Chapter 28

Role of Nanoparticles in Biofertilizers

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ABSTRACT

Modern cropping systems mostly rely on synthetic or chemical fertilizers to improve yields; however, persistent, long-term use of these fertilizers can have negative impacts on soil fertility, soil biota, and the environment. These problems highlight the need for extremely economical, highly effective fertilizers that can raise yields and have the least amount of harm to the environment. To maintain bio-safety in agriculture, bio- and nano-fertilizers are chosen over synthetic (chemical) fertilizers since they are non-toxic and environmentally benign. Regarding this, Nanotechnology has become a potentially game-changing invention in the twenty-first century. In light of population growth, this chapter focuses on the coupling of nanoparticles with biofertilizers to act as nano-biofertilizers (NBFs), which could guarantee global food security. When plants are injected with NBFs, their growth and stress tolerance are enhanced. NBFs that are based on microorganisms and metallic nanoparticles get over the restrictions of traditional chemical fertilizers. While the NBF application is still in its early stages, it appears to have greater potential than other methods for transforming traditional farming into high-tech, "smart" farming. This chapter examines the innovative application of nanotechnology in agriculture, which leverages the special properties of materials at the nanoscale to solve pressing issues including crop protection, nutrient delivery, and sustainable agricultural practices. Future directions and recent advancements in field farming with NBF formulations are also discussed.

KEYWORDS		
Nanozymes, Nanotechnology, Chemical fertilizers, Nanoparticles, Biofertilizers, Sustainable Agriculture, Ecofriendly	Received: 29-June-2024 Revised: 02-July-2024 Accepted: 07-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

The global economy has been built on agriculture, which is under great stress and cries for new answers to sustainable development. Food production must be increased by around 70% while conserving the environment in order to meet the requirements of an increasing population, projected to hit nine billion by the year 2050 (Godfray et al., 2010). Worse still, climate change affects crop yields negatively leading to reduced food security and a depreciating soil quality. Additionally, these challenges are again made worse by resource depletion with poor water quality, soil erosion and loss of arable land as a result of unsustainable agricultural practices. Chemicals from pesticides and synthetics fertilizers degrade soil health, reduce fertility and contaminate food with hazardous residues. This means that, for sure, we have to address socio-economic inequalities by dealing with rural poverty, limited resources, and bad infrastructures to make sure we achieve sustainable agriculture (Kopittke et al., 2019).

Sustainable agriculture pays main attention to biodiversity and maintenance of the ecosystem. Approaches like crop rotation, agroforestry, and integrated pest control not only guarantee soil fertility and biodiversity but also decrease dependence upon external inputs. Climate-resilient techniques—the use of drought-tolerant crops and water-efficient irrigation systems, in particular would help in limiting the consequences related to climate change while conserving the resources. It enhances soil health and ecosystem services through practices like cover cropping and reduced tillage, by improving fertility, carbon sequestration, and water retention. Organic farming, apart from producing better food with less synthetic chemicals, also protects biodiversity by minimizing environmental pollution (Thrupp et al., 2000). In rural areas it is important to invest in infrastructure reforms that will support farmers at small scale level or subsistence farming. This includes resource access fairness among others that help improve economic stability in rural communities towards a more food-secure region.

Agricultural practices have been transformed by conventional chemical fertilizers through increasing yields and food security. Growth and development can be enhanced through the instant release of nutrients that correct crop deficiencies. The use of chemical fertilizers in poor countries, mainly during the Green Revolution has led to increased agricultural output. They work towards precision agriculture through efficient utilization of resources as well as endorsing prevailing trends such as highly dense planting and modern irrigation systems (Pahalvi et al., 2021). Improved fertilizer formulation is aimed at enhancing efficiency while minimizing environmental pollution; however, misuse of this product leads to water pollution, soil degradation, and disarray in microbial community within soils. Mitigating these concerns involves accurate nutrient management strategies, sustainable farming methods and innovative technologies with biological and nanotechnology alternatives pointing to positive approaches for escalating agricultural adaptability and ecological conservationism.

Biofertilizers: Exploiting the Potential of Nature

Biofertilizers are the living microorganisms that contain natural compounds which improve development by increasing the provision or accessibility of essential nutrients to the host plant upon application to seeds, plants, or soil. Biofertilizers consist of different types of useful microorganisms such as nitrogen-fixing bacteria, phosphate-solubilizing microbes and growth promoting agents like enzymes and hormones. This rich composition promotes symbiotic connections between bacteria and plants, which improves soil fertility and nutrient uptake. Biofertilizers improve the quality of the soil by improving its texture, porosity, and soil moisture-holding capacity. They also enhance the formation of soil aggregates, promote organic matter decomposition, and foster improvement in soil fertility and plant growth. Besides, biofertilizers are environmentally friendly because they decompose and break down to non-toxic materials, leaving no harmful residues (Brahmaprakash and Sahu, 2012).

Mechanisms of Biofertilizers

Biofertilizers promote plant and soil health through several processes. They form associations like the Rhizobium-legume association, which are symbiotic in nature and enhance nutrient uptake and soil fertility. Auxins and other growth hormones like cytokinins secreted by microorganisms in the biofertilizers lead to root development and hence overall plant health. Apart from this, these biofertilizers excrete bioactive compounds that exert influence on plant development and microbial populations, hence enhancing the fertility of the soil and health of the plants, as reported by Hofmann et al. (2023). In addition to this, the products strengthen the constitutive immunity of the plants, rendering them resistant to infection by the induction of defense-related proteins and chemicals through a process called induced systemic resistance (Kannojia et al., 2019).

