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The use of conventional fertilizers and pesticides is not sustainable for a number of reasons, including high inefficiency of delivery and utilization, significant inputs of energy and water, and great potential for negative environmental implications. Achieving and sustaining global food security is a global grand challenge will require agricultural practices to be modified and perhaps revolutionized so as to effectively combat the negative pressure from a changing climate, increasing population and loss of arable land. Many ENMs have potential to enhance crop growth and increase yield, although an understanding of basic mechanistic processes is sorely lacking. It is widely known that robust plant nutrition can dramatically improve crop defense against pathogenic diseases. This review will highlight our current understanding on the use of nano-enabled fertilizers and pesticides to suppress crop disease and enhance food production. A discussion of key knowledge gaps and needed future direction will be included.

Recent advances in nano-enabled fertilizers and pesticides: A critical review of mechanisms of action

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Abstract

The use of nanomaterials in agriculture as nanofertilizers, nanopesticides, or nano-enabled sensors to increase crop yield is gaining increasing interest. Engineered nanomaterials (ENMs) can improve crop productivity by influencing fertilizer nutrient availability in soil and uptake by plants. These materials can suppress crop diseases by directly acting on pathogens through a variety of mechanisms, including the generation of reactive oxygen species (ROS). ENMs may also suppress disease indirectly by improving crop nutrition and enhancing plant defense pathways. Efficient use of ENMs may complement or replace conventional fertilizers and pesticides, subsequently reducing the environmental impact of agricultural practices. This review evaluates the current literature on ENMs used as pesticides and fertilizers, and highlights critical knowledge gaps that must be addressed to ensure sustainable application of nanotechnology in agriculture so as to achieve global food security.

1. Introduction

The rise in global population, combined with improved income and dietary changes, is driving an ever-increasing food demand that is expected to rise by 70% in 2050.[1] Agriculture is the major source of food and feed for humans and domestic animals. However, agricultural crop pests, climate change events such as drought, and low nutrient use efficiency are significant hindrances to achieving global food security.[2] Over 22,000 species of plant pathogens, weeds, insects and mites are attacking farm produce globally.[3] Annually, China and the United States utilize approximately 1,806 and 386 millions of kilograms of pesticides, respectively. Yet, economic losses caused by crop diseases and pests in the United States are estimated at several billions of dollars annually. In the United States, efforts to combat fungal pathogens alone exceed $600 million annually.[4,5] This level of economic loss and inefficiency in food production continue to confound efforts aimed at achieving and maintaining food security.[4] The management of plant diseases and pests is particularly challenging, both in terms of timely identification of disease and due to the limited number of management options.

The most successful approach among the conventional methods of disease management strategies is the development of host resistance crop varieties.[6] However, not all crops inherently possess resistance genes against pathogenic diseases and there continues to be significant societal unease over genetically modified foods. It is known that micronutrients such as Cu, Mn, and Zn are critical for the activation of enzymes and the synthesis of biomolecules involved in plant defense. However, the efficacy of conventional fertilizer-micronutrient amendments is hindered by low nutrient bioavailability in neutral to alkaline soils and poor basipetal transport in plants.[7,8] Similarly, the use of conventional pesticides (including insecticides and herbicides) is encumbered with the challenges of excessive use of the chemicals and unintended contamination of the environment. Hence, there is urgent need for sustainable alternative strategies to improve crop production and to manage plant pests and diseases. There has been interest in the use of nanotechnology in agriculture for nearly 15 years, although successful application has been somewhat elusive. Nevertheless, the use of engineered nanomaterials (ENMs) in plant disease management and soil fertilization has garnered increased interest recently, with various reports demonstrating significant potential. A number of ENMs have been reported to improve growth, enhance nutrient use efficiency, and suppress diseases in plants in greenhouse experiments and a small number of field trials.[9,10] In addition, the use of ENMs as a potential alternative in the protection of plants against pests and weeds is gaining interest, although few studies have been conducted in this area.[11] This review evaluates current opportunities for the application of ENMs in agriculture, focusing on nanotechnology-enabled fertilizers and pesticides (including microbes, insecticides and herbicides), henceforth referred to as nanofertilizers and nanopesticides. A number of the reported articles were critically evaluated based on the efficacy of ENMs employed in the research, the experimental design, potential environmental impacts, and relative comparison with conventional commercial products. In addition to surveying the existing literature, a discussion of potential mechanisms of action is included, as well as perspectives on knowledge gaps to be filled, prior to the successful and sustainable application of nanotechnology in agriculture.

2. Nanotechnology and agriculture

The application of nanotechnology cuts across important human endeavors including agriculture, medicine, cosmetics, electronics, pharmaceuticals, water treatment, and environmental remediation.[12] A robust literature on the toxicological interactions of ENMs with plants has developed, although we note that a significant fraction of these studies were focused on hazard assessment and as such, involved short term high dose exposures, often under model conditions. In contrast, there has been relatively less focus on the beneficial impact of ENMs on plants.[9] A tutorial review by Rodrigues *et al*.[13] identified various promising opportunities for applying nanotechnology to improve sustainable agri-food systems. These include improved technologies for controlled release and target delivery of agrochemicals (nanofertilizers and nanopesticides) to control pathogens and thus, increase food safety and security; and sensors for assessing specific conditions or analytes of interest in plant systems. Advances in pathogen and toxin detection in plants have also been reported.[13] These applications provide a promising platform, which invariably makes ENMs better alternatives to conventional fertilizers and pesticides. Moreover, ENMs may be incorporated into conventional fertilizers and pesticides to enhance product efficiency, with the ENMs being embedded within bulk formulations or as the sole active agent.[9,11] Several studies have evaluated the efficacy of different ENMs on plant growth and productivity, as well as on disease suppression and nutritional enhancement.[10] The most commonly studied ENMs include metalloids, metallic oxides, or non-metals. Specifically, metals or metallic oxides of silver (Ag), cerium (Ce), copper (Cu), manganese (Mn), titanium (Ti), and zinc (Zn) have been used.[7,14-19] Other organic-based biopolymeric nanoparticles, such as chitosan [20,21] and β-D-glycan,[22] have been used solely or amended with other ENMs to improve plant growth and/or combat plant diseases. Further, ENMs such as silica, Ag, Al_2O_3, TiO, and ZnO have been shown to have insecticidal activity,[23] and Ag, Cu, CuO, Fe, Mn, and Zn have shown promise as herbicides.[24] Overall, the observed outcomes varied across these ENMs, often based on dose, plant species, application mode, environmental conditions, and experimental/exposure design.

Depending on the material used, the effect of ENMs may be related to the improved nutritional status of treated plants, although some elements will directly act as a fungicide, bactericide or insecticide against pathogens or pests. Importantly, any use of ENMs in agricultural practices must be preceded by a thorough understanding of the environmental and human health implications. As previously noted, extensive work has been conducted to evaluate the fate and effects of ENMs in the environment. However, many of these studies were conducted under conditions not entirely relevant for proposed agricultural uses. For example, some ENMs are obviously phytotoxic at high concentration (>500 mg/L), but at lower concentrations (< 50 mg/L), beneficial effects become evident.[25,26]

3. Nanofertilizers

Crop nutrition and yield depend greatly on availability of essential elements.[27] Several long-term field studies have shown that 30 to 50% of crop yield can be attributed to nutrient input from commercial fertilizers.[27,28] Considering the advantages of ENMs, these nutrients can be supplied in nanosized forms to improve release and enhance efficiency of use so as to achieve greater improvement in plant crop with lower environmental impacts. Dimkpa and Bindraban[9], and Chhipa[27] have reviewed the use of nanofertilizers and their impacts on a range of crops. Nanofertilizers can be defined as ENMs that directly provide one or more required nutrients to plants. The definition can also be applied to ENMs that enhance the performance, availability, or utilization of conventional fertilizers.[29] As nanofertilizers, ENMs have been shown to improve plant productivity and enhance food safety through both soil and foliar applications.[9] Globally, the demand for chemical fertilizers to replenish nutrient levels in soils that are continuously used for crop production has increased dramatically over the last 40 years.[30] It has been reported that between 1970 and 2008, the amount of fertilizer needed to produce one ton of grain increased by over 300%.[31,32] In addition, it has also been estimated that 182.8, 186.7 and 199.4 Mt of fertilizers were used globally in 2013, 2014, and 2017, respectively.[31] The efficacy of conventional fertilizers is inherently limited by the low availability in soil of many nutrients required by plants. This may be caused by inefficient delivery to the target and underutilization by the crop at the target endpoint. Notably, over the past four decades the nutrient use efficiency of the most important elements required by plants, including nitrogen (N), phosphorus (P) and potassium (K), has remained low: 30-35%, 18-20%, and 35-40%, respectively.[32] Inefficiencies in nutrient delivery to and use by plants ensures that growers add excessive amounts, subsequently leading to environmental contamination from emissions, leaching, and run-off. Several studies have reported that nano-enabled fertilizers have the potential to increase efficiency of nutrient delivery to plants[27]. If this potential could be optimized, the economic and environmental benefits could be dramatic.[32] Accordingly, the intended use of ENMs as nanofertilizers is targeted at increasing nutrient use efficiency, decreasing immobilization of nutrients, and reducing agricultural waste and run-off of nutrients through leaching and volatilization.[29] Table 1 presents a selection of the most promising results of ENMs application as nanofertilizers. Since plants require different nutrients to different degrees, ENM products (similar to conventional products) can be classified into macro- and micro-nutrient nanofertilizers.[27]

3.1 Macronutrient nanofertilizers

Macronutrient nanofertilizers provide nutrients required by plants in relatively large amounts, and include N, P, K, Ca, Mg and S. It has been estimated that by 2050, the global demand for macronutrient fertilizers will increase to 263 Mt.[27,33] The high surface area and penetrability of ENMs make them potentially more efficient products in terms of nutrient use relative to conventional fertilizers. In this regard, controlled or slow release of macronutrients such as N has been achieved from materials such as nano-enabled urea-coated zeolite chips and urea-modified hydroxyapatite (HA).[27,34,35] Kottegoda *et al.*[34] demonstrated the efficacy of a nanocomposite of urea-modified hydroxyapatite encapsulated under pressure into *Gliricidia septum*. The nanocomposite yielded a biphasic pattern, with initial rapid release of N followed by subsequent slow release over 60 days. On day 60, in a sandy soil (pH 7) the nanocomposite released □78% more N than the commercial fertilizer. This temporal release pattern could effectively enhance N uptake efficiency in amended plants, thereby significantly improving plant yield compared to conventional fertilizer.[34] The above study demonstrates a promising nanotechnology-based macronutrient formulation that optimizes nutrient dosage through slow and sustainable release of N over time. Notably, a follow up laboratory and field trial by the authors[35] revealed efficient slow release of N, which can be correlated with significant increase in rice yield, even at 50% lower concentration than the conventional urea.[35] The nanocomposites were synthesized from urea-hydroxyapatite nanohybrid (6:1) with carbonyl and amine functional groups that are implicated in the effective slow release of N. Although the resultant crop yield increase is impressive, the authors did not account for the additional P and Ca in the urea-HA nanocomposite. This is important given that $Ca(OH)_2$ and H_3PO_4 were used as precursors of the HA. We note that the authors did apply P separately to the apparently P-deficient soil. However, it is likely that this P would be more susceptible to fixation in soil, relative to the P in the nanoformulation that was likely released in a controlled fashion similar to the N. Thus, together with Ca, the P in the formulation with controlled release is more likely to have contributed to plant growth than P added directly to the soil.

Abdel-Aziz *et al.*[36] reported that foliar exposure of wheat to a NPK-nanochitosan composite (10-100 mg/L) significantly shortened the plant lifecycle by 40 days, and increased the grain yield by 51 and 56%, relative to the control and conventional NPK, respectively. The nanochitosan used in this study was synthesized by polymerization of methacrylic acid and chitosan. However, the impact of surface-adsorbed NPK on the nanochitosan was not taken into consideration, and the results were not compared with a pure nanochitosan control.[9,36] Furthermore, the experiments were conducted in a controlled greenhouse environment; field trials are necessary to further validate the approach. Amirnia *et al.*[37] also investigated the plant growth enhancing potential of a PK-Fe nanofertilizer on saffron plants grown on a silty-loam soil. When exposed through the leaves, the nanofertilizer increased dry biomass by up to 270 g/ha relative to untreated plants. Unfortunately, an evaluation of the individual components of the nanofertilizer formulation was not reported, nor was the effect on the plant as compared to a conventional PK-Fe fertilizer.[37]

Hydroxyapatite nanomaterials have been reported to increase seed productivity (20%) and plant growth (33%) of *Glycine max*, compared to traditional P fertilizer.[38] Notably, the growth study was conducted in an inert medium. As such, the eutrophication potential of the P NMs in acidic *vs*. alkaline soil needs to be assessed so as to provide information on applicability in actual soils. Furthermore, although a soil column test indicated that the NMs had more controlled mobility to ensure improved nutrient delivery to plant roots compared to conventional water-soluble P, the preferred/optimum delivery strategy between foliar and root application needs to be determined.

In peanut (*Arachis hypogeae*), biosynthesized Ca ENMs increased shoot biomass by 15%, and enhanced the nutrient content in the roots (C; 0.32%, N; 0.43%, P; 0.04%, and K;

0.014%) and shoots (C; 0.72%, N; 1.3%, P; 0.08%, and K; 0.014%) as compared with a single application of nitric acid calcium.[39] The role of humic acid and organic manure was also implicated in the observed physiological improvements (branches, needles in the dust, leaf area and dry weight of the peanut) in the plant. In a separate study, foliar treatment of Ca-deficient peanut with nano-CaO increased the Ca accumulation and enhanced root development of the plants when compared to treatment with bulk CaO and $CaNO_3$.[40] Interestingly, this study demonstrated that Ca in nanoscale form can be transported through the phloem. However, the mechanism of its action is still unknown. Similarly, seed treatment of *Vigna mungo* with nano-$CaCO_3$ resulted in greater growth compared to conventional Ca as $CaCl_2$. Specifically, shoot water content, as well as fresh and dry biomass, were significantly increased by nano-$CaCO_3$ at 10 mM relative to untreated controls and $CaCl_2$ treated plants.[41] The nano-$CaCO_3$ was biosynthesized from the reaction of $CaCl_2$ with the stem extract of *Boswellia ovalifoliolata*.[41] Importantly, large scale production of biosynthesized NMs is still a challenge.

Treatment of black-eyed pea (*Vigna unguiculata*) with Mg ENMs at 500 mg/L in combination with normal Fe (500 mg/L) resulted in a significant (10%) increase in seed mass compared with the plants treated with normal Fe.[42] However, the study did not compare the outcomes with plants individually exposed to Fe and Mg NMs. Moreover, the concentrations of the combined ENMs used in this study were relatively high (Fe: 500 mg/L and Mg: 500 mg/L), with potential for negative implications on non-target soil biota. Biosynthesized nano-S from mixtures of extracts of Chinaberry (*Melia azedarach*) and Tree of Heaven (*Ailanthus altissima*) and sodium thiosulfate were shown to enhance the root and shoot growth of tomato (respectively, 127 and 78%)[43] and pumpkin (respectively, 133 and 220%)[44] compared with untreated controls, when applied to soil at 100-400 mg/kg, or at 150 kg/ha. These studies demonstrate the concentration-dependent growth promoting effect of nano-S. However, no comparisons were made with conventional S to demonstrate that the effects were nanoscale-specific. Moreover, growth inhibition occurred in tomato and pumpkin at concentrations higher than 300 and 600 mg/kg, respectively, indicating the need for judicious application.

It is widely known that excessive accumulation of NPK, Ca, Mg and S from conventional fertilizer sources poses a threat to agroecosystems. Run-off of these macronutrients can pollute bodies of water, leading to eutrophication and eventual damage to aquatic biota. The use of ENMs as alternative sources for these nutrients may minimize environmental impacts, as overall application amounts of these elements would be significantly reduced. This benefit is coupled with enhanced crop productivity through directed delivery, enhanced availability and targeted release both spatially and temporally.

3.2 Micronutrient nanofertilizers

In comparison to macronutrients, micronutrient fertilizers supply essential nutrients required by plants in relatively smaller amounts, usually less than 10 mg/kg of soil. Nanoscale nutrient forms can increase availability of these important elements, promoting plant metabolism and thereby enhancing growth, development and nutritional quality.[9] For example, exposure of black-eyed pea (*Pisum sativum*) and soybean (*Glycine max*) to a FeO nanofertilizer at 250-500 mg/L and 30-60 mg/kg, increased leaf chlorophyll content compared with untreated controls.[42,45] In addition, the number of branches (□15%) and root dry biomass (□33%) of peanut increased upon amendment with Fe_2O_3 NMs at 1000 mg/kg relative to the untreated control.[46] However, in addition to using relatively high doses of Fe, which may have questionable relevance to agriculture, these studies did not compare the findings with the appropriate conventional Fe fertilizer. Thus, field trials using the FeO NMs at relatively low concentrations, alongside efforts to understand the possible mechanisms of action, are necessary prior to further development of this micronutrient strategy.

Foliar exposure of mung bean (*Vigna radiata*) to Mn ENMs at 0.05 mg/L increased the root length (52%), shoot length (38%), rootlet number (71%) and biomass (38%), relative to treatment with bulk or $MnSO_4$.[47] Adhikari *et al.*[48] reported a significant increase (51%) in maize (*Zea mays*) growth when exposed to CuO NMs at 10 mg/L compared with untreated control. Similarly, maize exposed to ZnO NMs at 0.5 mg/L showed a significant increase in the shoot dry weight (177%) and height (83%) relative to untreated controls.[49] Subbaiah *et al.*[50] also reported a significant increase in maize growth and development, as well as grain yield, upon treatment with bare ZnO NMs at 50-1000 mk/kg as compared with $ZnSO_4$. Exposure in soil to weathered and fresh ZnO nanoparticles significantly increased wheat shoot height and grain yield compared to the control treatments.[51] Dimkpa *et al*.[52] also revealed that a composite of micronutrients nanoparticles (ZnO, B_2O_3, and CuO, at 2.8 mg Zn/kg soil, 0.6 mg B/kg soil and 1.3 mg Cu/kg soil, respectively) added under drought stress improved the growth (33%) and yield (36%) of soybean as compared with untreated control. Virtually all the plants treated with different nanomaterials at low concentrations exhibited positive results, except ZnO NMs, which at relatively high concentrations (1000 mk/kg) produced good results in maize.[50]

Overall, both macro- and micro nutrient nano-enabled formulations evaluated in the above studies demonstrated the potential for significant increases in biomass or grain/seed yields. For some of these studies, there are strong correlations between improved crop yield and nutrient acquisition from the nanofertilizers, when compared either to untreated controls or to conventional nutrient-fertilizers. The preliminary results suggest significant potential of the

ENMs as fertilizers. However, mechanistic evaluation of the underlying processes, as well as field scale studies under realistic treatment scenarios, are needed to fully understand the potential benefits of the ENMs.

