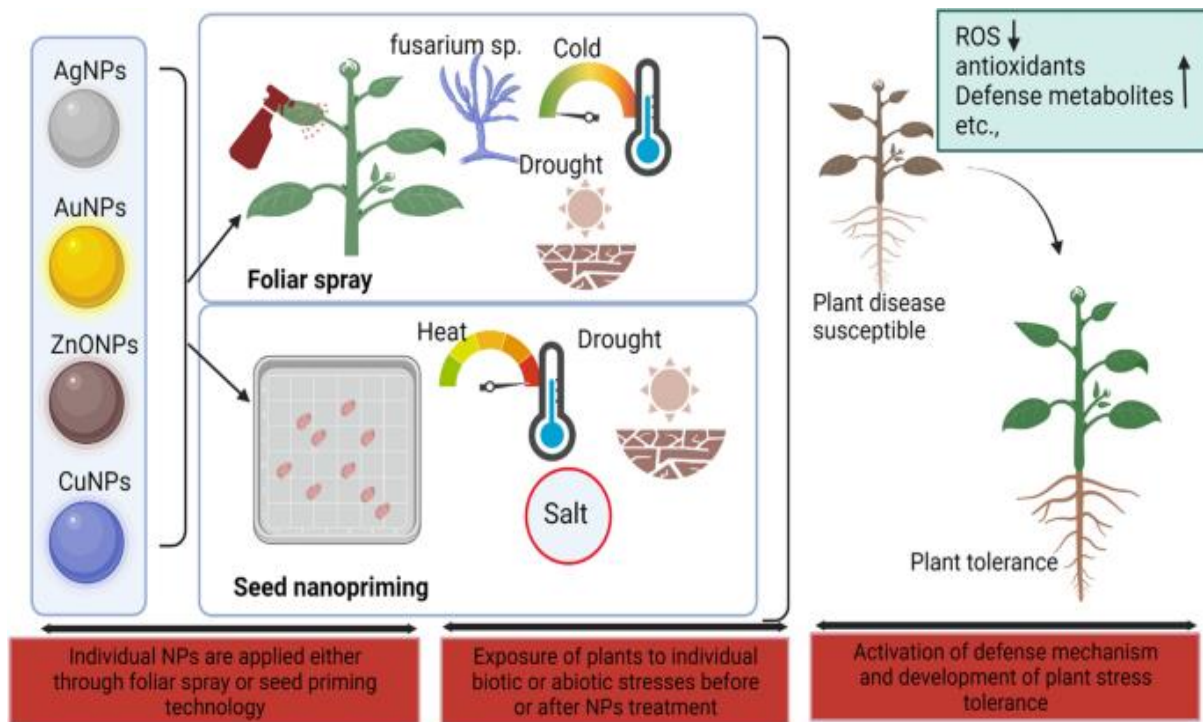


Fig. 4. Beneficial NPs application methods for exploring plant tolerance mechanisms against biotic and abiotic stresses

issue are green synthesis techniques using less expensive ingredients such as iron, copper, and zinc. However, steps need to be taken to lower the final nanotechnological product's cost so that farmers can use it on a big scale for agriculture. After reading this review, we were able to conclude that various NP formulations made with environmentally friendly processes and NP application techniques had beneficial impacts on plants. Nevertheless, there is insufficient proof to conclude that a particular NP type or application technique in agriculture is entirely safe for the wellbeing of the plants and soil. Thus, it is imperative to investigate these gaps in knowledge. Generally speaking, shorter durations of use of foliar spray and seed priming techniques with smaller sized nanoparticles at lower concentrations improve the physiological/biochemical state of plants under stress and non-stress circumstances (Fig. 4). Long-term use of nanoparticles and their effects on the food chain connected to crops are still mostly unknown. A crucial benchmark for bringing technologies from the lab to the field is NPs toxicity. Researchers have coated nanoparticles with various moieties to reduce the nanotoxicity; nevertheless, as of right now, no thorough report that addresses the short- and long-term impacts of nanoparticles on crops and the crop-assisted food chain is available. Since the nanotoxicity study may differ based on the type of nanomaterial, crop species, and agricultural settings, these constraints ought to be discussed in every study. It may be necessary to make extra efforts to evaluate any potential defences and resistance that plants may develop in response to these nanoparticles' impact after prolonged or excessive use. Another goal to be met while employing NPs in agriculture is soil fertility, as good plant growth is regulated by millions of microorganisms and all the necessary nutrients found in fertile soil. Consequently, regardless of the manner of NPs application, soil health should be observed for a considerable amount of time following the use of these nanoparticles to make sure that it does not influence soil fertility. Nanoparticles are a new and exciting technology in agriculture, despite all of these worries.

7. CONCLUSION AND POTENTIAL DIRECTIONS

There is a plethora of published research on the application of metallic nanoparticles in agriculture. Researchers have reported using a variety of metal nanoparticles (Cu, Ag, Au, and so on) with shapes (spherical, triangular, cubic,

and so on) and sizes (X to Y nm; this may be a general range from all cited papers in this review article) to improve crop/plant performance both under stress and in normal conditions. Numerous application techniques were used to apply these nanoparticles to plants both in the lab and in the field. However, in order to attain the highest level of sustainability in agriculture, the best technique and nano formulation must be used. Reviewing and contrasting the most recent techniques and their results is required to provide a response to this subject. In order to close this gap, we have made an effort to use foliar spray technology, seed priming, and smaller, less concentrated nanoparticles. These techniques are safer for plants and have been shown to improve plant performance in both stressed and unstressed environments. Furthermore, our article presented an urgent issue of regulatory issues to govern the use of nanotechnology in the agricultural industry, taking findings from the literature review. We also provided an overview of the use of nanomaterials to distribute biomolecules, which can be used as a safer and more environmentally friendly method of sustainable agriculture in place of chemical fertilisers.

If applied responsibly, nanotechnology may also improve the health of the soil. Additionally, the agro-industries that depend on better farming practices might function more effectively to generate food while meeting the world's rising population and food demand. With less space available for farming and more people living in the world than ever before, nanoparticle-mediated enhanced agriculture undoubtedly looks like a great path to a better future. However, there are other obstacles in the way, including the ecosystem's sensitivity to nanotoxicity, negative environmental effects including unhealthy soil, and a dearth of appropriate, powerful regulatory agencies to oversee the use of nanomaterials. The nanoparticles may provide a great means of sustaining green agriculture once these obstacles are overcome.

All things considered, our evaluation aids researchers working in this area in choosing the finest nano-formulation available in terms of physicochemical qualities and synthesis techniques. It also recommends the best way to use nano-formulation in agriculture with the least amount of phytotoxicity. Our paper also highlights the gaps in this area, such as (i) the long-term consequences of applying nanotechnology to the agricultural sector and (ii) regulatory issues. Thus, our attempts to compile

this can serve as a kind of manual for scholars working in this area.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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