Furthermore, biofertilizers purify heavy metals through biosorption and chelation done by some bacteria and fungi to ensure overall soil health and safety of crops. They are capable of degrading certain pollutants in an effort to use plants for remediation; this is able to enhance the capacity of plants to absorb harmful substances.

According to Anli et al. (2020), biofertilizers produce osmoprotectants that help the plants to balance the cells under dry and saline conditions, hence improving the stress tolerance. Through all these mechanisms, biofertilizers contribute to sustainable agriculture by improving soil fertility, promoting healthy crops, and creating a clean environment.

Application of Biofertilizers

In the present methodology, various modes of application may be involved in enriching the fertility of the soil and promoting plant growth by using biofertilizers. (Anand and Kamaraj, 2017).

a) Seed treatment: The seed treatment with bio-fertilizers will enhance faster colonisation of beneficial microorganisms which helps in developing healthy seedlings. Soil application: The inoculation of bio-fertilizers into the soil during the preparation of the field enriches microbial diversity and availability of nutrients resulting in enriched soil quality.

a) Exposing the roots in bio-fertilizer solutions prior to planting can stimulate microbial colonization, reduce transplanting shock and can enhance root growth. The foliar application promotes nutrition absorption through leaves by misting the moisture content on the plants that enhances nutrition uptake and also systematic disease resistance.

c) Drip irrigation of fertilizers economizes water while dispensing the fertilizers to the root zone in exactly the required quantity.

Inoculation of biofertilizers combined with irrigation as a method of application, namely fertigation, improves nutrient availability and enhances microbes' activity, resulting in enhanced plant growth and higher yield.

The environment-friendly nature of the biofertilizers is followed by the disadvantages such as slow release, variable quality feed due to environmental factors, special storage conditions, high upfront investment, contamination, narrow nutritional spectrum, and skill requirement make them less attractive for general use.

Nanozyme-based biofertilizers are far better than their traditional counterparts. Nanozymes act as enzyme mimics and provide the formulation of biofertilizers with enhanced activity and stability but do not compromise the good microbes. Therefore, such formulations ensure a controlled and slow release of nutrients, thereby increasing their uptake by plants, encouraging plant growth, and raising their resistance to the environment. Besides, nanozymes enhance nutrient delivery and absorption at the cellular level, thereby increasing the crop yield with less extra fertilizer application. This method is eco-friendly because it reduces the requirements of chemical fertilizers by not allowing them to runoff and promotes efficient use of the natural resource, hence fit for sustainable agriculture.

Nanozyme Coupled Biofertilizers

Such nanozymes, which can be considered as nanomaterials endowed with catalytic properties similar to enzymes, have been in the limelight for several applications, including agricultural. Among the most important characteristics that make nanozymes highly applicable in agriculture are their size-dependent catalytic activity, tunable surface functionalities, and biocompatibility. In comparison with natural enzymes, nanozymes exhibit enhanced stability, catalytic efficiency, and adaptability and can replicate a number of enzymatic functions involved in agricultural applications. It includes the functions of these genes in the areas of soil remediation, nitrogen management, pest control, improvement of the plant growth, and stress tolerance (Manjunatha et al., 2016).

Characteristics of Nanozymes

These nanozymes will offer a number of properties that make them act as active catalysts in many biological processes, mimicking the activities of peroxidase, catalase, and superoxide dismutase. They are more stable to the harsh conditions like a wide pH range or high temperatures, and thus can be used for longer (Ma et al., 2023). One way through which the adaptability of nanozymes can be understood is that changes in their composition, variations in size, surface functionalization, and structure can fine-tune their catalytic activities so that they are efficient and selective for certain processes (Ali et al., 2021). In this context, many types of nanozymes have been compatible with biological systems or degradable after use, making them very suitable for biomedical applications where low cytotoxicity and minimal environmental impact are desired. These dimensional modifications can, in turn, modulate the size-dependent activities of these catalysts.

Also, they are substrate-specific and selective and may be adjusted in composition or structure, making them more adapted for the process of catalysis (Huang et al., 2019). Nanozymes are also robust to stand up to shoals in drastic conditions, be stable in catalyzing across pH and thermally stable in high temperature (Zhao et al., 2022). Hence, owing to their stability and reprocessability, they make very good materials for long-term operation in a wide range of applications, from the development of biomimetic systems to bio-inspired technology (Jiang et al., 2019). If it were possible to functionalize their surfaces to interact differently with substrates or a target system, then their value in biological and medical applications would be correspondingly increased (Liu and Liu, 2017).

Classification of Nanozymes

These nanozymes differ in composition and functionalities, hence portray their ability to be versatile in acting as different catalysts.

A) Metal-based nanozymes, including AuNPs, AgNPs, and FeNPs, have different capabilities as enzymes because they have different surface topographies and catalytic abilities. These get the same things done as peroxidase, catalase or oxidase that's why they are called the mimetic enzymes (Liu et al., 2021). Hence the reason why their resistance and flexible catalytic properties have fascinated people from medicine to ecology to industry (Huang et al., 2019).