Analogous to vegetative and reproductive effects, the influence of ENM treatment on nutrient acquisition has also been reported. For instance, enhancement of Zn uptake was recorded in rice (*Oriza sativa*) when exposed to Mn ENMs.[53] In other studies, ZnO NMs at a range of concentrations (2-500 mg/kg) not only increased yield, but also enhance Zn uptake in different plants,[51,54-56,] in some cases with greater shoot-to-grain translocation efficiency by Zn NM compared to conventional Zn.[41] Rui *et al.*[46] reported a significant increase in Fe accumulation in the root (□33%) and shoot (□50%) compared with untreated control. Nanocomposites of ZnO, CuO and B_2O_3 significantly increased the uptake of N, K, Zn, and B, under drought stress relative to untreated controls.[52] Furthermore, findings such as those described by Dimkpa *et al*.[17,52,57] revealed that root or foliar exposure of sorghum and soybean to Zn NMs or to a composite of Zn, Cu, and B ENMs not only increased grain yield but also enhanced N and K accumulation. These findings indicate that fortifying N and K macronutrient fertilizers with nano-scale micronutrients can increase overall nutrient use efficiency. In the case of N, this potentially allows for mitigating the effect of N loss to greenhouse gas production. Similarly, Dimkpa *et al.*[19] reported that Mn NMs at 6 mg/kg did not significantly impact wheat grain yield, but did have subtle effects on nutrient acquisition by the plant.[19] These minimal positive impacts or clear negative outcomes could be attributed to dosage-related factors given the initial level of the Mn in the test soils.

3.3 Non-nutrient ENMs with fertilizer potential

A range of other ENMs not classified as plant nutrients have also been shown to have positive impacts on plants. This group of ENMs includes carbon nanotubes (CNTs), CeO_2, SiO_2 and TiO_2.[10] Although these are not nutritionally required by plants, these materials can stimulate growth and increase yield. Taha *et al.*[58] demonstrated that carbon nanotubes (CNTs) at 0.05-0.1 mg/L increased the shoot length of date palm (*Phoenix dactylifera*)[58] and at 5-500 mg/L, improved the growth of tobacco plant by 55-64%.[59] In a similar manner, CeO_2 NMs at 500 mg/kg increased the growth (9%), shoot biomass (12.7%), and grain yield (36.6%) of wheat (*Triticum aestivum L.*)[60] relative to untreated controls; a similar finding was reported for shoot biomass of barley (*Hordeum vulgare L.*).[61] Zhao *et al.*[62] also reported that CeO_2 NMs (400 mg/kg) increased the globulin content of cucumber (*Cucumis sativus*) by 76% compared to control. Globulins are water-insoluble proteins used for energy storage in the seeds of legumes and other plant species.[63] Although the precise mechanism of action leading to increased growth by CeO_2 NMs is unclear, it appears to be correlated with increased chlorophyll content, as well as enhanced levels of biomolecules such as amino acids, fatty acids, and mineral elements such as P, K, Ca, Mg, S, Fe, Zn, and Cu.[60,61] Notably, none of these studies compared their findings with equivalent respective salt concentrations as positive controls. It is noteworthy that some positive results were observed in plants treated with high concentrations of ENMs (>500 mg/L).[42-44,46,51] However, there are still some questions yet to be answered in terms of fate and environmental impact of ENMs. The overall impacts of ENMs on soil microbial communities are still not well understood, particularly under instances of chronic low dose exposure. In addition, one of the key drivers for proposing the use of ENMs as a novel strategy against conventional fertilizers is cost savings. Therefore, if large amounts of ENMs will be required to achieve the desired positive results, the concept of saving cost will be compromised and actual use in agriculture will not happen. Hence, studies of ENM effects on plants should be accompanied by cost-benefit analysis, as discussed by Dimkpa and Bindraban.[9]

3.4 Chitosan based NMs as nanofertilizers

Chitosan is a naturally-occurring, inexpensive and biodegradable cationic biopolymer.[64] The growth enhancement, antimicrobial, and agrochemical (micronutrient and pesticide) delivery potential of chitosan in plants is now being studied extensively. In agriculture, chitosan is generally known for its antimicrobial activities, even in its bulk form.[20,64] However, the efficacy of bulk chitosan is limited in biological systems due to its insolubility in aqueous media, which reduces its homogenous dispersability when applied to plants.[64] In an effort to improve its distribution on plant surfaces, chitosan is commonly prepared in acidic aqueous media and subsequently dialyzed to remove the salt and acidity. Incidentally, this causes the formulation to become more toxic to the target organism, increasing the inhibitory potential of bulk chitosan against microbes.[20] Compared to its bulk form, chitosan NMs (CNMs) are highly soluble in aqueous media, and have a high positive surface charge. A positive surface charge increases CNM affinity towards biological membranes, resulting in enhanced reactivity with biological systems. Relative to bulk chitosan, CNMs will have enhanced affinity to both organic and inorganic materials, especially metallic macro- and micronutrients. In addition, chitosan contains approximately 9-10% N, thereby serving as a good source of this macronutrient for plants. A number of research groups have synthesized chitosan-based NMs through physico-chemical modifications using various methods, including emulsion cross-linking/droplet coalescence, ionotropic gelation, precipitation, reverse micelles, sieving, and spray drying.[20] As with other

ENM exposures, the response of plants to CNMs is directly influenced by particle size, surface charge/zeta potential, the size distribution/polydispersity index (PDI), and encapsulated components with the CNM.[20] Studies have demonstrated that CNMs enhance seed germination, improve plant growth, enhance nutrient uptake, improve photosynthetic rate, and increase crop yield.[20,65] In a specific example, Van *et al.*[66] investigated the effect of size and concentration of high molecular weight chitosan nanoparticles (600 kDa, 300-3500 nm sizes) on the physiological parameters of Robusta coffee (*Coffea canephora Piere var Robusta*) in a greenhouse study. With stronger effect shown in plants treated with 10 mg/L, chitosan NM increased the chlorophyll content by 30-50%, photosynthetic rate by 30-60%, and nutrient uptake [N (9.8-27.4%), P (17.3-30.4%) and K (30–45%)] compared with untreated control.[66] However, no significant changes were observed in the plants exposed to CNMs within the size range of 420-970 nm. In addition, the role of chelation of CNMs to other organic and inorganic compounds in the soil was not considered. Although it is clear from this study that CNMs enhanced the uptake of N, P, and K, the authors did not compare the findings with equivalent conventional NPK treatments. In addition, the mechanism of action for chitosan NM remains unclear.

Saharan *et al.*[67] reported that stabilized Cu-chitosan NMs synthesized by ionic gelation at 0.08, 0.10, and 0.12% increased tomato seed germination by 4%, seedling height (29, 27, and 18%), and fresh (19, 22, and 16%) and dry weights (20, 27, and 13%), respectively, compared with the untreated controls.[67] Interestingly, sole chitosan NM (0.1%) lacking the Cu formulation exhibited a similar effect on the growth parameters as Cu containing CNM, compared with the untreated control. The zeta potential of Cu-CNMs (+22.6 mV) indicated a positively charged surface with higher affinity of the NMs towards biological membranes in an aqueous environment, which could explain the mechanism of action of the formulation. Furthermore, FTIR analysis revealed that the Cu-chitosan interaction induces greater dispersion of the NMs as compared with the typical CNMs.[67] Since the observations were based on foliar exposure, it is not clear how Cu-CNMs will behave in soil at various pH values, as well as their possible interaction with organic and inorganic soil materials. These complex soil interactions will most certainly impact the efficacy of CNMs in plants.

CNMs have also been evaluated as a seed conditioner. Pre-treatment of maize seeds with Cu-CNMs at 0.04, 0.08, and 0.12% for 4 h significantly increased the growth parameters as compared with untreated control and salt-treated ($CuSO_4$) seedlings.[68] However, there are no reports on the long-term effects of these treatments. Choudhary *et al.*[69] also reported that maize seed treatment and foliar application of Cu-CNMs in both pot experiments (0.12 to 0.16% concentrations) and field trials (12– 0.16% concentrations) significantly increased maize growth and yield, as well as chlorophyll a and b contents.

The impact of “green” or biologically synthesized CNMs has also been investigated. Foliar exposure of CNMs synthesized from fungal cell wall material increased the number of flowers and fruit yield in exposed tomato plants.[70] Sathiyabama and Parthasarathy[71] also demonstrated that biosynthesized spherical chitosan NMs increased the germination rate, seedling biomass, seedling vigor index, and root and shoot lengths of chickpea relative to the bulk counterpart.

Studies have indicated that CNMs, either solely or in its complexed form, can enhance the uptake of essential mineral nutrients. For instance, CNMs have been implicated in changes in the leaf mineral content of treated mango trees.[72] In addition, Cu-chitosan NMs increased the Cu content in maize seedlings, and caused changes in concentrations of Ca, Cu, Fe, K, Mg and Zn in tomato plant tissues compared with controls.[68,73] The alteration in the mineral content in most plant tissues can likely be attributed to the chelation activity of chitosan to most metals.

A number of studies investigating chitosan-based NMs as growth promoters in plants are highlighted in Table 1. Generally, it is assumed that the potential of CNM to serve as a growth promoter stems from its unique nanoscale properties. Overall, changes in size, concentration, surface charge and the specific nanoformulation components of the material contribute to enhance its function as a growth promoter in plants. Another promising aspect worthy of further investigation is the green synthesis of CNMs from other organic sources and the exploration of different biosynthesis options so as to fully understanding the mechanisms of action in different plant species. However, specific mechanisms of action of CNMs are addressed later in this review.

The most recently studied ENMs exhibiting nanofertilizer potential are shown in Table 1. Notably, among those that investigated the fertilization potential of ENMs in various plant species, 15 studies reported plant growth enhancement, compared with conventional macronutrient sources. [17,34-36,38-41,43,44,55,63-65,74] However, 53 studies lack relevant comparison with conventional fertilizers or other sources of the macronutrients,[19,37,42-54,56-62,66-73,75-97] and few studies were conducted under field situations.[35,37,42,55,63-65,69,72,82,85,89] In addition, concentrations used in some studies are considered high relative to the exposure time and plant life cycle.[42,45,46,50,55,56,59-62,83,84,86,88,92] This, therefore, does not alleviate concerns regarding the fate and environmental impact of the ENMs, as well as issues related to food safety. Overall, it is evident that certain nanofertilizers show better potential for improving crop yield and nutritional content than conventional means. However, there is need for better understanding of the mechanisms involved in order to elucidate the exact material properties and characteristics for optimizing these benefits, while simultaneously minimizing negative outcomes. This will require the expertise of material scientists and plant scientist to produce ENMs in commercial quantities and who can relate and predict material effects in biological systems under realistic agricultural production conditions. Ultimately, considering that different crops and soil types have unique nutritional or fertilization requirements; smart, responsive and tunable materials will be required. Crop- and soil-specific ENM and composites could be developed for specific environmental and climatic variabilities. As with conventional fertilizers, the common nanofertilizer exposure routes are through soil and foliar pathways. Although NMs have demonstrated potential for efficient delivery by both application routes, the chemical/physical properties that optimize outcomes will differ dramatically as a function of pathway, particularly considering differences in soil (e.g., pH, organic matter/clay content, CEC, and soil microbiome) and leaf surface (e.g., age, stomatal number, size and distribution) environments. Thus, formulations that meet the specific soil or foliar requirements for a given crop system are needed.

4. Engineered nanomaterials as pesticides or plant health products for controlling pathogenic diseases

Nanopesticides can be defined as any pesticide formulation or product containing engineered nanomaterials as active ingredients and having biocidal properties, either as a whole or part of the engineered structure.[98,99] The primary aim of any nanopesticide is to serve as a sustainable agricultural amendment with improved ability to prevent or suppress the severity of plant fungal, bacterial and oomycete disease. Thus, based on their nano-scale properties, it is anticipated that such amendments will be more potent, require lower application doses, and maintain, if not improve productivity, compared to conventional products of similar chemical composition. In fact, there has been recent increased interest in the use of ENMs as pesticides for protecting plants against a range of diseases. Notably, the ability to use ENM pesticides at lower rates than their conventional equivalents would reduce over application, run-off of the active ingredients into the environment, and thus, resultant environmental contamination. These benefits are in addition to reduced energy and water inputs that would be needed for material production. Taken together, this will also lower the economic cost of pesticide inputs by farmers.[27]

Studies have demonstrated the potential of ENMs to act as superior alternatives to conventional pesticides, further increasing interest in the production of antimicrobials that incorporate NMs, either polymers, or as stand-alone materials.[27] A variety of strategies have been employed in developing nano-enabled versions of conventional antimicrobials, including inorganic and organic polymeric materials with a variety of morphologies.[98-100] Nanospheres, nanocapsules, nanogels, and nanofibers are forms of polymer-based nanoformulations with varying degrees of biodegradability. The active ingredients are homogenously distributed into the polymeric matrix in nanospheres, whereas in nanocapsules they are located at the core and are surrounded by the polymer matrix.[99] In contrast, nanogels are cross-linked biopolymer networks, with pores filled with the active ingredient.[98,100] Nanogels containing pheromones, essential oils, or copper as active ingredients have been proposed to meet organic farming standards.[101] As noted, pesticides may also be encapsulated (nanoencapsulation) by manipulating the outer shell using nanoscale materials to engender slow or controlled release of the active ingredient over an extended period of time. Alternatively, the solubility and efficacy of antimicrobials can also be enhanced by using nanoemulsions of water or oil.[102] Overall, the design of nanoencapsulations are aimed at creating smart or responsive materials, effectively regulating the bioavailability of the active ingredient to the pathogen of interest, while reducing or preventing unintended effects on non-target organisms.

Certain elements have demonstrated antimicrobial activity. Notably, some of these elements are plant-required nutrients. This has resulted in the use of nutrient element-based ENMs to simultaneously suppress plant disease, increase yields, and enhance nutrient use efficiency. The efficacy of element-based ENMs in suppressing plant pathogenic diseases at the laboratory, greenhouse, and field scales is increasingly being evaluated. Of these, the most widely studied are metals and metallic oxides of Ag, Cu and Zn. However, others such as Mn, Ti and Ce, as well as biopolymers such as chitosan and β-D-glycan nanoparticles, have also been studied.[10,20,22,65] Table 2 provides a summary of the different types of ENMs that have been evaluated against plant diseases. The section below enumerates the antimicrobial potential of the recently evaluated ENMs.

4.1 Silver-based nanopesticides

Several *in vitro* studies have demonstrated the inhibitory activity of Ag NMs against the growth of different pathogens.[103] The mechanism of Ag NM toxicity is still not completely resolved; however, it appears to be largely derived from the release of ionic Ag^+. Ag ions are widely known to be highly toxic; membrane disruption of the pathogen by binding to cysteine-containing proteins on the plasma membrane is a well characterized mode of action.[6] In one report, exposure to biosynthesized Ag NMs exhibited *in vitro* antifungal activity by reducing spore count and biomass in *Alternaria solani* by 100% and 73% after 3 and 7 days, respectively.[104] Similarly, *in vitro* exposure to Ag nanoparticles at 2, 4 and 10 mg/L inhibited fungal conidial growth in *Bipolaris sorokiniana,* as compared with untreated control.[105] In addition, Ag nanoparticles prepared on double-stranded DNA and graphene oxide were shown to inhibit the activities of *Xanthomonas perforans* both *in vitro* (at 16 mg/L by 100%) and *in planta*.[106] The authors suggested that Ag nanoparticles and the Ag ions released from the nanoparticles reacted with functional groups (thiol, carboxyl, hydroxyl, amino, phosphate, and imidazole) on the bacterial cell, triggering inactivation and eventual death. Enhanced antimicrobial efficacy of nanoencapsulated Ag on gram negative bacteria (*Pseudomonas aeruginosa*) have been demonstrated, in which Ag (2.9 μg/mL in contained in a nanochitosan (193.3 μg/mL) carrier showed a minimum inhibitory concentration (MIC) that was lower than sole Ag NMs.[107] Liang *et al.*[108] also demonstrated inhibitory potential of a graphene oxide/Ag NM composite against a bacterial blight disease causative organism in rice (*Xanthomonas oryzae pv. Oryzae*). The nanocomposite was 4-fold more effective than Ag NP alone, and was prepared using graphene oxide powder, poly (N-vinyl-2-pyrrolidone) and 1 mM $AgNO_3$.[108] Notably, the environmental fate and toxicity of Ag from an ENM perspective is still a major concern. However, reports have suggested that Ag NP biotransformation in aqueous and soil environments to Ag_2S, AgCl, Ag^0, or Ag-cysteine, among others, can reduce toxicity and modulate any short-term unintended environmental impacts.[109,110]

Compared to direct *in vitro* assays, a limited number of studies have investigated the antimicrobial activity of ENMs in actual plant-pathogen systems under greenhouse or field conditions, with most conducted on fungal pathosystems. In one case, the antimicrobial potential of Ag nanoparticles was demonstrated by spraying biosynthesized particles (5 mg/L) on tomato plants to protect against early blight disease caused by *A. solani*.[104] The Ag nanomaterial reduced disease progression by □49% compared to untreated infested plants. Notably, with the moderate concentration, the chlorophyll content and fruit fresh weight increased by □24 and □33%, respectively. However, a non-nano Ag source or a conventional pesticides was not included in the study. In addition, biosynthesized Ag NMs (2-10 mg/L) demonstrated strong antifungal efficacy against spot blotch disease in wheat caused by *B. sorokiniana* (100%),[105] and collar rot disease caused by *Sclerotium rolfsii* in chickpea (50-95%).[111] The authors suggest that disease suppression was as a result of sclerotial rind disruption due to Ag^+ penetration, and subsequent accumulation inside the pathogen cells.[111] In another study, inhibition of *P. parasitica* and *P. capsici* in tobacco by Ag NMs synthesized from aqueous extract of *Artemisia absinthium* at 100 mg/L was as effective as the a commercial fungicide (mefenoxam) but more effective than untreated control.[112] In addition, another *in vivo* study demonstrated inhibitory activity of "Tween 80-stabilized" Ag NMs against tobacco wilt caused by *Ralstonia solanacearum* at 7 (97%), 14 (90%), and 21 (84%) days exposure, compared with untreated control.[113] Similarly, wilt disease in *Crossandra* spp. caused by *Fusarium incarnatum (*Desm*.)* Sacc. was reduced (19%) by foliar exposure to 800 mg/L of Ag NMs, relative to the untreated control.[114] The concentration of Ag NMs employed in this study was relatively high; thus, its effects on non-target microbes and the environmental health is a major concern. Strayer *et al*.[115] also revealed that an Ag-based nanocomposite (Ag-dsDNA-GO) at 75-100 mg/L more efficiently suppressed bacterial spot disease (68-84%) caused by copper-tolerant *Xanthomonas perforans* in tomato plant than did the conventional Cu-mancozeb and negative controls.

Taken together, these studies indicate that Ag ENMs may be more efficient against various plant pathogens. However, most of the studies did not compare the findings with conventional pesticides, and virtually all are conducted in controlled environments. Hence, field trials are necessary to ascertain the optimal conditions for efficient functions of the ENMs.

Importantly, the environmental implications and economic limitations of Ag NMs use in the suppression of plant disease are still a concern.

4.2 Copper-based nanopesticides

Similar to Ag, studies have demonstrated *in vitro* antimicrobial activity of Cu-based NMs. For example, using the Kirby–Bauer disc diffusion method, Cu NM at 20 µg/disc reduced the growth of the plant pathogens *Alternaria alternate, Curvularia lunata,* and *Phoma destructiva*.[120] In this technique, standard paper discs impregnated with Cu NM were placed at four corners of potato dextrose agar (PDA) plates inoculated with fungal spore suspensions in 6 mm diameter petri dish. In different studies, *in vitro* exposure to Cu NMs at high concentrations (440 mg/L) inhibited the growth of *Fusarium* sp. by 64% after 9 days,[121] and at 50, 100 and 200 mg/L, reduced the growth of *Botrytis cinerea* after 72 h exposure by 18, 17 and 13%, respectively, compared with untreated control.[122] In addition, Cu NMs also reduced the radial expansion of three common plant pathogenic *Fusarium sp., F. culmorum* (19 mm)*, F. oxysporum* (20 mm) and *F. equiseti* (25 mm)*,* relative to amphotericin used as a positive control.[123] Similarly, Zabrieke *et al*.[124] demonstrated that CuO NMs can inhibit the growth of pathogenic *Pythium* isolates of wheat; namely, *P. ultimum* and *P. aphanidermatum*. In that study, citrate was added in the growth media to enhance the release and efficacy of the Cu. At 250 and 500 mg/L, CuO NMs showed blue coloration in the plates, indicating the release of Cu^{2+} ions from the NMs. At 500 mg/L, CuO NMs reduced the growth of *P. ultimum* and *P. aphanidermatum* to □10% and □35% of untreated controls, respectively, showing species-dependent differences in the bio-response. It was also shown that the CuO NMs inhibited the activity of the mycelia-bound ferric reductase, an enzyme required to supply Fe to the pathogen.

Copper has historically been a component of many plant antimicrobial formulations.[125] Copper-based fungicides are widely used in United States. A 2012 EPA report revealed that about 3-5 million pounds of copper-based pesticides were used as active ingredient fungicides in United States.[126] Annually, about 7300 tons of copper pesticides were used in California alone. Copper-based pesticides generally contain 56% by weight organic Cu-compounds, 34% $CuSO_4$, 6% Cu_2O and 4% CuO.[125] Based on the scale of use of Cu products, interest in the use of nano-scale Cu, including $Cu(OH)_2$ and CuO, as microbicides has increased significantly.[126] Copper NMs are characterized by slow release of Cu^{2+}, which implies prolonged efficacy relative to $CuSO_4$. Compared to the negative control and the reference products (Kocide 2000 and Kocide Opti), CuO NMs were more effective at protecting tomato plants against *Phytophthora infestans*.[127] However, the authors did not clearly state the concentrations of Cu NMs used in the experiment. Applied foliarly, CuO NMs at 500-1000 mg/L suppressed Fusarium wilt disease caused by *Fusarium oxysporum f. sp niveum* in watermelon, more effectively than Cu-fungicides (Kocide 2000), with increased yield relative to untreated controls and other NMs (MnO, SiO, TiO, and ZnO).[128] In addition, the effect of bulk equivalents of various NPs concentrations when compared with the results proved to be less effective. However, the concentrations of Cu-based NMs used in the experiment was high. Biosynthesized Cu NMs at 2.5 mg/L were used in a field study to suppress red root-rot disease (by 80%) caused by *Poria hypolateritia* in tea plants. However, it was demonstrated that carbendazim was equally effective as the ENMs treatment.[129] Effective suppression of tomato bacterial spot disease caused by *Xanthomonas spp* was reported under greenhouse conditions, using an advanced Cu composite containing core-shell Cu, multivalent Cu, and fixed ammonium Cu.[130] Moreover, in field studies, the Cu nanocomposites significantly reduced disease incidence caused by copper-tolerant *X. perforans* when used at

20% of the Cu content of a commercial product, copper-mancozeb, with no significant increase in the yield.[130] This is an important finding considering that bacterial spot disease has become resistant to many conventional copper-based bactericides. The antimicrobial potential of Cu-based NMs, applied at low doses, can potentially serve as alternative strategy to replace or augment conventional pesticides in agricultural practice. Moreover, commercially available Cu-based pesticides are currently excessively applied in agricultural fields, resulting in the development of Cu-resistance among plant pathogens. Most of the studies demonstrated higher efficacy of Cu-based NMs or its composites against pathogens, with increased yield, often at relatively low concentrations.

4.3 Zinc-based nanopesticides

Green-synthesized ZnO nanoparticles have shown *in vitro* antimicrobial activity against bacterial (*Staphylococcus aureus, Serratia marcescens, Proteus mirabilis and Citrobacter freundii*) and fungal (*Aspergillus flavus, Aspergillus nidulans, Aspergillus niger, Botrytis Cinerea, Penicillium expansum, Fusarium graminearum, Trichoderma harzian and Rhizopus stolonifer*) pathogens.[6,131-134] Mechanistically, ROS released on the surface of the ZnO nanoparticles were thought to have severely damaged the microbial cell wall and subsequently, inactivating the organisms. More specifically, growth inhibition of *F. graminearum* was reported after a 7-day exposure in mung bean broth agar (75% inhibition) and in sand (63% inhibition) amended with ZnO NM (500 mg/L).[132] Similarly, growth inhibition of *A. flavus* and *A. niger* by ZnO NMs (25 mg/L) plate assays was reported, with maximum inhibitory zones of 19 and 22 mm observed for each fungus, respectively.[133] Also, ZnO NM at 3-12 mmol significantly inhibited the growth of *B. Cinerea* (63-80%) and *P. expansum* (61-91%).[134] *In vitro* studies have also demonstrated that ZnO nanoparticles reduced the radial expansion of *F. oxysporum* (at 1000 mg/L)[7] and inhibited *Pythium* isolates (at 50-500 mg/L)[124] compared with their respective controls. Taken together, these studies indicate that ZnO NMs can be a broad spectrum antimicrobial for inhibiting fungal, bacterial and oomycete pathogens. Moreover, based on the results from studies involving Ag-, Cu- and Zn-containing NMs, it appears that Zn-based NM are less toxic to plants, and may be a preferred option as a nanopesticide, with less expectation of negative environmental impacts.

Accordingly, there has been significant interest in the use of Zn in plant disease management, both because of its antimicrobial activity and as an essential nutrient in human nutrition. However, to date limited research has been conducted to evaluate its potential role in disease suppression in vivo. In both field and greenhouse trials, two different ZnO NM formulations, namely plate-like Zinkicide SG4 and particlulate-like Zinkicide SG6, were used in grapefruit to suppressed citrus canker lesion disease caused by *Xanthomonas citri* subsp. *Citri*.[135] In another study, Paret *et al.*[136] developed a light-activated TiO_2/Zn NM composite which was evaluated for disease suppression in an open-field pot experiment. The formulation (500-800 mg/L) reduced bacterial leaf spot disease caused by *Xanthomonas sp* by 62-71% in rose plants compared with untreated control. However, although the nanoformulation was effective in suppressing leaf spot disease, the concentration used was quite high. For this reason, the potential impact on non-target organisms needs to be assessed, and a comparative analysis with commercial pesticides should be conducted. Elmer and White[7] reported disease suppression of tomatoes with ZnO NMs (1 mg/L) in the greenhouse when plants were affected with wilt diseases. Generally, there are knowledge gaps in the research efforts to explore the full potential of Zn NMs as pesticides. Since the effect of Zn varies across plant species, there is a need for better understanding of appropriate conditions for beneficial use of Zn NMs in agriculture. Moreover, currently, most reported researches are mainly on vegetables. Perhaps, the research needs to be extended to flower and fruit bearing plants, and also the effect of Zn NMs in the presence of heavy metals and other organic contaminants needs to be examined. There is a limit to amount of zinc in human nutrition, hence, it is highly important to understand the trophic transfer of Zn across the food chain in order to avoid zinc toxicity in human foods.

4.4 Chitosan and other ENMs as pesticides

As stand-alone products, chitosan NMs (CNMs) can act as a potent antimicrobial towards pathogenic bacteria, fungi, and viruses.[20] Alternatively, they can be incorporated with other ENMs to form nanocomposites to both improve their function as microbicides and engender the slow delivery of nutrients and active ingredients to plants. An anionic protein solution isolated from a *Penicillium oxalicum* culture was added to chitosan to generate CNM. [71] Subsequently, the antifungal potency of the biologically synthesized CNM was evaluated against *Pyricularia grisea, A. solani, and F. oxysporum*.[6] The CNMs significantly inhibited the *in vitro* growth of all three pathogenic fungi, as well as improved the *in vivo* seed germination, seed vigor index and the biomass of chickpea.[71] Similarly, CNMs significantly reduced the mycelial expansion of *Ceratocystis fimbriata in vitro*, altering hyphal morphology and inducing irreversible membrane damage.[137] In addition, leakage of intracellular components, especially potassium ions, as a result of membrane permeability induced by the NMs was also observed. Moreover, a significant reduction in the number of viable cells revealed that the CNMs caused necrotic cell death, and within three hours of exposure, 70% of the spores were dead.[137] CNMs and its composite with silver (CAgNCs) were shown to damage the membrane structure of *F. oxysporum*. Both NMs caused morphological and ultrastructural changes in the pathogen, but the CAgNCs inhibited pathogen radial expansion to a greater extent than CNMs alone.[21] Xing *et al.*[138] reported similar impacts of oleoyl-chitosan nanocomposite on *Verticillium dahliae.* Furthermore, a bionanocomposite containing chitosan NMs and chitosan/pepper tree (*Schinus molle*) essential oil exhibited antifungal potency against *Aspergillus parasiticus* spores.[139] The nanocomposite at 12.5-200 µg/mL reduced the viability of *A. parasiticus* cells by 40-50%.[139] The authors suggested that the efficacy of the nanocomposite relies on the individual strength of the components, although the function of CNMs is suspected to play a vital role. Overall, most data from *in vitro* studies investigating the antimicrobial activity of these NMs suggest a mechanism based on ROS generation, clearly highlighting that these materials may act directly as bactericides or fungicides. However, for many of these materials it is unclear if such a direct mode of action is sufficient to protect plants against pathogens under field-based agricultural conditions.

To test this hypothesis, greenhouse and field experiments have been conducted using CNMs. In one example, pretreatment of wheat with CNMs at 1000 mg/L prior to pathogen inoculation effectively suppressed Fusarium head blight disease caused by *F. graminearum*.[140] However, the concentration of CNM used in the experiment was high, with unknown environmental impact. Moreover, the efficacy against the pathogen was less than that observed with conventional “Tilt” fungicide with propiconazole as an active ingredient. A detached leaf assay demonstrated that CNM, at 500 µl/leaf, effectively suppressed rice leaf blast disease caused by *Pyricularia grisea* by 50%, compared with untreated infested control.[141] Clearly, further study is required to fully understand the mechanism of action of chitosan in the rice-pathogen system. Similarly, Sathiyabama and Manikandan[142] reported that CNMs delayed the onset blast disease symptoms in infested finger millet from 15 days to 25 days and reduced the disease by 64% on day 50, compared with the untreated infested controls. The authors further reported increases in peroxidase activity and ROS production, which were plant responses ostensibly induced by the CNM treatment that led to disease suppression.[142] This finding suggests ROS generation as a possible mechanism by which CNMs may positively impact plant growth in diseased systems. In other plant-pathogen systems, the incidence of downy mildew disease caused by *Sclerospora graminicola* was significantly reduced (~82%) in millet seeds exposed to CNMs (250 mg/kg soil), compared with untreated infested control, but less effective than the commercial fungicide (metalaxyl, 92%).[143] The observed disease suppression was correlated with the expression of genes encoding ammonia lyase, catalase, peroxidase, polyphenol oxidase, phenylalanine and superoxide dismutase, all of which were upregulated in the treated plants.[143] Similarly, a Cu-chitosan nanocomposite was reported to boost the defense mechanisms of finger millet against *P. grisea*,[144] and of maize against *Curvularia lunata*[70] and *Fusarium verticillioide*s.[145] Significant yield improvement of these crops in the presence of the nanocomposite was noted. Notably, combining seed treatment and foliar application of the nanocomposite was more effective against *P. grisea* in finger millet when compared with foliar treatment only. The seed treatment was specifically suggested to protect the plant against invasion by the pathogen by enhancing a range of defense enzymes.

Observations on the disease suppression potential of CNMs and Cu-chitosan nanocomposites are summarized in Table 2. Clearly, there is still limited understanding of the mechanisms of action of chitosan-based NMs. However, in general and as with other ENMs, particle antimicrobial activity relies on material size, surface charge, exposure concentration, solubility, biodegradability and penetrability in living systems. It is also likely that additional modifications of the physicochemical properties of CNMs could further enhance their function as antimicrobial agents.

Other ENMs such as those based on carbon, non-nutrient metallic ENMs other than Ag, and composite ENMs have also been evaluated in a range of disease systems. With fullerene amendment (50 mg/L), *Botrytis cinerea* growth was reduced by 20% after 72 h inoculation.[123] Si NM reduced disease incidence caused by *Aspergillus niger* and *F. oxysporum* in maize.[146] However, the mechanisms of action of these ENMs remain poorly understood.[6,147] Under greenhouse conditions, the mesoporous alumina NM and tolclofos-methyl (a commercial fungicide) equally suppressed root rot disease caused by *F. oxysporum*, and yielded 20-fold greater survival of tomato plants compared with the untreated control.[148] Hao *et al.*[149] demonstrated that the proliferation of Turnip mosaic virus (TuMV) infection in tobacco (*Nicotiana benthamiana*) plants was inhibited upon foliar treatment with Fe_2O_3 NPs, TiO_2 NPs, or carbon-based NMs (MWCNTs and C_{60}).[149] As with human viruses, plant viruses are inherently more difficult to manage, and host nutrition is critical in this regard. In Hao *et al.*[149] study, fully developed new tobacco leaves were inoculated with the virus after pre-treatment of the plants with the ENMs (50 or 200 mg/L) for 21 days. After 5-day of inoculation with TuMV, proliferation of the virus on the leaf surface was significantly reduced by ENM treatment as compared to the untreated control. The metallic- (at 50 mg/L) and carbon-based (at 50 and 200 mg/L) ENMs increased tobacco fresh biomass of infected plants by 55% as compared to the untreated infected control.[149] Although there was accumulation of the metallic NMs in the plant chloroplast, cellular integrity of the plants was not compromised. Significant reduction in the amount of TuMV coat proteins and elevated (40%) phytohormone levels both point to the potential mechanisms of action of the ENMs in the observed disease suppression.[149] In another study with turmeric plants infested with rhizome rot disease, foliar treatment with β-D-glycan nanoparticles (0.1%, w/v) increased the activities of defense enzymes, including peroxidase, polyphenol oxidase, protease inhibitors and β-D-glucanase.[22] The increased activity of these enzymes resulted in a 77% reduction in disease incidence relative to the untreated control.[22]

Overall, although the evaluated studies demonstrated antimicrobial activities of several ENMs, this was often at high concentrations.[7,114,128,136,140] Moreover, a substantial number of the studies did not compare their findings to conventional pesticides to evaluate the potency of the tested ENMs,[7,15,69,104-106,11-114,118,123,141,142,144,146,149,151,152,154-156] and some studies[15,104-106,111-115,118,123,127,140-144,146,148,149,152,154-156] were conducted only in controlled environments without associated field trials. That being said, a limited number of studies utilized ENMs at moderate concentrations, and compared their findings with conventional pesticides, and were conducted under field conditions.[116,117,119,129,130,145,153] In spite of these promising findings, there are still substantial knowledge gaps with regard to the potential of ENMs as antimicrobial agents for controlling plant diseases. Of critical importance is a thorough understanding of the mechanisms of disease suppression or inactivation by ENMs to ensure efficacy, as well as an understanding of the fate and implications of ENMs in agroecosystems. Also, given the unique risks associated with food production and the narrow economic profit margin in agriculture, only sustainable strategies will be successfully deployed on a large scale. These facts highlight the importance for functionally optimized materials that achieve improved efficiency with responsive and tunable capability. In addition, one of the primary benefits behind the use of ENMs, relative to conventional agrichemicals, is the reduction in the overall load of material required for food production. These outcomes can be realized through several formulation-specific properties, including but not limited to: improved solubility, increased adhesion and absorption to plant leaves, controlled and responsive release of active ingredients, targeted delivery, enhanced bioavailability and biodegradability, and improved stability of active ingredients in the environment.[150] If the physicochemical properties of ENMs or nano-enabled agrichemicals can be manipulated and optimized to sustainably attain these characteristics, nano-enabled agrichemicals can become a critical component to achieve and maintain global food security.