B) Based on Zhu et al. (2022), carbon-based nanozymes like CNTs and graphene-based structures act in a similar manner as natural enzymes. Thus the electron transfer based on redox reactions can occur using these compounds that resemble oxidoreductases, peroxidases and hydrolases therefore useful for biosensing, drug delivery systems and environmental remediation (Lee and Kamruzzaman, 2023).Polymeric nanozymes made out of organic polymers or polymer based nanoparticles take advantage of their forms and surfaces to mimic enzymatic activities. Dendrimers such as polymer-coated nanoparticles suggest certain features of catalytic activities exhibited by proteases or even by peroxidases acts catalytically like *polymer coated NPs* which means it works like any other enzyme in human bodies. In such fields as biomedicine, sensing, and therapy, these materials have a lot of potential due to their biocompatibility, ease of production, and variable catalytic properties (Huang et al., 2023).

C) Quantum dot nanozymes (QDs) demonstrate peroxidase and catalase functions mimicking enzymes. These semiconducting nanoparticles have flexible dimensions for light-induced degradation resistant with effective catalytic capabilities enabling them to detect biological materials, visualize structure and deliver targeted drugs (Devi et al., 2021).

D) Liposomes and lipid-coated nanoparticles are structurally similar to cell membranes which are the same as enzymes such as lipases, phosphatases, esterases. Their bio-compatibility features and simplicity in terms of functionalization make them appropriate for drug delivery systems (Wang et al., 2023).

E) Nanozymes made from proteins resemble natural enzymes and they enable one to regulate catalytic processes with precision through their adjustable activity levels. There is an application of protein folding principles in nanomedicine as well as biotechnology and synthetic biology inspired nanostructures (Wang et al., 2023).

F) DNAzymes are artificial DNA sequences with different types of enzyme activities like nuclease or ligase that can include peroxidase.Their programmability, chemical resistance, and potential for biosensing, diagnostics, and nanotechnology-based treatments make them versatile tools in biomedical research and analytical chemistry (Zhou et al., 2023).

Nanozyme Mechanisms

Nanozymes work like redox catalysts through the imitation of peroxidases, catalases, and oxidases that help in breaking down reactive oxygen species (ROS) as well as promoting oxidation-reduction reactions. Some nanozymes also have hydrolytic properties and can break chemical bonds like hydrolases (Huang et al., 2019). This size dependence arises from quantum confinement effects (quantum size control); the changes in electronic states with sizes govern their distinctive intrinsic catalytic activity. An increased surface area to volume ratios leads to more active sites for catalytic activity relative solid-state of the materials. Some metallic nanozymes show LSPR (Localized Surface Plasmon Resonance) because the migration of unbound electrons increases catalytic activity due to the concentration of electromagnetic fields and facilitation of electron transfer processes (Wang et al., 2023). Bimetallic nanozymes exhibit synergistic interactions between the two metals that make up the system, which leads to increased catalytic capabilities or substrate selectivities (Zhou et al., 2023). Additionally, under different environmental conditions, the structure of nanozyme surfaces can undergo changes, which can positively impact catalytic activity by altering the surface (Pietrzak and Ivanova, 2021). When placed in external magnetic fields, magnetic nanozymes display modified catalytic behaviors, allowing for remote-controlled or magnet-directed catalysis (Afzal et al., 2021). The catalytic activity heavily depends on surface characteristics like shape and size, which strongly influence adsorption and reaction kinetics, especially near edges and corners (Dong et al., 2022). By incorporating nanozymes, it can overcome the limitation of heterogeneous nutrient release and poor absorption of the biofertilizer by encapsulating/modifying its components to ensure highly accurate nutrient release at a target point. A controlled release system reduces leaching and volatilization of the nutrients, thereby improving nutrient uptake and reducing environmental pollution. The nanozymes act as catalyzers, much like enzymes, breaking down complex organic compounds to simple ones, increasing nutrient availability in the biofertilizer. They are also known to stabilize the components of the biofertilizer, in effect acting as preservatives that maintain the effectiveness till use (Attia and Saad, 2001). Coupled nanozymes with biofertilizers thus can make farming much more ecofriendly, reducing environmental pollution, providing easier nutrient uptake, improving soil fertility, and promoting eco-friendly agriculture (Al-Mamun et al., 2021).

Synergistic Approach: Nanozymes with Biofertilizers

Such a combination of nanozymes and biofertilizers can exert a synergistic effect; that is, the digestion of organic molecules by nanozymes can increase the bioavailability of nutrients for the beneficial microorganisms in the biofertilizer. Therefore, this collaborative effect helps further strengthen the cycling of elements to create the most favorable soil environment for the growth and activities of microorganisms, hence enhancing nutrient uptake by plants. Nanozymes accelerate the conversion of complex chemical molecules into simpler forms that can be easily absorbed by the plants. Application of biofertilizers containing potent microorganisms could enhance nutrient mobilization and uptake in improving plant growth and yield. The combination of nanozymes and biofertilizers is a green strategy over conventional agrochemicals, and offers a solution to environmental pollution and soil degradation. High technology in sustainable agriculture supports the preservation of fertility and biodiversity in soils while reducing the negative impacts related to synthetic inputs on ecosystems.

Applications of Nanozyme-Coupled Biofertilizers

The Catalytic Potential of Nanoenzymes

Because of their gigantic catalytic properties, nanozymes can improve the efficiency of applied biofertilizers. For instance, nanozymes synthesized from metal NPs such as iron or copper oxides will take on the activity of enzymes and increase the speed at which important nutrients are available to plants in growth. Such nanomaterials increase the decomposition of organic matter, increasing the release of nutrients into the soil, therefore releasing more significant amounts of crucial elements such as nitrogen, phosphorus, and potassium. (Patel et al., 2023). Having an extremely high catalytic efficiency, these nanomaterials facilitate enzymatic reactions in the soil, stimulate microbial activity, and finally create a nutrient-rich environment that helps promote plant well-being.

Controlled Release Systems

On association with biofertilizers, nanozymes develop intricate mechanisms of controlled release. Nanozymes involve the trapping of the bioactive substances, enzymes, or growth regulators within the nanostructures with tunable features that enable controlled release over a longer period of time. For instance, the silica-based nanozymes loaded with plant growth-promoting substances, upon mixing with biofertilizers, release the growth stimulants into the soil in a steady and sustained manner. This will ensure that the plants grow steadily and progressively for a longer period of time (Vejan et al., 2021).

Manipulation of Soil Microbiota

The combination of nanozymes with biofertilizers alters the soil microbiome for the better, and this has a positive effect on plant health. Nanostructures, mainly composed of carbon-based nanotubes or graphene oxide in biofertilizers, influence microbial activity and diversity. According to Sambangi et al. (2022), these nanomaterials enhance the interaction between bacteria and root exudates, thus showing their positive effect on microbial populations. They can enhance the multiplication of microorganisms that are beneficial in enhancing the sharing of nutrients within the soil, improving soil structure, and increasing resistance to plant diseases by colonizing the rhizosphere.

Improved Resilience to Stress

The nanozymes' embedding into the biofertilizer is a very effective way to improve plants' resistance to stressful conditions. These nanoparticles contain antioxidants capable of reducing oxidative stress induced by a number of environmental factors such as drought, salinity, or metal ions. For instance, selenium or ceria nanozymes, when incorporated into bio-fertilizers, can function as antioxidants by clearing reactive oxygen species and alleviating stress-induced harm in plants (Husen et al., 2021)

Environmental Sustainability

Such combined applications of nanozymes and biofertilizers lower chemical dependencies, environmental contaminations, and promote sustainable practices of farming for better soil quality and productivity. Interactions of nanozymes and biofertilizers decrease the burden of applying synthetic fertilizers and agrochemicals, thereby solving environmental pollution, soil erosion, and other connected ecological risks. This is an integrated approach toward viable soil management, as it changes the structure of the soil, nutrient cycling, and the diversity of microbes. This reduces the quantity of chemicals used, hence their adverse effects on the health of the general ecosystem, and preserves the fertility of the soil in the long term (Zulfiqar et al., 2019).

Sustainable Precision Agriculture

In case of biofertilizers, nanozymes represent a more environment-friendly and it involves a very accurate form of farming called precision farming. It makes use of nanotechnology's precision and catalytic features to ensure efficiency in resource use, hence not overdosing on nutrients. This might make it possible for nanozyme-based biofertilizers to deliver in a tailored way, ecological footprint reduction, and sustainable agriculture with crop production balanced against environmental conservation. This new technique is part of the changing times of farming. It provides accurate, efficient, and environmentally sensitive solutions to the current challenges facing agriculture.

Sequential Synthesis of Nanozyme-Coupled Biofertilizers

Nanozymes and Biofertilizer Matrix Selection

The integration of nanotechnology with agronomic research is implemented in formulating

nanozyme conjugated biofertilizers through approaches that combine nanostructures with biofertilizer supports. Those nanozymes, as metal nanoparticles and carbon-based nanostructures, are chosen for their ability to replicate the essential enzymatic catalytic mechanisms that occur in nutrient transformation. The surface is then modified superbly towards enhancing stability by mimicking enzymes and corresponds to the carrier of biofertilizers. The matrix, usually composed of biopolymers or natural substrates, is formulated to favor the efficient incorporation of the nanozymes, which in turn assures that they perform in the best way possible in agricultural settings.

Synthesis and Modification of Nanozymes

By utilizing techniques of encapsulation, such as emulsion, co-precipitation, or layer-by-layer, the nanozymes assembly process with the carrier matrix is a crucial task. These methods ensure homogeneous dispersion and long-term entrapment of nanostructures within the biofertilizer matrix. Strategic optimization at this stage can guarantee precise control over it. This would further regulate the nanozymes' release rate and prevent possible inadvertent loss or aggregation (Sarkar et al., 2023).

Characterization and Quality Control

After synthesis, detailed investigation using advanced spectroscopic and imaging methods—including transmission electron microscopy, scanning electron microscopy, Fourier-transform infrared spectroscopy, and so on— enables one to obtain all the detailed information about physical and chemical characteristics, distribution, and interaction kinetics of obtained compounds, which are products of the interaction of nanozymes and biofertilizers. Strict control at all stages of their production ensures uniformity and structural soundness, which is required to be a batch of effectiveness and upscaling potential for the formulation.

After performing a reduction reaction detailed assessment at this stage with the aid of state-of-the-art spectroscopic and imaging tools including TEM, SEM, and FTIR will be possible which allows us to explore their interaction with the biofertilizers and their physical-chemical features.

Nanozyme complexes will require an extensive QC program that will prove consistency, uniformity, and structural integrity for each batch. This factor is key to the securing of stability and scalability of a formulation.