5. Postulated mechanisms of disease suppression by ENMs: ROS generation and essential nutrient biofortification

The nano-size scale, large surface area, and other unique features of ENMs result in significantly enhanced activity and functionality in biological systems. Different ENMs can be taken up and biotransformed differently in plant systems relative to their bulk or ionic counterparts.[157,158] However, the impact of ENMs on plants are also influenced by a range of biotic and abiotic factors. In the presence of pathogens, plant response to ENM exposure differs across different NMs and plant species. The study of El-Argawy *et al.*[152] presents plausible mechanisms of antimicrobial action of NMs. The authors discuss how cations such as Ag^{+}, Cu^{2+}, Zn^{2+}, and Ti^{4+}, among others, bind to sulfhydryl or other functional groups in proteins upon their release from ENMs.[152] This interaction alters the activity and function of important membrane proteins and disrupts the cellular membrane structure. Moreover, released ions and parent NMs could be genotoxic, interrupting the electron transport chain (ETC) and altering overall DNA structure/function. Together, these impacts can result in compromised cellular integrity and eventual pathogen death. This is the likely mode of action of chitosan-based NMs, where microbial cell wall and cell membrane destabilization are reported.[20] ENMs and released ions may also induce ROS generation, which may interfere a number of important processes in pathogenic organisms.[27,152] The interaction between ENMs and plants in the presence of bacterial and fungal pathogens is complex, and understanding the mechanisms of activity will be critical to successfully deploying ENMs as sustainable agrichemicals. ENMs can mitigate pathogenic diseases in crops by two primary pathways: direct antimicrobial action or indirect action based on nutrition-induced stimulation of plant defense and secondary metabolic processes.[6]

In the direct pathway, a variety of toxicity mechanisms are possible. Generally, ENMs can penetrate and accumulate in the microbial cell membrane, subsequently causing cell lysis.[159] A scanning electron microscopy image presented by Lamsal *et al*.[116,117] indicated severe morphological disruption in fungal mycelia of *Golovinomyces cichoracearum*, *Sphaerotheca fusca* and *Colletotrichum* spp due to Ag NMs. Furthermore, stress caused by ENMs may stimulate the generation of cellular ROS.[116] Elevated ROS production disrupts microbial cellular homeostasis, creating an oxidative burst that damages microbial cells at several levels, which can eventually lead to cell death (apoptosis).[160] ROS are natural intracellular byproducts of the diffusion of electrons onto O_2 from the electron transport reactions in cellular organelles such as mitochondria, chloroplast, and plasma membrane. However, ROS can also be generated via a range of metabolic processes in different cellular compartments.[161-163] ROS includes hydrogen peroxide (H_2O_2), superoxide anions (O_2^{*-}), hydroxyl radicals ($^{*}OH$), hydroxyl ions ($^{*}OH^{-}$) and molecular oxygen (O_2).[164,165] To maintain homeostasis, the level of cellular ROS must be low; at high concentrations, ROS induces toxicity. Specifically, excess ROS causes DNA damage, lipid peroxidation, enzyme inhibition and cellular apoptosis. Under normal conditions, cells must balance the processes by which ROS are generated and scavenged (Figure 2). Indeed, a well-coordinated ROS scavenging pathway from different cellular compartments has evolved, which, under normal metabolic conditions, gives rise to low concentrations of potentially harmful RO intermediates in most cells.[161] ROS at low or moderate concentrations can be described as secondary messengers in many biomolecular processes in cells, conferring tolerance to different biotic and abiotic stresses in plants and other organisms. Such response has been described for intracellular hormone-mediated signaling cascades, including apoptosis, stomatal closure, gravitropism, and plant responses to biotic and abiotic stresses.[166-169] Notably, pathogen infection typically triggers significant generation of intracellular ROS in plants. The presence of pathogens in plant systems is often recognized by generation in the apoplast of O_2^{*-}, or its dismutation product, H_2O_2.[170] Relative to the control, higher concentrations of H_2O_2 and MDA have been detected in the leaves of *Vicia faba* upon infection with yellow mosaic virus, which was indicative of ROS stimulation by pathogen infection.[171] As previously noted, most cells possess complex anti-oxidative defense mechanisms to mitigate ROS generation, including increasing the level of endogenous antioxidant defense[172] through non-enzymatic or enzymatic pathways (Figures 3 and 4). The non-enzymatic component of plant cellular antioxidant defense involves phytochemicals such as phenolic compounds, carotenoids, and tocopherol, as well as essential cellular redox buffers, including glutathione and ascorbic acid. Apart from their role in plant defense, these secondary metabolites interact with many cellular components, or act as antioxidants and enzyme cofactors, all of which positively influence plant growth and development.[161,173] Various studies have implicated ENMs in the stimulation of secondary metabolites and involvement in the suppression of diseases in plants.[104-106] For example, the maximum total phenol content was found in Ag NM-treated tomato plants, ostensibly serving as the first line of plant defense against the pathogen *A. solani*.[106] Alternatively, the enzymatic components of ROS response include antioxidants such as ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), guaiacol peroxidase (GPX), and superoxide dismutase

(SOD), among others.[174] Plants respond to oxidative stress by concerted protein synthesis from different cellular compartments. Notably, plant antioxidant defense mechanisms against pathogens involving ROS can be manipulated using external analytes.[161] Several studies have reported significant increase in enzyme/antioxidant activities in plants exposed to ENMs.[15,22,79,107,130,175] For example, Shah *et al.*[175] reported that the activity of antioxidative enzymes was elevated in metal-stressed plants. Thus, pre-treating plants with ENMs could contribute to alleviating ROS generated by pathogen infection (Figure 5). Moreover, ENMs such as CeO_2 have been reported to exhibit redox state-dependent catalase activity.[17,176] Specifically, the antioxidant potential of CeO_2 NMs relies on its mimetic catalase activity at either +3 or +4 oxidation states in plant cells, in addition to its superoxide scavenging activity.[176] Indeed, these characteristics may account for the antimicrobial activity of CeO_2 NMs, whereby exposure suppressed Fusarium wilt disease in tomato plants.[15]

As a biologically derived nano-formulation, it is interesting to better understand the mechanism of action of chitosan-based nanopesticides. The potential modes of action of CNMs as an antimicrobial agent have been highlighted.[20] The authors noted that the positively charged surface of CNMs, rendered by the presence of amino functional groups present in the natural biopolymer, confer antimicrobial activity to the CNM. The positively charged surface of chitosan enhances its affinity towards anionic surfaces on the microbial cell membrane and also increases chelation with metals present in the cellular environment.[20] The electrostatic interaction between polycationic CNMs and anionic components of the pathogens can cause cell membrane permeability, leakage of intracellular materials, and eventual cell lysis.[20,137] Based on a similar mechanism as that in bacteria, chitosan NMs can also act electrostatically against fungi by disrupting the cell wall/membrane.[134] Such action is achieved by direct inhibition of enzymes involved in the biosynthesis of glucans, an important biomolecule involved in strengthening the fungal cell wall.[177] Furthermore, CNMs can also interact with fungal DNA, inhibiting mRNA and protein synthesis.[140] The binding of amino functional groups of CNMs to the negatively charged phosphate groups and anionic amino acids of DNA can cause deactivation of enzymes involved in the biosynthesis of important proteins in the target organisms.[140]

Biofortification of plants with ENM-derived nutrients has been explored as an indirect strategy for disease management. Plants, like all biological species, can be protected against pathogenic infections through a robust nutritional regimen. Evidence[6,7,10] suggests that plants treated with a more balanced nutrient composition are healthier than those treated with less-balanced nutrient composition, and thus, are better able to resist pathogen infection. However, obtaining sufficient nutrients from soil, particularly under stressed conditions such as during infection or drought, is often confounded by low element availability as a function of soil chemistry. Moreover, plants are generally less able to basipetally transport metal ions if supplied via foliar application.[6] If a sufficient amount and diversity of nutrients are available, the mechanism of plant response to pathogen attack involves the triggering of a sequence of biochemical reactions which lead to the synthesis of secondary metabolites that ultimately confer resistance against the pathogen. Notably, many of the reactions involved in the synthesis of these secondary metabolites are catalyzed by enzymes requiring micronutrients as cofactors. Specifically, Mn, Cu, and Zn have been implicated in the activation of enzymes involved in plant defense systems.[6] The aforementioned nutrients, when presented to infected or stressed plants in nanoscale form, have been demonstrated to enhance plant defense mechanisms,[7,10] presumably via greater availability/activity that results in greater in planta content of these nutrients. It is noteworthy that the amount of micronutrients present in the plant cellular compartments can influence the synthesis of these defense metabolites during pathogen infection. However, as indicated above, the availability of these elements may be restricted by different factors in the soil.[6,178] In several instances, the use of ENMs as nanoscale micronutrients as a comprehensive agricultural amendment has been shown to increase the bioavailability of elements upon infection. In those instances, infected plants were better able to suppress disease progression, resulting in greater yields in spite of disease pressure.[6,7,15,128] Clearly, these are very promising advances. Overall, most studies indicate that ENMs have significant advantage over conventional pesticides by improving efficacy against pathogens, increasing yield, enhancing mineral nutrients uptake, directed delivery of active ingredients, all with lower amount applied to reduce environmental impact. However, continued validation under field conditions where plants may be simultaneously perturbed by a broader range of biotic and abiotic stresses is required. In addition, the tuning or functionalization of these nanoscale nutrients may lead to the development of responsive advanced materials that are more effective both temporarily and spatially, as well as being applicable against broader range of plant-pathogen systems.

5. Use of ENMs as insecticides and herbicides

There is growing interest in the use of ENMs in plant production as pesticides against insect pests and pathogenic nematodes, and as herbicides to control weed species. However, much less work has been done in this area compared to ENM use as antimicrobials. Currently, a limited number of available reports suggest that ENM platforms may provide effective strategies for the control and management of insect pests and weed species. Nano-enabled formulations involving the incorporation of ENMs into conventional insecticides/herbicides such as through nano-emulsification and nano-encapsulation can be used to enhance active ingredient solubility, penetrability, stability, and controlled-release properties against target species.[179,180] Similar to nanofertilizers and nano-enabled antimicrobials, increasing the accuracy and precision of active ingredient delivery will reduce the load of material released to the environment, thereby limiting unintended impacts on non-target species and the environment.[31]

One novel strategy in this regard is the use of ENM for the delivery of genetic material and other active ingredients directly into host plant tissues to protect against insect pests, as has been reported for *Callosobruchus maculatus, Drosophila melanogaster Sitophilus oryzae* and *Rhyzopertha dominica*.[181] A second example is the slow release of active ingredients from a nano-emulsion containing polyethylene glycol (PEG) coated NMs embedded in garlic essential oils; this platform enhanced the insecticidal efficacy of PEG against adult *Tribolium castaneum* by 80%.[182] One of the early studies demonstrating the insecticidal potential of NMs involved use of nanostructured alumina against two major insect pests, *S. oryzae* and *R. dominica*.[183] After 3 days of continuous exposure of wheat plants to alumina NM, significantly greater pest mortality was recorded (LD_{50} 127-235 mg/kg) as compared with a commercial insecticidal dust.[183] Subsequent laboratory and field bioassays have further demonstrated the insecticidal activity of nano-structured alumina against other insects, including the leaf-cutting ant *Acromyrmex lobicornis.*[184] In this study, nanostructured alumina (0.08-0.5 mg/g) showed an LC_{50} of 0.14 mg/g, with stronger toxicity against *A. lobicornis* when compared with diatomaceous earth (LC_{50} = 0.36 mg/g). Nanostructured alumina is an amorphous material with strong sorptive properties, which coupled with its unique NM properties in terms of size and shape, enhances its insecticidal efficacy. Although nanostructured alumina is effective compared to the respective controls in the above-mentioned study, it should be noted that the study was conducted in a controlled environment. Thus, additional studies in actual crop production scenarios in which other environmental factors are at play are required to fully understand the potential of nanostructured alumina in agriculture. In addition, the ecotoxicological risks of nanostructured alumina need to be characterized. Similar to alumina, formulations containing silica NM have been shown to exhibit slow release of the active ingredient, thereby protecting plant seeds from insect attacks. This has been demonstrated in pigeon pea (*Cajanus cajan*), horse gram (*Macrotyloma uniflorum),* black gram (*Vigna mungo*), green gram (*Vigna radiata*), chick pea (*Cicer arietinum*), cowpea (*Vigna unguiculata*), wheat (*Triticum aestivum L.*) and barley (*Hordeum vulgare*) against different insects species, including *Callosobruchus maculatus*, *R. dominica* F. and *Tribolium confusum* Jacquelin du Val .[185-189] Insects utilize a variety of cuticular lipids to protect their water barrier against desiccation.[189] Mechanistically, silica NMs are absorbed into the insect cuticular lipid, disrupting the structures and causing desiccation. In Europe, use of stable aggregated synthetic amorphous SiO_2 NMs with size >1 μm as an insecticide has been approved.[99] Other metal-based NMs including Ag, Al_2O_3, TiO, and ZnO NMs have also been shown to be effective in the control of insects such as rice weevil *(Sitophilus oryzae)*.[20] Similarly, a recent study[190] revealed the insecticidal potential of nanostructured CuO and CaO against cotton leafworm (*Spodoptera littoralis*), with higher mortality rates from CuO NM after 3 days (LC_{50} =232.75 mg/L) compared to CaO NM (LC_{50} = 129.03 mg/L) after 11 days of exposure.[190] The difference in the mode of action of the NMs was attributed to their unique physicochemical properties and interactions with the insect midgut and cuticle layer. Unfortunately, the authors did not compare the NMs with a positive control such as a known commercial insecticide. Moreover, the relatively high concentrations of the NMs (150-600 mg/L) used in the experiment may have negative impacts on non-target organisms.

Biologically derived ENMs have also been evaluated for their insecticidal ability. For example, Sahab *et al*.[191] reported the insecticidal activity of Chitosan-g-poly (acrylic acid) (PAA) NMs against three insect pests of soybean (*Aphis gossypii, Callosobruchus maculatus*, and *Callosobruchus maculatus*). Another study[192] investigated the insecticidal activity of CNM-based formulations against *Helicoverpa armigera*. The CNM composites, with 32-90 nm size range, were synthesized by separately crosslinking CNM with glutaraldehyde (GLA) or tripolyphosphate (TPP), or by interacting CNM with a patented botanical pesticide, PONNEEM®. The CNMs, prepared in different combinations, were all highly stable, and. at 0.1, 0.2, and 0.3% exposure levels, exhibited toxicity against the insects. At 0.3% exposure level, specific combinations of the nano-formulation demonstrated significant anti-feedant (CNMs-TPP-PONNEEM, 20%), or larvicidal (CNMs-TPP-PONNEEM, 18%; and CNMs- GLA-PONNEEM, 15%) activity against *H. armigera* relative to the commercial neem pesticide (0.15% emulsifiable concentrate) as a reference control.[192] The nano-formulations also distorted the growth of the insect at the larval stage. Taken together, the study of Paulraj *et al.*[192] clearly demonstrates efficient pesticidal activity against a pest species using a relatively low concentration (0.3%) of the nano-formulations, regardless of the crosslinking agent (TPP or GLA) involved.

ENMs have also been investigated for the control of soil nematodes. For example, Yin *et al.*[101] demonstrated higher nematicidal efficacy of lansiumamide B against *Bursaphelenehus xylophilus* and second stage juveniles of *Meloidogyne incognita*.[101] Subsequently, the nanocapsules reduced the root knot disease incidence in water spinach (*Ipomoea aquatica*) by 68% compared with untreated control. Lansiumamide B has low solubility in water[101] and nanogels can be used to significantly enhance loading and release of active ingredients. Notably, as stated above, a limited number of studies have been conducted on this aspect of ENMs utilization. Among the reported data, few[184,191] were conducted in real agricultural fields, and evaluated the insecticidal potential of the NMs relative to conventional insecticides.[101,183,184,192]

Similarly, nano-enabled herbicides can play an important role in the sustainable eradication of unwanted weed species.[98,99] The long-term use of conventional herbicides may lead to accumulation of residues in the soil, and to herbicide resistance in the target species. For instance, atrazine is one of the most widely used herbicides in the United States, with strong potential to contaminate agricultural soils and drinking water sources. Currently, there is debate over the potential of atrazine to affect human/animal health through endocrine disruption.[193] However, encapsulation of herbicides in polymeric NMs can be a strategy to enhance the environmental safety and efficacy of current herbicides such as atrazine, significantly reducing the rate of application .[23] Herbicides can be embedded in specific ENM formulations for targeted delivery into the roots of weed species. Due to high penetrability facilitated by the ENMs, the herbicide can be taken up into the weed root tissues and transferred to target cells, where obstruction of significant metabolic pathways such as glycolysis can lead to toxicity and death.[23] Schnoor *et al*.[194] evaluated the herbicidal efficacy of synthesized poly (lactic-co-glycolic-acid) (PLGA) loaded with atrazine, using potato as the test plant. Controlled slow release of the atrazine was achieved, with only 15% of the compound released after 72 h of application on the potato plants. The nano-formulation significantly inhibited potato root length *in vitro*. Based on this finding, the authors concluded that the formulation can be presented as a nano-herbicide for controlling weed growth. However, the formulation should be evaluated on an actual weed species, preferably in the presence of a non-target food crop and with a non-nanoscale form for comparison. In addition, the interaction of PLGA, atrazine, and solvents can be improved if the mechanism of toxicity of the nano-formulation against weed species is better understood. Similarly, a nanocapsule of poly (ε-caprolactone) containing ametryn and atrazine was reported to be more effective against algae *(Pseudokirchneriella subcapitata)* as compared to the free herbicides (ametryn and atrazine).[195] Although the study showed that the nanocomposite was relatively toxic to the algae, the herbicidal potential should also be assessed on other weed species, preferably under field conditions. Similar to atrazine, glyphosate-based herbicides (GBHs) are also heavily used in United States, with about 109 million kilograms used in 2014.[196,197] However, there is growing concern about glyphosate accumulation and persistence in crops due to continuous exposure, with environmental, and possibly, human health implications.[198] Recently, glyphosate has been considered a probable carcinogen by the World Health Organization's International Agency for Research on Cancer.[197] However, it remains an open question whether the rise in glyphosate resistance and societal discomfort with genetically modified glyphosate-tolerant crop plants will foster the development and use of nano-enabled herbicides as alternatives. Along this line, the herbicidal potential of a nanocomposite containing glyphosate, amino silicon oil (ASO), attapulgite (ATP), azobenzene (AZO), and biochar was investigated in a greenhouse experiment.[199] The nanocomposite significantly promoted light-responsive controlled release performance against *Bermuda* weeds. The authors proposed strong adhesion of the nanocomposite on the leaf surface as a mechanism enhancing its efficacy. Ostensibly, enhanced adhesion reduced glyphosate run-off and loss, potentially decreasing unwanted environmental impacts. Although biochar-ATP was added in the formulation as a carrier for Glyphosate and ASO, the complex interactions among the different components need to be further examined, considering that ATP contains elements (Mg and Si) that can evoke plant responses contrary to the herbicidal effects. Moreover, the possible impact of the formulation on non-targeted plant species also needs to be assessed.

Herbicidal activity of encapsulated Ag-chitosan NMs has also been shown to form necrotic lesions on the plant *Eichhornia crassipes.*[200] Slow release of the active ingredient from the formulation was observed, with 90% release during 24 h. Notably, soil nutrient (macro and micro) levels, soil enzymes, microflora seedling emergence, and growth of black gram (*Vigna mungo)* were unaffected when compared with untreated control.[200] Other studies have also demonstrated enhanced controlled release of herbicides using CNMs. For instance, Yu *et al*.[164] reported efficient controlled release of diuron by glutathione-responsive stably loaded carboxymethyl chitosan NMs (250 nm). Interestingly, at neutral pH the diuron-loaded NMs exhibited herbicidal activity against *Echinochloa crusgalli*, with no growth inhibitory effect observed on non-targeted plant species (*Zea mays*). This study demonstrated that glutathione at high concentration (2-3 mM) slowed the release of diuron from the nano-formulation, thus enhancing herbicidal efficacy. Similarly, Maruyama *et al*.[165] demonstrated smart and efficient delivery of encapsulated herbicides (imazapic and imazapyr) in highly stable alignate-chitosan and chitosan-TPP NMs. Cytotoxicity and genotoxicity assays using the nano-formulations demonstrated efficacy exceeding 60% against *Bidens pilosa* (black-jack), with reduced toxicity towards non-target soil microorganisms.[165] The nano-formulations, when applied on the target weed species at the same concentration (400 g/ha) used in the field, significantly inhibited root and shoot growth as compared with the untreated control and free imazapic and imazapyr.[165] This study highlights the use of nano-formulations as promising alternative herbicidal products, with reduced toxicity against non-target organisms.

Nanoformulations containing other NMs (Ag, Cu, CuO, Fe, Mn and Zn) have also been shown to possess herbicidal activity against *Allium cepa (L.), Cucurbita pepo, Raphanus sativus, Lolium perenne, Lolium rigidum, Fagopyrum esculentum, Elodea densa* and *Cucumis sativus*.[23] Overall, none of the reported studies[164,165,194,195,199,200] evaluated the herbicidal potential of ENMs in real agricultural fields, and few[165,194,195,199] that compared the findings with conventional herbicides. Despite these impressive advances, continuous evaluation of these novel nano-based herbicide is necessary, bearing in mind the effect of dosage, and impact on non-target plant and animal species. In addition, molecular evaluation of the mechanism of toxicity is needed to characterize any nanoscale-specific effects.

8. Limitations, knowledge gap and future directions

Although ENMs have demonstrated potential in a wide array of applications in agriculture, significant limitations and knowledge gaps remain. For example, as noted above, more soil and field-based studies are necessary to demonstrate the efficacy and, importantly, the reproducibility of ENM effects under realistic agricultural conditions. Furthermore, the beneficial effects of ENMs as plant disease suppressing agents are dependent on multiple factors, including material properties (size/morphology/charge/coating), exposure concentration, plant species, pathogen presence, and timing of application. Thus, the selection of appropriate ENM type, dosage, and application regime are critical for ensuring beneficial outcomes. It must also be noted that the majority of ENMs are metallic in nature. As such, large-scale application of such ENMs could potentially lead to metal contamination of soil. Thus, careful consideration of application dose and establishment of an efficient and targeted delivery strategy are required prior to utilization.[65,201-203] Clearly, it is necessary to ensure that nanopesticides do not affect non-target plant growth-promoting microbes or other symbiotic microbial species, in the plant, or soil. Furthermore, considering the evolutionary pressure driving microbial pathogens to develop resistance upon repeated application of conventional pesticides, critical assessment of similar tendency towards ENMs-based nanopesticides should be made.

Undoubtedly, nanofertilizers and nanopesticides hold great promise for nano-enabled agriculture. Yet, more research is needed prior to their widespread use. Understanding the basic mechanisms of action of ENMs will enable a more clear understanding of the material properties driving positive (or negative) effects. This, in turn, will enable the synthesis and manipulation of materials with desired properties, resulting in highly tunable, responsive and smart nano-enabled agrochemicals that can be applied on a situational basis. For instance, coating ENMs' surface with certain agents may improve their antimicrobial properties and change their bio-interactions.[204] Considering the anticipated goal of reducing environmental impact of ENMs as agrochemicals, developing standard strategies for measuring their physicochemical properties will be crucial.[201] Ultimately, the economic viability, societal acceptance, and regulatory compliance must all be considered in order to realize the goal of commercialization of nano-fertilizers and nano-pesticides for large-scale agricultural application.

9. Conclusion

Collectively, the evidence provided in this review strongly indicate that nanotechnology has immense potential to improve the efficacy of agrochemical delivery and utilization by crops. As a result of extensive research on plant exposure to nanoparticles, it is clear that ENMs can have detrimental effects at higher concentrations. However, lower dose applications of select ENMs under specific conditions will yield beneficial effects, including enhanced delivery of nutrients, antimicrobial and disease suppression, and insecticidal and herbicidal applications. One very significant development associated with ENMs is that they can significantly reduce the amount of metals/agrichemicals being released into the environment, when compared to conventional formulations. ENM-based soil or foliar fertilization can be achieved through macro- and micronutrient amendments; whereas, the suppression of plant pathogens in infected systems can be attributed to *in vivo* generation of ROS and activation of antioxidant enzymes by ENMs and other secondary metabolites. Enhanced nutrition through nano-fertilizers could promote inherent plant defense and systemic resistance pathways. Notably, studies often compare ENM effects against untreated controls, with claims of positive outcomes. It is important that all assessment of ENM effects on plants as nano-fertilizers or nano-pesticides involve the corresponding conventional equivalents.[9,144,201,203] Also, in all cases, a cost-benefit analysis of the use of ENMs should be conducted. Without such comparisons, accurate claims of the efficacy and cost effectiveness of nano-enabled agrochemicals cannot be made. Given the small profit margin associated with agriculture/food production, novel strategies will have to be equally effective to conventional approaches, both in terms of economics and efficacy. If developed and applied properly, nanoenabled agricultural approaches such as those described in this review will be a critical component in achieving and sustaining global food security and safety.

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References

1. Bindraban, P. S., Dimkpa, C. O., Angle, S., & Rabbinge, R. (2018). Unlocking the multiple public good services from balanced fertilizers. *Food Security*, 1-13.

2. Kagan, C. R. (2016) "At the nexus of food security and safety: opportunities for nanoscience and nanotechnology." *ACS Nano.*10, 2985-2986.

3. Zhang, W. (2018). Global pesticide use: Profile, trend, cost/benefit and more. *Proceedings of the International Academy of Ecology and Environmental Sciences*, *8*(1), 1.

4. Oerke, E. C., & Dehne, H. W. (2004). Safeguarding production—losses in major crops and the role of crop protection. Crop protection, 23(4), 275-285.

5. FAO News. (2015). FAO report: Keeping plant pests and diseases at bay: experts focus on global measures at annual meeting of the Commission on Phytosanitary Measures (CPM); http://www.fao.org/news/story/en/item/280489/icode/ (Accessed February 27, 2018)

6. Servin, A., Elmer, W., Mukherjee, A., De la Torre-Roche, R., Hamdi, H., White, J. C., ... & Dimkpa, C. (2015). A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. *Journal of Nanoparticle Research*, *17*(2), 92.

7. Elmer, W. H., & White, J. C. (2016). The use of metallic oxide nanoparticles to enhance growth of tomatoes and eggplants in disease infested soil or soilless medium. *Environmental Science: Nano*, *3*(5), 1072-1079.

8. Wang, Z., Xie, X., Zhao, J., Liu, X., Feng, W., White, J. C., & Xing, B. (2012). Xylem-and phloem-based transport of CuO nanoparticles in maize (Zea mays L.). *Environmental science & technology*, *46*(8), 4434-4441.

9. Dimkpa, C. O., & Bindraban, P. S. (2017). Nanofertilizers: New products for the industry? *Journal of agricultural and food chemistry*, 66: 6462–6473.

10. Elmer, W. H., & White, J. C. (2018). The future of nanotechnology in plant pathology. *Annual Review phytopathology*, 56, 6.1-6.23.

11. Prasad, R., Bhattacharyya, A., & Nguyen, Q. D. (2017). Nanotechnology in sustainable agriculture: recent developments, challenges, and perspectives. *Frontiers in microbiology*, *8*, 1014.

12. Verma, S. K., Das, A. K., Patel, M. K., Shah, A., Kumar, V., & Gantait, S. (2018). Engineered nanomaterials for plant growth and development: A perspective analysis. *Science of the Total Environment*, *630*, 1413-1435.

13. Rodrigues, S. M., Demokritou, P., Dokoozlian, N., Hendren, C. O., Karn, B., Mauter, M. S., ... & Welle, P. (2017). Nanotechnology for sustainable food production: promising opportunities and scientific challenges. Environmental Science: Nano, 4(4), 767-781.

14. White, J. C., Gardea-Torresdey, J. L. (2018). Achieving food security through the very small. *Nature Nanotechnology* 13, 621-629.

15. Adisa, I. O., Pullagurala, V. L., Rawat, S., Hernandez-Viezcas, J. A., Dimkpa, C., Elmer, W. H., ... & Gardea-Torresdey, J. L. (2018). Role of cerium compounds in Fusarium wilt suppression and growth enhancement in tomato (Solanum lycopersicum). *Journal of Agricultural and Food Chemistry*.

16. Borgatta, J., Ma, C., Hudson-Smith, N., Elmer, W., Plaza Pérez, C. D., De La Torre-Roche, R., ... & Hamers, R. J. (2018). Copper Based Nanomaterials Suppress Root Fungal Disease in Watermelon (Citrullus lanatus): Role of Particle Morphology, Composition and Dissolution Behavior. *ACS Sustainable Chemistry & Engineering*, *6*(11), 14847-14856.

17. Dimkpa, C. O., White, J. C., Elmer, W. H., & Gardea-Torresdey, J. (2017). Nanoparticle and Ionic Zn Promote Nutrient Loading of Sorghum Grain under Low NPK Fertilization. *Journal of agricultural and food chemistry*, *65*(39), 8552-8559.

18. Pullagurala, V. L. R., Adisa, I. O., Rawat, S., Kalagara, S., Hernandez-Viezcas, J. A., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2018). ZnO nanoparticles increase

photosynthetic pigments and decrease lipid peroxidation in soil grown cilantro (Coriandrum sativum). *Plant Physiology and Biochemistry*, *132*, 120-127.

19. Dimkpa, C., Singh, U., Adisa, I., Bindraban, P., Elmer, W., Gardea-Torresdey, J., & White, J. (2018). Effects of manganese nanoparticle exposure on nutrient acquisition in wheat (Triticum aestivum L.). *Agronomy*, *8*(9), 158.
20. Kumaraswamy, R. V., Kumari, S., Choudhary, R. C., Pal, A., Raliya, R., Biswas, P., & Saharan, V. (2018). Engineered chitosan based nanomaterials: Bioactivity, mechanisms and perspectives in plant protection and growth. *International journal of biological macromolecules*.
21. Dananjaya, S. H. S., Erandani, W. K. C. U., Kim, C. H., Nikapitiya, C., Lee, J., & De Zoysa, M. (2017). Comparative study on antifungal activities of chitosan nanoparticles and chitosan silver nano composites against Fusarium oxysporum species complex. *International journal of biological macromolecules*, *105*, 478-488.
22. Anusuya, S., & Sathiyabama, M. (2015). Foliar application of β-D-glucan nanoparticles to control rhizome rot disease of turmeric. *International journal of biological macromolecules*, *72*, 1205-1212.
23. Duhan, J. S., Kumar, R., Kumar, N., Kaur, P., Nehra, K., & Duhan, S. (2017). Nanotechnology: The new perspective in precision agriculture. *Biotechnology Reports*, *15*, 11-23.
24. Goswami, A., Roy, I., Sengupta, S., & Debnath, N. (2010). Novel applications of solid and liquid formulations of nanoparticles against insect pests and pathogens. *Thin solid films*, *519*(3), 1252-1257.

25. Pullagurala, V. L. R., Adisa, I. O., Rawat, S., Kim, B., Barrios, A. C., Medina-Velo, I. A., ... & Gardea-Torresdey, J. L. (2018). Finding the conditions for the beneficial use of ZnO nanoparticles towards plants-A review. *Environmental Pollution*.

26. Reddy, P. V. L., Hernandez-Viezcas, J. A., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2016). Lessons learned: are engineered nanomaterials toxic to terrestrial plants?. *Science of the Total Environment*, *568*, 470-479.

27. Chhipa, H. (2017). Nanofertilizers and nanopesticides for agriculture. *Environmental Chemistry Letters*, *15*(1), 15-22.

28. Marchiol, L. (2018). Nanotechnology in Agriculture: New Opportunities and Perspectives. In New Visions in Plant Science. IntechOpen.

29. Liu, R., & Lal, R. (2015). Potentials of engineered nanoparticles as fertilizers for increasing agronomic productions. *Science of the total environment*, *514*, 131-139.

30. Peralta-Videa, J.R. (2018). Are nanomaterials a real solution for sustainable agriculture? Acta Scientific Agriculture 2(3), 16-17.

31. Yin, J., Wang, Y., & Gilbertson, L. M. (2018). Opportunities to advance sustainable design of nano-enabled agriculture identified through a literature review. *Environmental Science: Nano*.

32. Subramanian, K. S., Manikandan, A., Thirunavukkarasu, M., & Rahale, C. S. (2015). Nano-fertilizers for balanced crop nutrition. In *Nanotechnologies in Food and Agriculture* (pp. 69-80). Springer, Cham.

33. Alexandratos, N., & Bruinsma, J. (2012). *World agriculture towards 2030/2050: the 2012 revision* (Vol. 12, No. 3). FAO, Rome: ESA Working paper.

34. Kottegoda, N., Munaweera, I., Madusanka, N., & Karunaratne, V. (2011). A green slow-release fertilizer composition based on urea-modified hydroxyapatite nanoparticles encapsulated wood. *Current science*, 73-78.

35. Kottegoda, N., Sandaruwan, C., Priyadarshana, G., Siriwardhana, A., Rathnayake, U. A., Berugoda Arachchige, D. M., ... & Amaratunga, G. A. (2017). Urea-hydroxyapatite nanohybrids for slow release of nitrogen. *ACS nano*, *11*(2), 1214-1221.

36. Abdel-Aziz, H. M., Hasaneen, M. N., & Omer, A. M. (2016). Nano chitosan-NPK fertilizer enhances the growth and productivity of wheat plants grown in sandy soil. *Spanish Journal of Agricultural Research*, *14*(1), 0902.

37. Amirnia, R.; Bayat, M.; Tajbakhsh, M. (2014). Effects of nano fertilizer application and maternal corm weight on flowering at some saffron (Crocus sativus L.) ecotypes. *Turkish J. Field Crops* 19, 158−168.

38. Liu, R., & Lal, R. (2014). Synthetic apatite nanoparticles as a phosphorus fertilizer for soybean (Glycine max). *Scientific reports*, *4*, 5686.

39. Liu, X., Zhang, F., Zhang, S., He, X., Wang, R., Fei, Z., & Wang, Y. (2005). Responses of peanut to nano-calcium carbonate. *Plant Nutrition and Fertitizer Science*, *11*(3), 385-389.

40. Deepa, M., Sudhakar, P., Nagamadhuri, K. V., Reddy, K. B., Krishna, T. G., & Prasad, T. N. V. K. V. (2015). First evidence on phloem transport of nanoscale calcium oxide in groundnut using solution culture technique. *Applied Nanoscience*, *5*(5), 545-551.

41. Yugandhar, P., Savithramma, N. (2013). Green synthesis of calcium carbonate nanoparticles and their effects on seed germination and seedling growth of Vigna mungo (L.) Hepper. *Int. J. Adv. Res.* 1, 89−103

42. Delfani, M., Baradarn Firouzabadi, M., Farrokhi, N., & Makarian, H. (2014). Some physiological responses of black-eyed pea to iron and magnesium nanofertilizers. *Communications in soil science and plant analysis*, *45*(4), 530-540.

43. Salem, N. M., Albanna, L. S., Abdeen, A. O., Ibrahim, Q. I., & Awwad, A. M. (2016). Sulfur nanoparticles improves root and shoot growth of tomato. *Journal of Agricultural Science*, *8*(4), 179.

44. Salem, N. M., Albanna, L. S., Awwad, A. M., Ibrahim, Q. M., & Abdeen, A. O. (2015). Green synthesis of nano-sized sulfur and its effect on plant growth. *Journal of Agricultural Science*, *8*(1), 188.

45. Ghafariyan, M. H., Malakouti, M. J., Dadpour, M. R., Stroeve, P., & Mahmoudi, M. (2013). Effects of magnetite nanoparticles on soybean chlorophyll. *Environmental science & technology*, *47*(18), 10645-10652.

46. Rui, M., Ma, C., Hao, Y., Guo, J., Rui, Y., Tang, X., ... & Zhu, S. (2016). Iron oxide nanoparticles as a potential iron fertilizer for peanut (Arachis hypogaea). *Frontiers in plant science*, *7*, 815.

47. Pradhan, S., Patra, P., Das, S., Chandra, S., Mitra, S., Dey, K. K., ... & Goswami, A. (2013). Photochemical modulation of biosafe manganese nanoparticles on Vigna radiata: a detailed molecular, biochemical, and biophysical study. *Environmental science & technology*, *47*(22), 13122-13131.

48. Adhikari, T., Sarkar, D., Mashayekhi, H., & Xing, B. (2016). Growth and enzymatic activity of maize (Zea mays L.) plant: solution culture test for copper dioxide nano particles. *Journal of plant nutrition*, *39*(1), 99-115.

49. Adhikari, T., Kundu, S., Biswas, A. K., Tarafdar, J. C., & Subba Rao, A. (2015). Characterization of zinc oxide nano particles and their effect on growth of maize (Zea mays L.) plant. *Journal of plant nutrition*, *38*(10), 1505-1515.

50. Subbaiah, L. V., Prasad, T. N. V. K. V., Krishna, T. G., Sudhakar, P., Reddy, B. R., & Pradeep, T. (2016). Novel effects of nanoparticulate delivery of zinc on growth, productivity, and zinc biofortification in maize (Zea mays L.). *Journal of agricultural and food chemistry*, *64*(19), 3778-3788.

51. Dimkpa, C. O., Singh, U., Bindraban, P. S., Elmer, W. H., Gardea-Torresdey, J. L., White, J. C. (2018). Exposure to weathered and fresh nanoparticle and ionic Zn in soil promotes grain yield and modulates nutrient acquisition in wheat (*Triticum aestivum* L.) Journal of Agricultural and Food Chemistry, doi 10.1021/acs.jafc.8b03840

52. Dimkpa, C. O., Bindraban, P. S., Fugice, J., Agyin-Birikorang, S., Singh, U., & Hellums, D. (2017). Composite micronutrient nanoparticles and salts decrease drought stress in soybean. *Agronomy for Sustainable Development*, *37*(1), 5.

53. Yuvaraj, M., & Subramanian, K. S. (2015). Controlled-release fertilizer of zinc encapsulated by a manganese hollow core shell. *Soil science and plant nutrition*, *61*(2), 319-326.

54. Watson, J. L., Fang, T., Dimkpa, C. O., Britt, D. W., McLean, J. E., Jacobson, A., & Anderson, A. J. (2015). The phytotoxicity of ZnO nanoparticles on wheat varies with soil properties. *Biometals*, *28*(1), 101-112.

55. Singh, D., & Kumar, A. (2016). Impact of irrigation using water containing CuO and ZnO nanoparticles on Spinach oleracea grown in soil media. *Bulletin of environmental contamination and toxicology*, *97*(4), 548-553.

56. Pullagurala, V. L. R., Adisa, I. O., Rawat, S., Kalagara, S., Hernandez-Viezcas, J. A., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2018). ZnO nanoparticles increase photosynthetic pigments and decrease lipid peroxidation in soil grown cilantro (Coriandrum sativum). Plant physiology and biochemistry, 132, 120-127.

57. Dimkpa, C. O., Singh, U., Bindraban, P. S., Adisa, I. O., Elmer, W. H., Gardea-Torresdey, J. L., & White, J. C. (2019). Addition-omission of zinc, copper, and boron nano and bulk oxide particles demonstrate element and size-specific response of soybean to micronutrients exposure. *The Science of the total environment*, *665*, 606.

58. Taha, R. A., Hassan, M. M., Ibrahim, E. A., Baker, N. H. A., & Shaaban, E. A. (2016). Carbon nanotubes impact on date palm in vitro cultures. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *127*(2), 525-534.

59. Khodakovskaya, M. V., De Silva, K., Biris, A. S., Dervishi, E., & Villagarcia, H. (2012). Carbon nanotubes induce growth enhancement of tobacco cells. *ACS nano*, *6*(3), 2128-2135

60. Rico, C. M., Lee, S. C., Rubenecia, R., Mukherjee, A., Hong, J., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2014). Cerium oxide nanoparticles impact yield and modify nutritional parameters in wheat (Triticum aestivum L.). *Journal of agricultural and food chemistry*, *62*(40), 9669-9675.