Expansion and Implementation

Compatibility tests indicate that nanozymes retain their catalytic activity throughout the biofertilizer matrix and are resistant to some type of environmental stress. In addition, stability studies in different conditions will answer how robust the formulation is in terms of agricultural longevity. Therefore, field trials are so important to evaluate the real efficiency of enzyme-enhanced biofertilizers. These trials assess their effect on crop yields, soil health, and environmental sustainability to refine formulations for best performance across multiple sites.

Working of NBF Application, and Its Uptake and Translocation in Plants

NBFs can be applied by seed priming, foliar spraying, or soil (Sharma et al., 2023). Applying NBFs to the soil can increase plant growth and replenish soil fertility. Heterogeneous aggregation between NPs and soil particles substantially limits the activity, mobility, and bioavailability of NBF in soil. Foliar spraying NBF would be a more effective way to enable NPs to enter plant tissues quickly (Bairwa et al., 2023). A pre-planting technique called "seed priming" entails soaking seeds in NBFs to hasten seed germination, increase seedling growth, and use less fertilizer. Stress resistance genes are upregulated, and chemicals that reduce reactive oxygen species and promote plant development are stimulated (Nile et al., 2022).

After coming into touch with the surfaces of seeds, roots, and leaves, nanoparticles cling to plant surfaces by hydrophobic, electrostatic, and Vander Waals forces (Bashir et al., 2022). NPs are mostly absorbed by roots through physiologically active lateral roots and root hairs, as opposed to the leaf, where they enter largely through stomata but also through trichomes. After entering roots and leaves, NPs move upward through the xylem (apoplastic transport) and downward through phloem tissues (symplastic transport). NPs enter plant cells and travel between cells by a variety of pathways, including plasmodesmata, endocytosis, ion transporters, and cell wall pores (Rani et al., 2023). The processes that control NPs' entry and migration into plant cells are greatly influenced by their chemical makeup, size, shape, and aggregation state. Since different plants have different receptors, it also depends on the species of plant. A plant can act as an accumulator for some types of NP and as an excluder for others (Masarovicova and Kralova 2013).

Significance of NBFs

To throw a light on the significance of NBFs in the agrochemical systems, following are the three case studies that carried out in three different region of the world on three different plants. These studies reveal that plants show a better growth when they are exposed to NBFs.

Peanut *(Arachis hypogea L.)*

Peanut *(Arachis hypogea L.)* is an essential and inexpensive crop with high oil, protein-based content as well carbohydrate in its multiple grown tropical subtropical regions of the world (Shahid et al. 2010). Plants need Boron (B) and Calcium (Ca) from peg emergence to pod fill, since B is necessary for protein synthesis, and meristematic tissue growth/cell elongation/maturity. Ca and B deficiencies result in a high rate of aborted seeds and underfilled pods, greatly lowering yields and shelling percentages (Walker, 1975; Yang et al., 2020). Abdelghani et al. (2022) investigated the effect of B and Ca nanoparticles (NPs) and biofertilizers on nutrient uptake in 2020 and 2021. The study assessed the impact of these treatments on nitrogen, phosphorous and potassium (NPK) uptake from sandy soil along with peanut seed oil and protein yield. Mycorrhiza and combination of nano-B with mycorrhiza had the highest percentage of nitrogen (3.9%) and phosphorus (1.4%), respectively as shown in Table 1, for two seasons. In the first season, mycorrhiza and nano-Ca or phosphorine and nano-Ca had the highest potassium concentration (0.3%), followed by mycorrhiza and nano-B in the second season (0.4%). In the first season, phosphorine and foliar nano Ca+B yielded the maximum oil content (45.6%), followed by mycorrhiza and nano-B in the second season (48.9%). Mycorrhiza and nano-B produced the highest protein content in both seasons (25.1% and 25.2%), respectively. Mycorrhizal distribution enhances plant growth and nutrient absorption. (Moradi et al., 2020).

Table 1: Effects of interaction between biofertilizer and nanoparticle treatments on peanut seed biochemical traits across season 2021(Abdelghani et al., 2022).

Bio fertilizers	Nano Particles	Nitrogen		Phosphorous		Potassium		Oil		Protein	
		2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
	Control	2.2^{e}	2.6^{d}	1.1^{d}	1.1^{f}	0.1^{cde}	0.1^{c}	29.1^{d}	29.1^{f}	18.3^{f}	18.3^{f}
Control	Ca	2.2^{e}	3.4^{abc}	1.2^{bcd}	1.2^{def}	0.1^{cde}	0.1^{bc}	33.6^{c}	32.6^{ef}	20.6^{def}	19.4^{f}
	B	2.6^{de}	3.7^{ab}	1.1^{d}	1.1^{ef}	0.3^{a}	0.2^{c}	^{Cd}	38.6^{cd}	19.3^{ef}	20.5^{cdef}
	Ca+B	2.3^{e}	3.5^{abc}	1.3^{ab}	1.3^{abc}	0.1^{cde}	0.1^{c}	32.4^{cd}	32.4^{ef}	21.6^{cde}	19.9^{def}
	Control	3.0^{cd}	3.0^{cd}	1.1^{cd}	1.1^{f}	0.1^{fg}	0.1^{c}	35.1^{c}	35.1^{de}	20.9^{de}	20.3^{cdef}
Mychorrihza	Ca	3.2^{bcd}	3.2^{bc}	1.3^{ab}	1.2^{def}	0.3^{a}	0.3^{a}	39.8^{b}	41.5^{bc}	22.2^{bcd}	24.1^{ab}
	B	3.9^{a}	3.9^{a}	1.4^{a}	1.4^{ab}	0.1^{def}	0.4^{a}	39.2^{b}	48.9^{a}	25.1^{a}	25.1^{a}
	Ca+B	3.7^{ab}	3.6^{abc}	1.4^{a}	1.4^{a}	0.1^{c}	0.1^{c}	44.9^{a}	47.2^{a}	23.6^{abc}	23.0^{abc}
	Control	3.5^{abc}	3.0^{cd}	1.1^{d}	1.1^{f}	0.1^{cd}	0.1^{c}	32.9^{cd}	32.9^{ef}	19.3^{ef}	19.3^{ef}
Phosphorine	Ca	3.2^{bcd}	3.2^{bcd}	1.3^{ab}	1.3^{bcd}	0.3^{b}	0.3^{ab}	33.9^{c}	33.6^{e}	21.0^{de}	21.4^{de}
	B	3.8^{a}	3.8^{a}	1.1^{d}	1.2^{def}	0.1^{efg}	0.1^{c}	39.6^{b}	39.6^{c}	24.3^{ab}	24.3^{ab}
	Ca+B	3.5^{abc}	3.5^{abc}	1.3^{bc}	1.2^{cde}	0.1^{g}	0.1^{c}	45.6^{a}	45.6^{ab}	22.7^{bcd}	22.7^{abcd}

Different lowercase letters on error bars indicate statistically significant differences between treatments ($p \leq 0.05$), as performed by the least significant difference (Fisher's LSD) test.

Cotton (*GossypiumhirsutumL.*)

Plant-eating insects are major global threats to world crop production, *Spodoptera littoralis* one among them feeds on cotton and other crops substantially. Metwally et al. (2022) explored the combination of *Beauvaria bassiana* silicon nanoparticles (Si NPs), arbuscular mycorrhizal (AM) fungus as novel approaches to enhance defense in cotton against *S.*

littoralis. Table 2 shows how *S. littoralis* infestation affected cotton plant growth parameters. Under regulated conditions, AM cotton plants had a considerably greater shoot fresh weight (Fwt) (4.9 g/plant) than non-AM plants (3.6 g/plant). Cotton plants infected with *S. littoralis* were treated with AM fungi and either *B. bassiana* Si NPs or Chlorpyrifos insecticide, resulting in an increase in both shoot Fwt and root dry weight. This revealed that AM fungi would improve immunity when challenged with pests by triggering the nitrogen uptake and boosting immunological responses in comparison to non-AM plants (Abdelhameed et al., 2021). On the other side, phenotypic traits of cotton plants treated with Chlorpyrifos manifested as a slight decrease suggesting stress and also changes in cellular activity The edible residues for male *C. medinalis* are according to FAO from 0-2 days (summer) or >1 day (winter). These data indicated that AM fungi potentially promoted the development of cotton and resistance against insect, which magnified their application in IPM.

Table 2: Fresh (Fwt) and dry weights (Dwt) of *mycorrhizal* (AM) and non-mycorrhizal shoots and roots of cotton plants reared with (+) or without (−) S. littoralis under different treatments (Metwally et al., 2022)

	Fwt (g/plant)			Dwt (g/plant)			MD%
Treatments	Shoot	Root	Total	Shoot	Root	Total	
-S. littoralis	3.638^{bc}	0.539^{ab}	4.178^{bc}	1.044^{ab}	0.145^{b}	1.189^{ab}	23.07^{c}
-S. littoralis + AM	4.906^{a}	0.674^{a}	5.581^{a}	1.342^{a}	0.204^{ab}	1.546^{a}	
+S. littoralis	3.020^{c}	0.520^{ab}	3.540^{c}	0.710^{bc}	0.113^{b}	0.823^{bc}	24.48^{b}
+S. littoralis + AM	4.501^{ab}	0.591^{ab}	5.092^{ab}	0.950^{abc}	0.140^{b}	1.090^{abc}	
+S. littoralis + B. bassiana Si NPs	2.953^{c}	0.360^{b}	3.313^{c}	0.675^{bc}	0.072^{b}	0.747^{bc}	34.50^{a}
+S. littoralis +B. bassiana Si NPs +AM	4.43^{ab}	0.413^{b}	4.843^{ab}	0.700^{bc}	0.440^{a}	1.140^{abc}	
+S. littoralis + Chlorpyrifos	2.961^{c}	0.450^{ab}	3.411^{c}	0.590^{c}	0.104^{b}	0.694^{c}	22.31^{d}
+S. littoralis + Chlorpyrifos+ AM	4.303^{ab}	0.492^{ab}	4.795^{ab}	0.651^{bc}	0.243^{a}b	0.894^{bc}	

Wheat (*Triticum aestivum L.*)

Drought stress in plants causes lower grain yield and oxidative damage from reactive oxygen species (ROS). Sharifi *et al.* used a combination of nanofertilizers (nano zinc oxide, nano iron oxide, and nano Zn-Fe oxide at 1.5 g L^{-1}) and biofertilizers (*Azotobacter*, *Azosperilium*, and *Pseudomonas*) on wheat (*Triticum aestivum* L.) at different irrigation levels to offset these impacts. Table 3 reveals that under extreme water constraints, *Azotobacter* with nano Zn-Fe oxide boosted grain production by 88%. Normal watering produced the maximum yield (331.7 g/m^{-2}). *Azotobacter* inoculation and nano Zn-Fe oxide resulted in the lowest yield (98.5 g m^{-2}) under severe water scarcity without fertilizer. The applications of oxide nanoparticles in plants raised peroxidase activity by 27%. Under normal irrigation, the supplementation of azotobacter and nano Zn-Fe oxide results in the highest level of chlorophyll, 4.59 mg g −1 FW, while the lowest under severe water scarcity conditions, 1.43 mg g −1 FW, in the absence of fertilizers. This mixture increases ROS scavenging and improves plants' tolerance to stress.