61. Rico, C. M., Barrios, A. C., Tan, W., Rubenecia, R., Lee, S. C., Varela-Ramirez, A., ... & Gardea-Torresdey, J. L. (2015). Physiological and biochemical response of soil-grown barley (Hordeum vulgare L.) to cerium oxide nanoparticles. *Environmental Science and Pollution Research*, *22*(14), 10551-10558.

62. Zhao, L., Peralta-Videa, J. R., Rico, C. M., Hernandez-Viezcas, J. A., Sun, Y., Niu, G., ... & Gardea-Torresdey, J. L. (2014). CeO_2 and ZnO nanoparticles change the nutritional qualities of cucumber (Cucumis sativus). *Journal of agricultural and food chemistry*, *62*(13), 2752-2759.

63. Derbyshire, E., Wright, D. J., & Boulter, D. (1976). Legumin and vicilin, storage proteins of legume seeds. *Phytochemistry*, *15*(1), 3-24.

64. Malerba, M., & Cerana, R. (2016). Chitosan effects on plant systems. *International journal of molecular sciences*, *17*(7), 996.

65. Kashyap, P. L., Xiang, X., & Heiden, P. (2015). Chitosan nanoparticle based delivery systems for sustainable agriculture. *International journal of biological macromolecules*, *77*, 36-51.

66. Van, S. N., Minh, H. D., & Anh, D. N. (2013). Study on chitosan nanoparticles on biophysical characteristics and growth of Robusta coffee in green house. Biocatalysis and Agricultural Biotechnology, 2(4), 289-294.

67. Saharan, V., Sharma, G., Yadav, M., Choudhary, M. K., Sharma, S. S., Pal, A., ... & Biswas, P. (2015). Synthesis and in vitro antifungal efficacy of Cu–chitosan nanoparticles against pathogenic fungi of tomato. *International journal of biological macromolecules*, *75*, 346-353.

68. Saharan, V., Kumaraswamy, R. V., Choudhary, R. C., Kumari, S., Pal, A., Raliya, R., & Biswas, P. (2016). Cu-chitosan nanoparticle mediated sustainable approach to enhance seedling growth in maize by mobilizing reserved food. *Journal of agricultural and food chemistry*, *64*(31), 6148-6155.

69. Choudhary, R. C., Kumaraswamy, R. V., Kumari, S., Sharma, S. S., Pal, A., Raliya, R., ... & Saharan, V. (2017). Cu-chitosan nanoparticle boost defense responses and plant growth in maize (Zea mays L.). Scientific reports, 7(1), 9754.

70. Sathiyabama, M., & Charles, R. E. (2015). Fungal cell wall polymer based nanoparticles in protection of tomato plants from wilt disease caused by Fusarium oxysporum f. sp. lycopersici. *Carbohydrate polymers*, *133*, 400-407.

71. Sathiyabama, M., & Parthasarathy, R. (2016). Biological preparation of chitosan nanoparticles and its in vitro antifungal efficacy against some phytopathogenic fungi. Carbohydrate polymers, 151, 321-325.

72. Zagzog, O. A., Gad, M. M., & Hafez, N. K. (2017). Effect of nano-chitosan on vegetative growth, fruiting and resistance of malformation of mango. *Trends Hortic. Res*, *6*, 673-681.

73. Juarez-Maldonado A, Ortega-Ortiz H, Perez-Labrada F, Cadenas-Pliego G, Benavides-Mendoza A. (2016). Cu nanoparticles absorbed on chitosan hydrogels positively alter morphological, production, and quality characteristics of tomato. J Appl Bot Food Qual. 89:183–9.

74. El-Batal, A. I., Gharib, F. A. E. L., Ghazi, S. M., Hegazi, A. Z., & Hafz, A. G. M. A. E. (2016). Physiological responses of two varieties of common bean (Phaseolus vulgaris L.) to foliar application of silver nanoparticles. *Nanomaterials and Nanotechnology*, *6*, 13.

75. Venkatachalam, P., Malar, S., Thiyagarajan, M., Indiraarulselvi, P., & Geetha, N. (2017). Effect of phycochemical coated silver nanocomplexes as novel growth-stimulating compounds for plant regeneration of Alternanthera sessilis L. *Journal of applied phycology*, *29*(2), 1095-1106.

76. Cao, Z., Rossi, L., Stowers, C., Zhang, W., Lombardini, L., & Ma, X. (2018). The impact of cerium oxide nanoparticles on the physiology of soybean (Glycine max (L.) Merr.) under different soil moisture conditions. *Environmental Science and Pollution Research*, *25*(1), 930-939.

77. Gui, X., Rui, M., Song, Y., Ma, Y., Rui, Y., Zhang, P., ... & Liu, L. (2017). Phytotoxicity of CeO 2 nanoparticles on radish plant (Raphanus sativus). *Environmental Science and Pollution Research*, *24*(15), 13775-13781.

78. Rico, C. M., Johnson, M. G., Marcus, M. A., & Andersen, C. P. (2017). Intergenerational responses of wheat (*Triticum aestivum L.*) to cerium oxide nanoparticles exposure. *Environmental Science: Nano*, *4*(3), 700-711.

79. Singh, A., Singh, N. B., Hussain, I., & Singh, H. (2017). Effect of biologically synthesized copper oxide nanoparticles on metabolism and antioxidant activity to the crop plants Solanum lycopersicum and Brassica oleracea var. botrytis. *Journal of biotechnology*, *262*, 11-27.

80. Apodaca, S. A., Tan, W., Dominguez, O. E., Hernandez-Viezcas, J. A., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2017). Physiological and biochemical effects of nanoparticulate copper, bulk copper, copper chloride, and kinetin in kidney bean (Phaseolus vulgaris) plants. *Science of the Total Environment*, *599*, 2085-2094.

81. Hernández, H. H., Benavides-Mendoza, A., Ortega-Ortiz, H., Hernández-Fuentes, A. D., & Juárez-Maldonado, A. (2017). Cu Nanoparticles in chitosan-PVA hydrogels as promoters of growth, productivity and fruit quality in tomato. *Emirates Journal of Food and Agriculture*, *29*(8), 573-580.

82. Sheykhbaglou, R., Sedghi, M., Shishevan, M. T., & Sharifi, R. S. (2010). Effects of nano-iron oxide particles on agronomic traits of soybean. *Notulae Scientia Biologicae*, *2*(2), 112-113.

83. Jeyasubramanian, K., Thoppey, U. U. G., Hikku, G. S., Selvakumar, N., Subramania, A., & Krishnamoorthy, K. (2016). Enhancement in growth rate and productivity of spinach grown in hydroponics with iron oxide nanoparticles. *Rsc Advances*, *6*(19), 15451-15459.

84. Shankramma, K., Yallappa, S., Shivanna, M. B., & Manjanna, J. (2016). Fe_2O_3 magnetic nanoparticles to enhance S. lycopersicum (tomato) plant growth and their biomineralization. *Applied Nanoscience*, *6*(7), 983-990.

85. Rawat, M., Nayan, R., Negi, B., Zaidi, M. G. H., & Arora, S. (2017). Physio-biochemical basis of iron-sulfide nanoparticle induced growth and seed yield enhancement in B. juncea. *Plant Physiology and Biochemistry*, *118*, 274-284.

86. Marchiol, L., Mattiello, A., Pošćić, F., Fellet, G., Zavalloni, C., Carlino, E., & Musetti, R. (2016). Changes in physiological and agronomical parameters of barley (Hordeum vulgare) exposed to cerium and titanium dioxide nanoparticles. *International journal of environmental research and public health*, *13*(3), 332.

87. Tiwari, M., Sharma, N. C., Fleischmann, P., Burbage, J., Venkatachalam, P., & Sahi, S. V. (2017). Nanotitania Exposure Causes Alterations in Physiological, Nutritional and Stress Responses in Tomato (Solanum lycopersicum). *Frontiers in plant science*, *8*, 633.

88. Singh, P., Singh, R., Borthakur, A., Srivastava, P., Srivastava, N., Tiwary, D., & Mishra, P. K. (2016). Effect of nanoscale TiO 2-activated carbon composite on Solanum lycopersicum (L.) and Vigna radiata (L.) seeds germination. *Energy, Ecology and Environment*, *1*(3), 131-140.

89. Yassen, A., Abdallah, E., Gaballah, M., & Zaghloul, S. (2017). Role of Silicon Dioxide Nano Fertilizer in Mitigating Salt Stress on Growth, Yield and Chemical Composition of Cucumber (Cucumis sativus L.). *International Journal of Agricultural Research*, *12*, 130-135.

90. Yousefi, R., & Esna-Ashari, M. (2017). The effect of micro-and nanoparticles of silicon on concentration of macro-and microelements and silicon content of strawberry plant in soilless culture conditions. *Journal of Science and Technology of Greenhouse Culture*, *8*(1).

91. Faizan, M., Faraz, A., Yusuf, M., Khan, S. T., & Hayat, S. (2018). Zinc oxide nanoparticle-mediated changes in photosynthetic efficiency and antioxidant system of tomato plants. *Photosynthetica*, *56*(2), 678-686.

92. López-Moreno, M. L., de la Rosa, G., Cruz-Jiménez, G., Castellano, L., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2017). Effect of ZnO nanoparticles on corn seedlings at different temperatures; X-ray absorption spectroscopy and ICP/OES studies. *Microchemical Journal*, *134*, 54-61.

93. Xu, J., Luo, X., Wang, Y., & Feng, Y. (2018). Evaluation of zinc oxide nanoparticles on lettuce (Lactuca sativa L.) growth and soil bacterial community. *Environmental Science and Pollution Research*, *25*(6), 6026-6035.

94. Venkatachalam, P., Priyanka, N., Manikandan, K., Ganeshbabu, I., Indiraarulselvi, P., Geetha, N., ... & Sahi, S. V. (2017). Enhanced plant growth promoting role of phycomolecules coated zinc oxide nanoparticles with P supplementation in cotton (Gossypium hirsutum L.). *Plant Physiology and Biochemistry*, *110*, 118-127.

95. Rajiv, P., & Vanathi, P. (2018). Effect of parthenium based vermicompost and zinc oxide nanoparticles on growth and yield of arachis hypogaea l. in zinc deficient soil. *Biocatalysis and Agricultural Biotechnology*.

96. Deshpande, P., Dapkekar, A., Oak, M. D., Paknikar, K. M., & Rajwade, J. M. (2017). Zinc complexed chitosan/tpp nanoparticles: A promising micronutrient nanocarrier suited for foliar application. *Carbohydrate polymers*, *165*, 394-401.

97. Behboudi, F., Tahmasebi Sarvestani, Z., Kassaee, M. Z., Modares Sanavi, S. A. M., Sorooshzadeh, A., & Ahmadi, S. B. (2018). Evaluation of Chitosan Nanoparticles Effects on Yield and Yield Components of Barley (Hordeum vulgare L.) under Late Season Drought Stress. *Journal of Water and Environmental Nanotechnology*, *3*(1), 22-39.

98. Kah, M., & Hofmann, T. (2014). Nanopesticide research: current trends and future priorities. *Environment international*, *63*, 224-235.

99. Iavicoli, I., Leso, V., Beezhold, D. H., & Shvedova, A. A. (2017). Nanotechnology in agriculture: opportunities, toxicological implications, and occupational risks. *Toxicology and applied pharmacology*, *329*, 96-111.

100. Kookana, R. S., Boxall, A. B., Reeves, P. T., Ashauer, R., Beulke, S., Chaudhry, Q., ... & Lynch, I. (2014). Nanopesticides: guiding principles for regulatory evaluation of environmental risks. *Journal of agricultural and food chemistry*, *62*(19), 4227-4240.

101. Yin, Y. H., Guo, Q. M., Yun, H. A. N., Wang, L. J., & Wan, S. Q. (2012). Preparation, characterization and nematicidal activity of lansiumamide B nano-capsules. *Journal of Integrative Agriculture*, *11*(7), 1151-1158.

102. Tong, Y., Wu, Y., Zhao, C., Xu, Y., Lu, J., Xiang, S., ... & Wu, X. (2017). Polymeric Nanoparticles as a Metolachlor Carrier: Water-Based Formulation for Hydrophobic

Pesticides and Absorption by Plants. *Journal of agricultural and food chemistry*, *65*(34), 7371-7378.

103. Dutta, P., & Kaman, P. K. (2017). Nanocentric Plant Health Management with Special Reference to Silver. *Int. J. Curr. Microbiol. App. Sci*, *6*(6), 2821-2830.

104. Kumari, M., Pandey, S., Bhattacharya, A., Mishra, A., & Nautiyal, C. S. (2017). Protective role of biosynthesized silver nanoparticles against early blight disease in Solanum lycopersicum. *Plant Physiology and Biochemistry*, *121*, 216-225.

105. Mishra, S., Singh, B. R., Singh, A., Keswani, C., Naqvi, A. H., & Singh, H. B. (2014). Biofabricated silver nanoparticles act as a strong fungicide against Bipolaris sorokiniana causing spot blotch disease in wheat. *PLoS One*, *9*(5), e97881.

106. Ocsoy, I., Paret, M. L., Ocsoy, M. A., Kunwar, S., Chen, T., You, M., & Tan, W. (2013). Nanotechnology in plant disease management: DNA-directed silver nanoparticles on graphene oxide as an antibacterial against Xanthomonas perforans. *Acs Nano*, *7*(10), 8972-8980.

107. Sharma, S. (2017). Enhanced antibacterial efficacy of silver nanoparticles immobilized in a chitosan nanocarrier. *International journal of biological macromolecules*, *104*, 1740-1745.

108. Liang, Y., Yang, D., & Cui, J. (2017). A graphene oxide/silver nanoparticle composite as a novel agricultural antibacterial agent against Xanthomonas oryzae pv. oryzae for crop disease management. *New Journal of Chemistry*, *41*(22), 13692-13699.

109. Levard, C., Hotze, E. M., Lowry, G. V., & Brown Jr, G. E. (2012). Environmental transformations of silver nanoparticles: impact on stability and toxicity. *Environmental science & technology*, *46*(13), 6900-6914.

110. Massarsky, A., Trudeau, V. L., & Moon, T. W. (2014). Predicting the environmental impact of nanosilver. *Environmental toxicology and pharmacology*, *38*(3), 861-873.

111. Mishra, S., Singh, B. R., Naqvi, A. H., & Singh, H. B. (2017). Potential of biosynthesized silver nanoparticles using Stenotrophomonas sp. BHU-S7 (MTCC 5978) for management of soil-borne and foliar phytopathogens. Scientific reports, 7, 45154.

112. Ali, M., Kim, B., Belfield, K. D., Norman, D., Brennan, M., & Ali, G. S. (2015). Inhibition of Phytophthora parasitica and P. capsici by silver nanoparticles synthesized using aqueous extract of Artemisia absinthium. Phytopathology, 105(9), 1183-1190.

113. Chen, J., Li, S., Luo, J., Wang, R., & Ding, W. (2016). Enhancement of the antibacterial activity of silver nanoparticles against phytopathogenic bacterium Ralstonia solanacearum by stabilization. Journal of Nanomaterials, 2016.

114. Mallaiah, B. (2015). Integrated approaches for the management of crossandra (Crossandra infundibuliformis L. Nees) wilt caused by Fusarium incarnatum (Desm.) Sacc (Doctoral dissertation, Tamil Nadu Agricultural University, Madurai).

115. Strayer, A., Ocsoy, I., Tan, W., Jones, J. B., & Paret, M. L. (2016). Low concentrations of a silver-based nanocomposite to manage bacterial spot of tomato in the greenhouse. Plant disease, 100(7), 1460-1465.

116. Lamsal, K., Kim, S. W., Jung, J. H., Kim, Y. S., Kim, K. S., & Lee, Y. S. (2011). Inhibition effects of silver nanoparticles against powdery mildews on cucumber and pumpkin. *Mycobiology*, *39*(1), 26-32.

117. Lamsal, K., Kim, S. W., Jung, J. H., Kim, Y. S., Kim, K. S., & Lee, Y. S. (2011). Application of silver nanoparticles for the control of Colletotrichum species in vitro and pepper anthracnose disease in field. *Mycobiology*, *39*(3), 194-199.

118. Nejad, M. S., Bonjar, G. H. S., Khatami, M., Amini, A., & Aghighi, S. (2016). In vitro and in vivo antifungal properties of silver nanoparticles against Rhizoctonia solani, a common agent of rice sheath blight disease. *IET nanobiotechnology*, *11*(3), 236-240.

119. Zakharova, O. V., Gusev, A. A., Zherebin, P. M., Skripnikova, E. V., Skripnikova, M. K., Ryzhikh, V. E., ... & Krutyakov, Y. A. (2017). Sodium tallow amphopolycarboxyglycinate-stabilized silver nanoparticles suppress early and late blight of Solanum lycopersicum and stimulate the growth of tomato plants. *BioNanoScience*, *7*(4), 692-702.

120. Kanhed, P., Birla, S., Gaikwad, S., Gade, A., Seabra, A. B., Rubilar, O., ... & Rai, M. (2014). In vitro antifungal efficacy of copper nanoparticles against selected crop pathogenic fungi. *Materials Letters*, *115*, 13-17.

121. Viet, P. V., Nguyen, H. T., Cao, T. M., & Hieu, L. V. (2016). Fusarium antifungal activities of copper nanoparticles synthesized by a chemical reduction method. *Journal of Nanomaterials*, *2016*, 6.

122. Bramhanwade, K., Shende, S., Bonde, S., Gade, A., & Rai, M. (2016). Fungicidal activity of Cu nanoparticles against Fusarium causing crop diseases. *Environmental chemistry letters*, *14*(2), 229-235.

123. Hao, Y., Cao, X., Ma, C., Zhang, Z., Zhao, N., Ali, A., ... & Xing, B. (2017). Potential Applications and Antifungal Activities of Engineered Nanomaterials against Gray Mold Disease Agent Botrytis cinerea on Rose Petals. *Frontiers in plant science*, *8*, 1332.

124. Zabrieski, Z., Morrell, E., Hortin, J., Dimkpa, C., McLean, J., Britt, D., & Anderson, A. (2015). Pesticidal activity of metal oxide nanoparticles on plant pathogenic isolates of Pythium. *Ecotoxicology*, *24*(6), 1305-1314.

125. Keller, A. A., Adeleye, A. S., Conway, J. R., Garner, K. L., Zhao, L., Cherr, G. N., ... & Ji, Z. (2017). Comparative environmental fate and toxicity of copper nanomaterials. *NanoImpact*, *7*, 28-40.

126. Atwood, D., & Paisley-Jones, C. (2017). Pesticides Industry Sales and Usage: 2008–2012 Market Estimates. *United States Environmental Protection Agency: Washington, DC, USA*.

127. Giannousi, K., Avramidis, I., & Dendrinou-Samara, C. (2013). Synthesis, characterization and evaluation of copper based nanoparticles as agrochemicals against Phytophthora infestans. *RSC Advances*, *3*(44), 21743-21752.

128. Elmer, W., De La Torre-Roche, R., Pagano, L., Majumdar, S., Zuverza-Mena, N., Dimkpa, C., ... & White, J. C. (2018). Effect of Metalloid and Metal Oxide Nanoparticles on Fusarium Wilt of Watermelon. *Plant Disease*, PDIS-10.

129. Ponmurugan, P., Manjukarunambika, K., Elango, V., & Gnanamangai, B. M. (2016). Antifungal activity of biosynthesised copper nanoparticles evaluated against red root-rot disease in tea plants. *Journal of Experimental Nanoscience*, *11*(13), 1019-1031.

130. Strayer-Scherer, A., Liao, Y. Y., Young, M., Ritchie, L., Vallad, G. E., Santra, S., ... & Paret, M. L. (2018). Advanced Copper Composites Against Copper-Tolerant Xanthomonas perforans and Tomato Bacterial Spot. *Phytopathology*, PHYTO-06.

131. Gunalan, S., Sivaraj, R., & Rajendran, V. (2012). Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. *Progress in Natural Science: Materials International*, *22*(6), 693-700.

132. Dimkpa, C. O., McLean, J. E., Britt, D. W., & Anderson, A. J. (2013). Antifungal activity of ZnO nanoparticles and their interactive effect with a biocontrol bacterium on growth antagonism of the plant pathogen Fusarium graminearum. *Biometals*, *26*(6), 913-924.

133. Jayaseelan, C., Rahuman, A. A., Kirthi, A. V., Marimuthu, S., Santhoshkumar, T., Bagavan, A., ... & Rao, K. B. (2012). Novel microbial route to synthesize ZnO nanoparticles using Aeromonas hydrophila and their activity against pathogenic bacteria and fungi. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *90*, 78-84.

134. He, L., Liu, Y., Mustapha, A., & Lin, M. (2011). Antifungal activity of zinc oxide nanoparticles against Botrytis cinerea and Penicillium expansum. *Microbiological research*, *166*(3), 207-215.

135. Graham, J. H., Johnson, E. G., Myers, M. E., Young, M., Rajasekaran, P., Das, S., & Santra, S. (2016). Potential of Nano-formulated zinc oxide for control of citrus canker on grapefruit trees. *Plant disease*, *100*(12), 2442-2447.

136. Paret, M. L., Palmateer, A. J., & Knox, G. W. (2013). Evaluation of a light-activated nanoparticle formulation of titanium dioxide with zinc for management of bacterial leaf spot on rosa 'Noare'. *HortScience*, *48*(2), 189-192.

137. Xing, K., Xing, Y., Liu, Y., Zhang, Y., Shen, X., Li, X., ... & Qin, S. (2018). Fungicidal effect of chitosan via inducing membrane disturbance against *Ceratocystis fimbriata*. *Carbohydrate polymers*, *192*, 95-103.

138. Xing, K., Liu, Y., Shen, X., Zhu, X., Li, X., Miao, X., ... & Qin, S. (2017). Effect of O-chitosan nanoparticles on the development and membrane permeability of *Verticillium dahliae*. *Carbohydrate polymers*, *165*, 334-343.

139. Luque-Alcaraz, A. G., Cortez-Rocha, M. O., Velázquez-Contreras, C. A., Acosta-Silva, A. L., Santacruz-Ortega, H. D. C., Burgos-Hernández, A., ... & Plascencia-Jatomea, M. (2016). Enhanced antifungal effect of chitosan/pepper tree (Schinus molle) essential oil bionanocomposites on the viability of Aspergillus parasiticus spores. *Journal of Nanomaterials*, *2016*, 38.

140. Kheiri, A., Jorf, S. M., Malihipour, A., Saremi, H., & Nikkhah, M. (2016). Application of chitosan and chitosan nanoparticles for the control of Fusarium head blight of wheat (*Fusarium graminearum*) in vitro and greenhouse. *International journal of biological macromolecules*, *93*, 1261-1272

141. Manikandan, A., & Sathiyabama, M. (2016). Preparation of chitosan nanoparticles and its effect on detached rice leaves infected with *Pyricularia grisea*. *International journal of biological macromolecules*, *84*, 58-61.

142. Sathiyabama, M., & Manikandan, A. (2016). Chitosan nanoparticle induced defense responses in fingermillet plants against blast disease caused by Pyricularia grisea (Cke.) Sacc. *Carbohydrate polymers*, *154*, 241-246.

143. Siddaiah, C. N., Prasanth, K. V. H., Satyanarayana, N. R., Mudili, V., Gupta, V. K., Kalagatur, N. K., ... & Singh, B. P. (2018). Chitosan nanoparticles having higher degree of acetylation induce resistance against pearl millet downy mildew through nitric oxide generation. *Scientific reports*, *8*(1), 2485.

144. Sathiyabama, M., & Manikandan, A. (2018). Application of Copper-Chitosan Nanoparticles Stimulate Growth and Induce Resistance in Finger Millet (Eleusine coracana Gaertn.) Plants against Blast Disease. *Journal of agricultural and food chemistry*, *66*(8), 1784-1790.

145. Choudhary, M. K., Joshi, A., Sharma, S. S., & Saharan, V. (2017). Effect of Laboratory Synthesized Cu-Chitosan Nanocomposites on Control of PFSR Disease of Maize caused by Fusarium verticillioids. *Int. J. Curr. Microbiol. Appl. Sci.*, *6*, 1656-1664.

146. Suriyaprabha, R., Karunakaran, G., Kavitha, K., Yuvakkumar, R., Rajendran, V., & Kannan, N. (2013). Application of silica nanoparticles in maize to enhance fungal resistance. *IET nanobiotechnology*, *8*(3), 133-137.

147. Datnoff, L.E., Elmer, W.H., Huber, D.M. (2007). Mineral nutrition and plant disease. *The Ame. Phytopathol. Soc.* St. Paul, Minnesota, USA.

148. Shenashen, M., Derbalah, A., Hamza, A., Mohamed, A., & El Safty, S. (2017). Antifungal activity of fabricated mesoporous alumina nanoparticles against root rot disease of tomato caused by Fusarium oxysporium. *Pest management science*, *73*(6), 1121-1126.

149. Hao, Y., Yuan, W., Ma, C., White, J., Zhang, Z., Adeel, M., ... & Xing, B. (2018). Engineered nanomaterials suppress Turnip mosaic virus infection in tobacco (Nicotiana benthamiana). *Environmental Science: Nano*.

150. Kah, M., Kookana, R. S., Gogos, A., & Bucheli, T. D. (2018). A critical evaluation of nanopesticides and nanofertilizers against their conventional analogues. *Nature nanotechnology*, 1.

151. Savi, G. D., Piacentini, K. C., de Souza, S. R., Costa, M. E., Santos, C. M., & Scussel, V. M. (2015). Efficacy of zinc compounds in controlling Fusarium head blight and deoxynivalenol formation in wheat (Triticum aestivum L.). *International journal of food microbiology*, *205*, 98-104.

152. El-Argawy, E., Rahhal, M. M. H., El-Korany, A., Elshabrawy, E. M., & Eltahan, R. M. (2017). Efficacy of some nanoparticles to control damping-off and root rot of sugar beet in El-Behiera Governorate. *Asian J Plant Pathol*, *11*, 35-47.

153. Hamza, A., El-Mogazy, S., & Derbalah, A. (2016). Fenton reagent and titanium dioxide nanoparticles as antifungal agents to control leaf spot of sugar beet under field conditions. *Journal of plant protection research*, *56*(3), 270-278.

154. Imada, K., Sakai, S., Kajihara, H., Tanaka, S., & Ito, S. (2016). Magnesium oxide nanoparticles induce systemic resistance in tomato against bacterial wilt disease. *Plant Pathology*, *65*(4), 551-560.

155. Nandini, B., Hariprasad, P., Prakash, H. S., Shetty, H. S., & Geetha, N. (2017). Trichogenic-selenium nanoparticles enhance disease suppressive ability of Trichoderma against downy mildew disease caused by Sclerospora graminicola in pearl millet. *Scientific reports*, *7*(1), 2612.

156. Kalagatur, N. K., Ghosh, O. S. N., Sundararaj, N., & Mudili, V. (2018). Antifungal activity of chitosan nanoparticles encapsulated with Cymbopogon martinii essential oil on plant pathogenic fungi Fusarium graminearum. *Frontiers in Pharmacology*, *9*.

157. Pullagurala, V. L. R., Rawat, S., Adisa, I. O., Hernandez-Viezcas, J. A., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2018). Plant uptake and translocation of contaminants of emerging concern in soil. *Science of The Total Environment*, *636*, 1585-1596.

158. Rawat, S., Pullagurala, V. L., Adisa, I. O., Wang, Y., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2018). Factors affecting fate and transport of engineered nanomaterials in a terrestrial environment. *Current Opinion in Environmental Science & Health*.

159. Salem, H. F., Kam, E., & Sharaf, M. A. (2011). Formulation and evaluation of silver nanoparticles as antibacterial and antifungal agents with a minimal cytotoxic effect. *International Journal of Drug Delivery*, *3*(2), 293.

160. Gill, S. R., Fouts, D. E., Archer, G. L., Mongodin, E. F., DeBoy, R. T., Ravel, J., ... & Dodson, R. J. (2005). Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant Staphylococcus aureus strain and a biofilm-producing methicillin-resistant Staphylococcus epidermidis strain. *Journal of bacteriology*, *187*(7), 2426-2438.

161. Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of botany*, *2012*.

162. Heyno, E., Mary, V., Schopfer, P., & Krieger-Liszkay, A. (2011). Oxygen activation at the plasma membrane: relation between superoxide and hydroxyl radical production by isolated membranes. *Planta*, *234*(1), 35-45.

163. Blokhina, O., & Fagerstedt, K. V. (2010). Reactive oxygen species and nitric oxide in plant mitochondria: origin and redundant regulatory systems. *Physiologia Plantarum*, *138*(4), 447-462.

164. Yu, Z., Sun, X., Song, H., Wang, W., Ye, Z., Shi, L., & Ding, K. (2015). Glutathione-responsive carboxymethyl chitosan nanoparticles for controlled release of herbicides. *Materials Sciences and Applications*, *6*(06), 591.

165. Maruyama, C. R., Guilger, M., Pascoli, M., Bileshy-José, N., Abhilash, P. C., Fraceto, L. F., & De Lima, R. (2016). Nanoparticles based on chitosan as carriers for the combined herbicides imazapic and imazapyr. *Scientific reports*, *6*, 19768.

166. Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in plant science*, *7*(9), 405-410.

167. Yan, J., Tsuichihara, N., Etoh, T., & Iwai, S. (2007). Reactive oxygen species and nitric oxide are involved in ABA inhibition of stomatal opening. *Plant, cell & environment*, *30*(10), 1320-1325.

168. Joo, J. H., Bae, Y. S., & Lee, J. S. (2001). Role of auxin-induced reactive oxygen species in root gravitropism. *Plant Physiology*, *126*(3), 1055-1060.

169. Miller, G., Shulaev, V., & Mittler, R. (2008). Reactive oxygen signaling and abiotic stress. *Physiologia Plantarum*, *133*(3), 481-489.

170. Grant, J. J., Yun, B. W., & Loake, G. J. (2000). Oxidative burst and cognate redox signalling reported by luciferase imaging: identification of a signal network that functions independently of ethylene, SA and Me-JA but is dependent on MAPKK activity. *The Plant Journal*, *24*(5), 569-582.

171. Radwan, D. E. M., Fayez, K. A., Mahmoud, S. Y., & Lu, G. (2010). Modifications of antioxidant activity and protein composition of bean leaf due to Bean yellow mosaic virus infection and salicylic acid treatments. *Acta Physiologiae Plantarum*, *32*(5), 891-904.

172. Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2010). Oxidative stress and antioxidative defense system in plants growing under abiotic stresses. *Handbook of plant and crop stress*, *3*.

173. De Pinto, M. C., & De Gara, L. (2004). Changes in the ascorbate metabolism of apoplastic and symplastic spaces are associated with cell differentiation. *Journal of Experimental Botany*, *55*(408), 2559-2569.

174. Noctor, G., & Foyer, C. H. (1998). Ascorbate and glutathione: keeping active oxygen under control. *Annual review of plant biology*, *49*(1), 249-279.

175. Shah, K., Kumar, R. G., Verma, S., & Dubey, R. S. (2001). Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. *Plant Science*, *161*(6), 1135-1144.

176. Pirmohamed, T., Dowding, J. M., Singh, S., Wasserman, B., Heckert, E., Karakoti, A. S., ... & Self, W. T. (2010). Nanoceria exhibit redox state-dependent catalase mimetic activity. *Chemical communications*, *46*(16), 2736-2738.

177. Aranda-Martinez, A., Lopez-Moya, F., & Lopez-Llorca, L. V. (2016). Cell wall composition plays a key role on sensitivity of filamentous fungi to chitosan. *Journal of basic microbiology*, *56*(10), 1059-1070.

178. Sims, J. T. (1986). Soil pH Effects on the Distribution and Plant Availability of Manganese, Copper, and Zinc 1. *Soil Science Society of America Journal*, *50*(2), 367-373.

179. Nuruzzaman, M., Rahman, M. M., Liu, Y., & Naidu, R. (2016). Nanoencapsulation, nano-guard for pesticides: a new window for safe application. *Journal of agricultural and food chemistry*, *64*(7), 1447-1483.

180. Singh, H. B., Mishra, S., Fraceto, L. F., & de Lima, R. (Eds.). (2018). *Emerging Trends in Agri-nanotechnology: Fundamental and Applied Aspects*. CABI.

181. Prasad, R., Kumar, V., & Prasad, K. S. (2014). Nanotechnology in sustainable agriculture: present concerns and future aspects. *African Journal of Biotechnology*, *13*(6), 705-713.

182. Yang, F. L., Li, X. G., Zhu, F., & Lei, C. L. (2009). Structural characterization of nanoparticles loaded with garlic essential oil and their insecticidal activity against

Tribolium castaneum (Herbst)(Coleoptera: Tenebrionidae). *Journal of agricultural and food chemistry*, *57*(21), 10156-10162.

183. Stadler, T., Buteler, M., & Weaver, D. K. (2010). Novel use of nanostructured alumina as an insecticide. *Pest management science*, *66*(6), 577-579.

184. Buteler, M., Lopez Garcia, G., & Stadler, T. (2018). Potential of nanostructured alumina for leaf-cutting ants Acromyrmex lobicornis (Hymenoptera: Formicidae) management. *Austral Entomology*, *57*(3), 292-296.

185. Rouhani, M., Samih, M. A., & Kalantari, S. (2013). Insecticidal effect of silica and silver nanoparticles on the cowpea seed beetle, Callosobruchus maculatus F.(Col.: Bruchidae).

186. Ziaee, M., & Ganji, Z. (2016). Insecticidal efficacy of silica nanoparticles against Rhyzopertha dominica F. and Tribolium confusum Jacquelin du Val. *Journal of plant protection research*, *56*(3), 250-256.

187. Arumugam, G., Velayutham, V., Shanmugavel, S., & Sundaram, J. (2016). Efficacy of nanostructured silica as a stored pulse protector against the infestation of bruchid beetle, Callosobruchus maculatus (Coleoptera: Bruchidae). *Applied Nanoscience*, *6*(3), 445-450.

188. Barik, T. K., Sahu, B., & Swain, V. (2008). Nanosilica—from medicine to pest control. *Parasitology research*, *103*(2), 253.

189. Rai, M., & Ingle, A. (2012). Role of nanotechnology in agriculture with special reference to management of insect pests. *Applied microbiology and biotechnology*, *94*(2), 287-293.

190. Ayoub, H. A., Khairy, M., Elsaid, S., Rashwan, F. A., & Abdel-Hafez, H. F. (2018). Pesticidal activity of nanostructured metal oxides for generation of alternative pesticide formulations. *Journal of agricultural and food chemistry*, *66*(22), 5491-5498.

191. Sahab, A. F., Waly, A. I., Sabbour, M. M., & Nawar, L. S. (2015). Synthesis, antifungal and insecticidal potential of Chitosan (CS)-g-poly (acrylic acid)(PAA) nanoparticles against some seed borne fungi and insects of soybean. *Int. J. Chem. Tech. Res*, *8*, 589-598.

192. Paulraj, M. G., Ignacimuthu, S., Gandhi, M. R., Shajahan, A., Ganesan, P., Packiam, S. M., & Al-Dhabi, N. A. (2017). Comparative studies of tripolyphosphate and glutaraldehyde cross-linked chitosan-botanical pesticide nanoparticles and their agricultural applications. *International journal of biological macromolecules*, *104*, 1813-1819.

193. Hayes, T. B., Anderson, L. L., Beasley, V. R., de Solla, S. R., Iguchi, T., Ingraham, H., ... & Luque, E. H. (2011). Demasculinization and feminization of male gonads by atrazine: consistent effects across vertebrate classes. *The Journal of steroid biochemistry and molecular biology*, *127*(1-2), 64-73.

194. Schnoor, B., Elhendawy, A., Joseph, S., Putman, M., Chacón-Cerdas, R., Flores-Mora, D., ... & Salvador-Morales, C. (2018). Engineering Atrazine Loaded Poly (lactic-co-glycolic Acid) Nanoparticles to Ameliorate Environmental Challenges. *Journal of agricultural and food chemistry*, *66*(30), 7889-7898.

195. Clemente, Z., Grillo, R., Jonsson, M., Santos, N. Z. P., Feitosa, L. O., Lima, R., & Fraceto, L. F. (2014). Ecotoxicological evaluation of poly (ε-caprolactone) nanocapsules containing triazine herbicides. *Journal of nanoscience and nanotechnology*, *14*(7), 4911-4917.

196. Grube A DD, Kiely T, Wu L. Pesticides Industry Sales and Usage, 2006 and 2007 Market Estimates, United States Environmental Protection Agency, EPA 733-R-11-001, 34 p. 2011. http://1.usa.gov/1VN2QDo. Accessed 03 February 2016.

197. Guyton, K. Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., ... & Straif, K. (2015). International Agency for Research on Cancer Monograph Working Group ILF. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. *Lancet Oncol*, *16*(5), 490-491.

198. Myers, J. P., Antoniou, M. N., Blumberg, B., Carroll, L., Colborn, T., Everett, L. G., ... & Vandenberg, L. N. (2016). Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. *Environmental Health*, *15*(1), 19.

199. Chen, C., Zhang, G., Dai, Z., Xiang, Y., Liu, B., Bian, P., ... & Cai, D. (2018). Fabrication of light-responsively controlled-release herbicide using a nanocomposite. *Chemical Engineering Journal*, *349*, 101-110.

200. Namasivayam, K. R. S., Aruna, A., & Gokila. (2014). Evaluation of silver nanoparticles-chitosan encapsulated synthetic herbicide paraquate (AgNp-CS-PQ) preparation for the controlled release and improved herbicidal activity against Eichhornia crassipes. *Research Journal of Biotechnology*, *9*(9), 19-27.