Table 3: Effects of irrigation, biofertilizer, and nanooxide on grain yield, chlorophyll and peroxidase production. (Sharifi *et al.*, 2020)

Treatment		Grain Yield (gm^{-2})				Chlorophyll				Peroxidase			
I	B	N_o	N_1	N_2	N_3	N_o	N_1	N_2	N_3	N_o	N_1	N_2	N_3
I_1	F_o	158.33^{no}	205.41^{ghi}	194.16^{g-k}	225^{ef}	2.05^{pq}	2.65^{jkl}	2.91^{hi}	3.21^{efg}	2.36^{w}	2.43^{vw}	2.55^{uvw}	2.76^{t-w}
	F_1	230.41^{de}	285.41^{b}	256.66^{c}	331.66^{a}	3.52^{d}	3.80^{c}	4.30^{b}	4.59^{a}	3.18^{q-t}	3.42^{n-s}	4.25^{ijk}	4.56^{i}
	F_2	204.16^{ghi}	246.25^{cd}	210.00^{fg}	288.33^{b}	3.23^{efg}	3.50^{d}	3.78^{c}	3.90^{c}	3.12^{rst}	3.06^{stu}	3.68^{i-q}	3.79^{k-p}
	F_3	162.91^{no}	244.16^{cd}	199.16^{g-j}	279.16^{b}	2.61^{jkl}	3.15^{fgh}	3.43^{de}	3.42^{de}	3.31^{o-s}	2.94^{s-v}	3.82^{k-o}	4.14^{i-l}
I_2	F_o	127.08^{stu}	155.83^{nop}	146.66^{opq}	172.50^{lmn}	1.77^{Rst}	1.91^{Rq}	2.31^{no}	2.58^{klm}	3.13^{rst}	3.30^{p-s}	4.27^{ijk}	4.00^{j-m}
	F_1	186.25^{jkl}	210.00^{fg}	209.16^{fgh}	243.75^{cd}	2.57^{klm}	2.83^{lj}	3.11^{gh}	3.37^{def}	5.22^{g}	5.19^{g}	6.18^{de}	6.27^{de}
	F_2	166.66^{mn}	192.08^{h-k}	190.41^{ijk}	225.00^{ef}	2.34^{mn}	2.50^{Lmn}	2.81^{ijk}	2.83^{ij}	4.61^{Hi}	5.09^{Gh}	5.34^{g}	5.98^{ef}
	F_3	147.91^{opq}	181.25^{klm}	155.83^{nop}	199.58^{g-j}	2.08^{opq}	2.20^{nop}	$2.43lmn$	2.79^{ijk}	3.91^{k-n}	4.44^{lj}	4.56^{l}	3.87^{k-n}
I_3	F_o	98.95^{w}	109.89^{uvw}	104.79^{vw}	119.37^{tuv}	1.43^{u}	1.50^{u}	1.49^{u}	1.63^{stu}	3.62^{m-q}	3.79^{k-p}	3.20^{df}	6.04^{ef}
	F_1	140.00^{p-s}	182.91^{j-m}	146.25^{o-}r	186.25^{jkl}	2.35^{mn}	$2.50lmn$	3.13^{fgh}	3.33^{d-g}	6.92^{c}	7.00^{bc}	7.50^{b}	8.89^{a}
	F_2	121.66^{tuv}	132.91^{q-t}	129.16^{rst}	170.83^{lmn}	1.86^{Qrs}	2.02^{q}	2.32^{no}	2.85^{ij}	6.62^{cd}	6.84^{c}	6.85^{c}	7.06^{bc}
	F_3	109.58^{vw}	128.75^{st}	120.83^{tuv}	161.25^{no}	1.50^{stu}	1.61^{tu}	2.06^{pq}	2.57^{klm}	5.53^{fg}	5.99^{ef}	6.67^{cd}	6.67^{cd}

I_1, I_2, and I_3 are normal irrigation, moderate water limitation, and severe water limitation respectively. F_0, F_1, F_2 and F_3 indicate noninoculation, inoculation with *Azotobacter, Azosperilium, Pseudomonas* respectively. N_0: without nanofertilizer (as control), N_1: nano Fe oxide; N_2: nano Zn oxide; N_3: nano Zn-Fe oxide. Means followed by different alphabets indicate significant difference between treatments at P ≤ 0.05 by using the Least Significant Difference test.

Potential Risks Associated with NBFs in Agriculture

The persistent nature of nanomaterials and their potential to accumulate in soil and water system makes them a concern for ecological sustainability. Thus, they can change soil microbial composition and impair plant growth and development, therefore altering ecological equilibrium. Hence, their long-term environmental distribution and bioaccumulation and potential toxicity need to be thoroughly investigated. Moreover, humans could be exposed to such materials during synthesis, application, or food chains ingestion, leading to potential health problems after inhalation or skin contact due to cutaneous sensitivity or respiratory problems. Finally, the production of ROS and non-selective cellular death that occasionally results in apoptosis, exhibited by nanomaterials may be harmful to plant growth aspect. Therefore, they need to be assessed for dosage reaction and their effects if mortality mechanisms in biological systems are not comprehended. Several dosage metrics can reduce the adverse reactions of such nanoparticles, e.g. particle charge, concentration, and surface area and dimension size and toxicity. Furthermore, some nanoparticles may alter the optimal configuration of beneficial soil bacteria. Some of these nanoparticles, such as AgNPs, will impair the viability of essential soil bacteria at lower doses, directly affecting plant growth. Therefore, to prevent environmental degradation when using nanoparticles as biofertilizers, compatibility is among the key selection criterions.

Although ecotoxicological aspects of various biofertilizers can be studied using post-nano formulations, there is significant potential for these technologies in agriculture, from processing to final delivery.

Nanotechnology is addressing environmental challenges with innovative solutions. Despite recent advances in nanotechnology improving biomedical diagnostics and treatments, understanding plant interactions with nanomaterials is still emerging. Research on developing environmentally friendly biofertilizers is limited. Further investigation into key parameters will enable biofertilizers to support sustainable agriculture and become a cutting-edge tool for promoting agricultural development.

Future Perspective

Nanotechnology presents immense potential to revolutionize agriculture but necessitates a cautious approach due to inherent risks. Rigorous risk assessment, standardized monitoring protocols, robust regulations, and ongoing research are paramount to harness the benefits of nanomaterials while mitigating potential environmental and health risks associated with their use in agriculture. A balanced approach, involving collaboration among researchers, policymakers, industry, and the public, is crucial to ensure the responsible and sustainable integration of nanotechnology into agricultural practices. Following are some points through which the potential of nanotechnology in agrochemical system can be enhanced.

i. A versatile NBF appropriate for a variety of crops must be developed.

ii. Cutting-edge technology, protocols, machine learning, and artificial intelligence (AI) must be combined to produce NBF that is of the highest quality, has a longer shelf life, is inexpensive, and is easy to use.

iv. NBF needs to be carefully investigated to ascertain how they affect human health and the environment.

v. Further research is needed to determine how NBF affects the physicochemical, biochemical, and molecular systems of plants at the cellular and molecular levels in both ambient and stressful environments.

vi. Research is required to determine how NBFs affect the agricultural ecology over the course of their long life cycle.

vii. Farmers need to be informed about the disadvantages of chemical fertilizers and the ways in which NBFs can save expenses and yield long-term gains.

Table 4: Comparison of Nanofertilizers and Conventional Fertilizers Applications (Nagula and Usha, 2016)

	Properties	Nano fertilizers	Conventional fertilizers
1	Solubility and dispersion of ‘mineral micronutrients	Reduce soil absorption and fixation, increase soil bioavailability, and improve the solubility and dispersion of insoluble nutrients.	Reduced solubility and big particle size resulting in decreased bioavailability for plants
2	Nutrient uptake efficiency	Might improve the absorption ratio of soil nutrients and fertiliser efficiency in crop production while conserving fertiliser resources.	For roots, bulk composite is unavailable and reduces efficiency.
3	Controlled-release modes	Encapsulating water soluble fertilizers in envelope forms allows for fine control over the release pattern and rate of nutrients.	Overuse of fertilizers can lead to toxicity and upset the soil's natural equilibrium.
4	Effective duration of nutrient release	The effective time that fertilizers give nutrients to the soil can be increased with nanofertilizers.	Utilised by the plants at the time of delivery, the remainder is transformed by the soil into insoluble salts.
5	Loss rate of fertilizer nutrients	Lower the rate at which fertilizers nutrients seep or drain into the soil.	High rate of loss due to drift, rain, and leaching.

Conclusion

Nanotechnology is unique because of its new ways to solve agriculture problems. Because they show enhanced plant growth, targeted nutrient delivery in soils and a strong systemic resistance compared to NPs or biofertilizers alone, these crop sustainability effects may make an NBF open up new possibilities. NBFs have a lot to offer agriculture; hence it is imperative that they be manufactured industrially using stable formulas and eco-friendly methods. The green synthesis of NBFs is an intriguing endeavor since a lot of work is being done to use natural resources and biological synthesis processes, which have several established advantages including being easy to scale up, affordable, and ecologically beneficial. However, we really do not understand how plants sense and interact with nanoparticles. Nanobiofertilizer Formulations could also be a solution to make agriculture sustainable. These also are non-toxic nanocarriers for efficient nano biofertilizer formulations. Still, further research is required to know the effect of NMs on the growth meantime need for strength and rationality in nanotechnology that could be used better fertilizers for maximum agricultural production. Although NMs have a lot of positive effects on plants, phytotoxicity is one major problem. Further investigations of NMs' functions in both normal and stressed plant environments are required to provide a better understanding because they can modulate the cellular and molecular levels on plants. Even though the exact process that NMs interact with plants is not known, it can be helpful for future studying in this area. A fruitful and necessary enhancement of interdisciplinary collaboration strategies will fill the gap of knowledge on NMs applications in agriculture, especially for plant science-related issues. Ilotan and Regep are key NBFs because they could help usher in a thriving agro-economy through better output, efficient resource savings, increased safety of the environment from pollutants (which is an ongoing challenge) as well deal with unpredictable weather due to climate change.

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