201. Kumar, S., Nehra, M., Dilbaghi, N., Marrazza, G., Hassan, A. A., & Kim, K. H. (2018). Nano-based smart pesticide formulations: Emerging opportunities for agriculture. *Journal of Controlled Release*.

202. Worrall, E., Hamid, A., Mody, K., Mitter, N., & Pappu, H. (2018). Nanotechnology for Plant Disease Management. *Agronomy*, *8*(12), 285.

203. Gao, X., & Lowry, G. V. (2018). Progress towards standardized and validated characterizations for measuring physicochemical properties of manufactured nanomaterials relevant to nano health and safety risks. *NanoImpact*, *9*, 14-30.

204. Medina-Velo, I. A., Adisa, I., Tamez, C., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2017). Effects of surface coating on the bioactivity of metal-based engineered nanoparticles: lessons learned from higher plants. In Bioactivity of Engineered Nanoparticles (pp. 43-61). Springer, Singapore.

Table 1. Summary of different types of ENMs exhibiting nanofertilizer potential.

Nanomaterial	Concentration	Plant	Application Type	Details	Reference
Ag/Ag-Gum acacia	0-60 mg/L	Common bean (*Phaseolus vulgaris* L.)	Foliar	All tested concentrations increased plant height, root length, number of leaves and leaf area, total fresh and dry weight, and bean yield. Improved phytohormone balance in the two varieties of beans, compared with untreated control.	[74]
Ag-photochemical loaded	2.5 mg/L	*Alternanthera sesselis*	Petri dish	Significantly enhanced 100% multiple shoot bud regeneration, and 2-fold shoot elongation	[75]
CeO_2/ PVP-CeO_2	100 mg/kg	Soybean (*Glycine max* L.)	Root	Both coated and uncoated NMs increased plant yield (70%) and improved the WUE and photosynthetic activity in highly wet soil, compared with untreated control.	[76]
CeO_2	10-00 mg/kg	Radish (*Raphanus sativus*)	Root	Increased fresh biomass (2-fold), chlorophyll content (12.5%) and enhanced antioxidant activity, compared with the untreated control.	[77]
	125 mg/kg	Wheat (*Triticum aestivum* L.)	Root	Stimulated the plant growth at 2nd generation and altered the nutrient accumulation in above ground tissues, compared with untreated control.	[78]
CuO	0.02-8 mg/L	Maize (*Zea mays* L.)	Root and Foliar	Both solution culture and foliar exposure enhanced maize growth (51%) and regulated different enzyme activities, compared with untreated control.	[48]
	10-500 mg/L	Tomato (*Solanum lycopersicum*) and	Root	Root length (18%), chlorophyll (14%) and sugar (7%) contents increased in tomato plant at 10 mg/L, compared with the	[79]

		Cauliflower (*Brassica oleracea var. botrytis*)		untreated control. Concentration dependent increase in antioxidant enzyme activities, and lignin deposition observed in both plants.	
Cu/Kinetin	50, 100 mg/kg	Kidney bean (*Phaseolus vulgaris*)	Root	50 and 100 mg/kg increased the pod biomass by 140 and 30%, independent of kinetin, compared with untreated control.	[80]
Cu-chitosan-PVA	0.02-10 mg/kg	Tomato	Root	At 10 mg/kg, tomato yield (17%), stem diameter (13%) and dry biomass (30%) increased. At 0.02 mg/kg, the lycopene content, and antioxidant capacity (10%) increased, compared to untreated control.	[81]
Cu-chitosan	0.06 g/L	Tomato	Root	Enhanced the plant growth (21-29%) and yield (30%), stomata conductance (7%), and increased the leaf catalase (462%) and fruit lycopene content (12%), compared with untreated control.	[73]
Fe_2O_3	0.25-1 g/L	Soybean	Foliar	Increased the grain yield by 48%, compared with untreated control	[82]
	100-200 mg/L	Spinach (*Spinacea oleracea*)	Root	At 200 mg/kg, the plant biomass (~340%), and Fe uptake (~100%) increased in the plant, compared with untreated control.	[83]
	2-1000 mg/L	Peanut (*Arachis hypogaea*)	Root	Increased root and shoot length, biomass, and chlorophyll content. Regulated the phyto-hormone content and the antioxidant enzyme activity. Enhanced Fe uptake in the plant, compared with the control.	[46]
	50-800 mg/L	Tomato	Root	Enhanced seed germination and increased the plant growth and total biomass, compared with untreated control.	[84]

FeS	2-10 mg/L	Mustard (*Brassica junceae*)	Foliar	Induced growth and yield of the plant, and increased the antioxidant enzyme activities, compared with untreated control.	[85]
TiO_2	500, 1000 mg/kg	Barley (*Hordeum vulgare* L.)	Root	Stimulated the plant growth by increasing germination rate, number of tiller, and plant height, compared with nCeO treated and untreated control.	[86]
	0.5-4 g/L	Tomato	Root	Significantly enhanced the plant growth by 50%, and increased the photosynthetic parameters at low concentrations (0.5-2 g/L), compared with untreated control.	[87]
TiO_2-activated Carbon composite	0-500 mg/L	Tomato and Mungbean (*V. radiata*)	Presoaked seed	Increased the germination rate by 10-20% in both plants, compared with untreated control.	[88]
SiO_2	15-120 mg/L	Cucumber (*Cucumis sativus* L.)	Foliar	Increased plant height, leaf size, number of fruit, yield and total biomass, more significant at 60 mg/L, compared with untreated control.	[89]
	20-80 mg/L	Strawberry (*Fragaria* × *ananassa*)	Root and foliar	Increased the uptake of macro and micro nutrients; K, Ca, Mg, Fe, Mn, and Si in the plant, compared with the untreated control. Better results obtained at 60 mg/L.	[90]
ZnO	6 mg/kg	Sorghum (*Sorghum bicolor*)	Root and foliar	Improved plant productivity and stimulated grain nutritional values and N use efficiency, compared with untreated control.	[17]
	2-16 mg/L	Tomato	Root	At 8 mg/L, shoot length (35.8%), root length (28.6%), leaf area (27.9%), antioxidant activities;	[91]

				CAT (70%), POX (650%), SOD (80%), proline content (65%) and photosynthetic rate increased, compared with control.	
	50-1600 mg/L	Corn	Germination study	At different temperature, ZnO NMs increased the root growth, Zn accumulation, and ascorbate peroxidase activity in the seedlings.	[92]
	1-100 mg/kg	Lettuce (*Lactuca sativa* L.)	Root	ZnO NP at 10 mg/L increased the biomass (6%) and photosynthetic rate (6%), compared with untreated control.	[93]
	25-200 mg/L	Cotton (*Gossypium hirsutum* L.)	Root	Significantly increased the growth (131%), total biomass (131%), total chlorophyll (~139%), carotenoids (139%), total soluble protein contents (179%), and SOD (264%) and POX (183%) activities, compared with untreated control.	[94]
ZnO-compost	100-500 mg/L	Peanut *Arachis hypogaea*	Foliar	In Zn-deficient soil, the NMs (most effective at 300 mg/L) improved the germination and growth rates of the plant. Increased the total biomass, yield, chlorophyll content, total phenol, and total sugar.	[95]
Zn-chitosan	20 mg/g (w/w)	Wheat (*Triticum durum)*	Foliar	Enhanced Zn uptake in the plant grown in Zn-deficient soil. About 27 and ~42% increase in the two wheat varieties, compared to the control.	[96]
Chitosan	10-100 mg/L	Wheat	Foliar	Increased the growth rate, total biomass, and grain yield, compared with untreated control.	[36]
				Increased the leaf area,	

	30-90 mg/L	Barley	Root and foliar	chlorophyll content, number of grain per spike, grain yield and harvest index, compared with untreated control.	[97]

Table 2: Summary of different types ENMs used as pesticides to manage plant diseases.

Nanomaterials	Conc.	Plant	Application Type	Pathogen	Disease Suppression (+/-)	Details	Reference
Ag	5 mg/L	Tomato	Foliar	*Alternaria solani*	+	Reduced early blight disease, increased plant fresh weight (32.58%) and chlorophyll content (23.52%), compared with untreated control.	[104]
	10, 30, 50, 100 mg/L	Cucumber and pumpkin	Foliar	*Golovinomyces cichoracearum or Sphaerotheca fusca*	+	Maximum powdery mildew disease suppression (25%) recorded at the highest concentration in cucumber and pumpkin leaves, compared with the control and more effective than commercial fungicides. Mycelia and conidia growth distorted.	[116]
	100 mg/L	Pepper	Foliar, pretreated	*Colletotrichum sp.*	+	Mycelia growth distorted. Suppressed pepper anthracnose in the field more effective than the commercial fungicides.	[117]
Ag	2, 4, 10 mg/L	Wheat	Detached leaf assay	*Bipolaris. Sorokiniana*	+	Spot blotch disease was suppressed, and conidia germination was inhibited (100%) by all treatments,	[105]

						compared with untreated control.	
	800 mg/L	*Crossandra spp.*	Foliar	*Fusarium incarnatum (Desm.) Sacc*	+	Reduced the disease incidence from 75% to 55%, compared with untreated control.	[114]
	50 mg/L	Rice	Foliar	*R. solani*	+	Efficiently reduced lesion development on the leaves. Increased the fresh and dry weight of the rice plant, compared with untreated control.	[118]
DNA-Directed Ag on graphene oxide composite	100 mg/L	Tomato	Foliar	*Xanthomonas perforans*	+	Effectively suppressed the bacterial spot disease by 32%, compared with untreated control.	[106]
Ag-Na Tallowate	100 mg/L	Tomato	Foliar	*Phytophthora infestans* and *A. solani*	+	Suppressed both pathogens, increased leaf surface area (+41 cm^2/plant), tomato yield (35%) and antioxidant enzymes, relative to untreated control.	[119]
$CuO/Cu/Cu_2O$	150-350 mg/L	Tomato	Foliar	*P. infestans*	+	CuO NP decrease leaf lesions by ~40%, 3 days after application to ~61%, 7 days after application, compared with untreated control and the reference products.	[127]

CuO	500-1000 mg/L	Watermelon	Foliar	*Fusarium oxysporum f. sp niveum*	+	Reduced the Fusarium wilt incidence (25%) and increased tomato yield (21-53%), compared with untreated control and commercial fungicides.	[128]
	2.5 mg/L	Tea plant	Foliar	*Poria hypolateritia*	+	Suppressed red root-rot disease in tea plants in a field study (80%) and increased the total leaf yield (30%), compared with untreated infested control.	[129]
Cu-composite	100 mg/L	Tomato	Foliar	*Xanthomonas spp.*	+	Decreased bacterial spot disease in tomato plants more effectively than Cu NP.	[130]
CuO	1000 mg/L	Tomato	Foliar	*Fusarium oxysporum*	+	Suppressed Fusarium wilt disease and increased yield more effective than ZnO and MnO NP.	[7]
	1000 mg/L	Eggplant	Foliar	*Verticilium dahliae*	+	Suppressed Verticilium wilt disease and increased yield more effective than ZnO and MnO NP.	[7]
	0.1% w/v	Finger millet	Presoaked seed/Foliar	*Pyricularia grisea*	+	Reduced blast disease incidence by 75%. Increased the number of leaves by 22% (foliar) and 33% (combined	[144]

						exposure). Increased activities of defense enzymes observed.	
Cu-chitosan	0.04-16% w/v	Maize	Foliar	*Culvularia lunata*	+	Reduced leaf spot disease incidence in the plant. Increased grain yield, shoot length, chlorophyll content, and the antioxidant and defense enzymes activities in both greenhouse and field trials.	[69]
ZnO	100mM	Wheat	Foliar	*Fusarium graminearum*	+	Significantly reduced disease incidence and reduced the mycotoxin in the grain.	[151]
ZnO NM formulations (pale-like Zinkicide SG4 and particlulate-like Zinkicide SG6)	100 mg/L	Sugar beet	Presoaked seeds	*F. oxysporum f. sp., betae, S. rolfsii and R. solani*	+	Reduced root rot fungal disease severity by 86%, compared with untreated control. Increased the plant growth by 45%, sugar content and PPO activity.	[152]
TiO/Zn	31-250 mg/L	Grapefruit trees	Foliar	*Xanthomonas citri subsp. citri.*	+	Decreased citrus canker lesion disease incidence in greenhouse and field studies.	[135]
	500-800 mg/L	Rose	Foliar	*Xanthomonas sp*	+	Suppressed bacterial leaf spot disease on rose by 99% at day 15, compared with non-coated control.	[136]

TiO_2	0.1 mg/L	Sugar beet	Foliar	*Cercospora beticola*	+	Significantly reduced disease at 1st (57%) and 2nd (51%) seasons, compared with untreated infested control.	[152]
	100 mg/L	Sugar beet	Presoaked seeds	*F. oxysporum f. sp., betae, S. rolfsii and R. solani*	+	Reduced root rot fungal disease severity by 95% mean, compared with untreated control. Increased the plant growth by 85%, the sugar content and the PPO activity.	[153]
CeO_2	50-250 mg/L	Tomato	Root and foliar	*Fusarium oxysporum*	+	Suppressed Fusarium wilt disease at 250 mg/L (root, 53% and foliar, 57%), improved the productivity, and altered the defense and stress enzyme activities.	[15]
MgO	5 mg/L	Tomato	Root	*Ralstonia solanacearum*	+	Reduced the disease incidence significantly by 30%, compared with untreated infested control..	[154]
	100 mg/L	Sugar beet	Presoaked seeds	*F. oxysporum f. sp., betae, S. rolfsii and R. solani*	+	Reduced the root rot fungal disease severity by 92% mean, compared with untreated control. Increased the plant growth by 60%, the sugar content and the PPO activity.	[153]

Se/Trichoderma asperellum	150-250 mg/L	Pearl millet	Foliar	*Sclerospora gra minicola*	+	Suppressed downy mildew disease in pearl millet and improved early plant growth	[155]
Fe_2O_3/TiO_2 or WCNTs/ C_{60}	50 or 200 mg/L	Tobacco plant (*Nicotiana benthamiana*)	Foliar	Turnip mosaic virus	+	Inhibited virus growth and reduced the disease incidence. Increased the shoot biomass by 50%.	[149]
β-D-glycan	0.1% w/v	Turmeric plant	Foliar	*Pythium aphanidermatum*	+	Suppressed rhizome rot disease and increased activities of defense enzymes such as peroxidases, polyphenol oxidases, protease inhibitors and β-D-glucanases.	[22]
Chitosan	100-1000 mg/L	Maize (*Zea may*)	Grain exposure	*Fusarium graminearum*	+	Induced resistance against the disease and reduced the mycotoxin in the maize grain.	[156]
	1000 mg/L	Wheat	Foliar	*Fusarium graminearum*	+	Suppressed the pathogen attack by 41.8% after 4 weeks of inoculation, relative to control.	[140]
	0.1% w/v	Rice	Detached leaf assay	*Pyricularia grisea*	+	Reduced the disease incidence 100% on day 10.	[141]
	0.1%		Presoaked	*Pyricularia grisea*		Suppressed the blast disease	

	w/v	Finger millet	seeds		+	in treated plants by 64% at day 50 after inoculation.	[142]

Figure Legends

Fig 1. Comparative effects of ENMs exposure on infected plants

Fig 2. Effect of ROS and antioxidant levels in plant homeostasis

Fig 3. Mechanism of ROS generation and role of enzymatic and non-enzymatic antioxidants

Fig 4. Impact of the ROS-based stress and its potential toxicity to plant cells (Modified from Reddy *et al.,* 2018)

Fig 5. Proposed anti-bacterial mechanisms of engineered nanomaterials

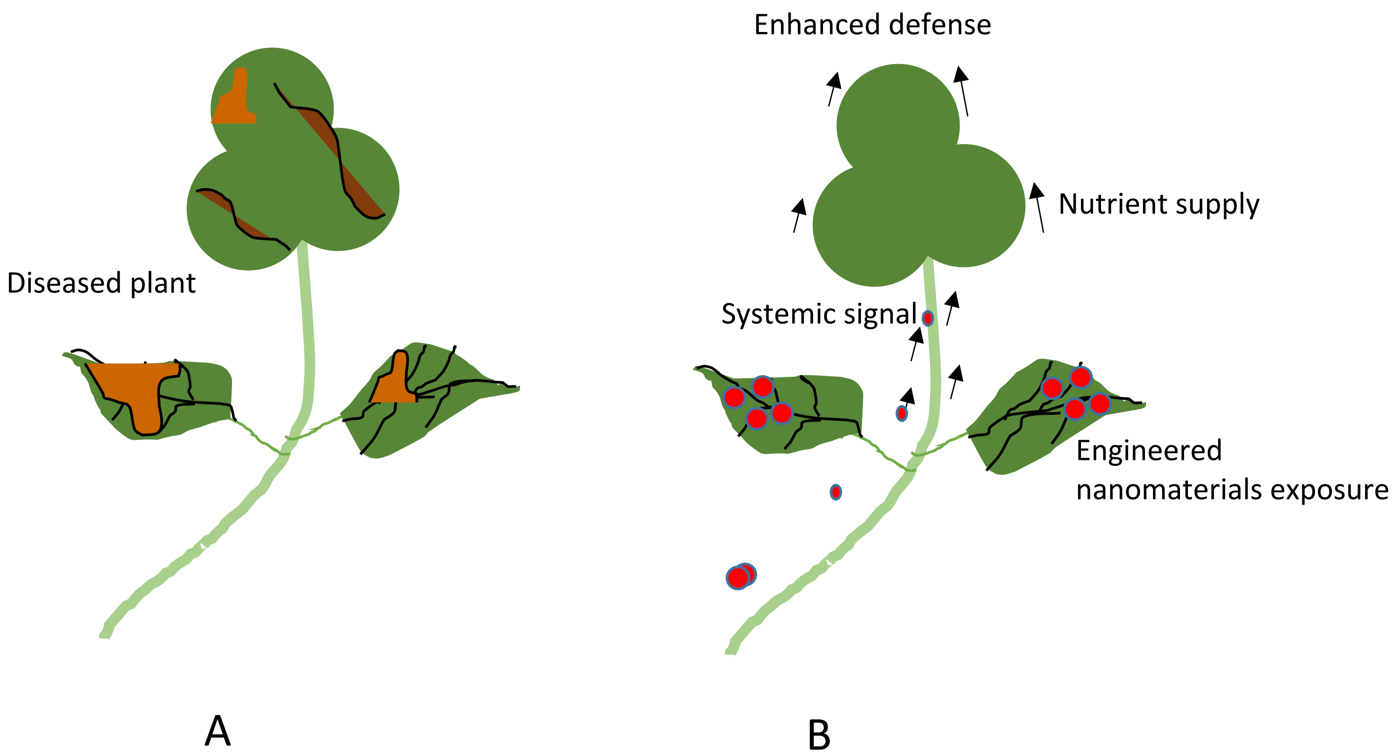
Diseased plant
A
Enhanced defense
Nutrient supply
Systemic signal
Engineered nanomaterials exposure
B

Fig 1

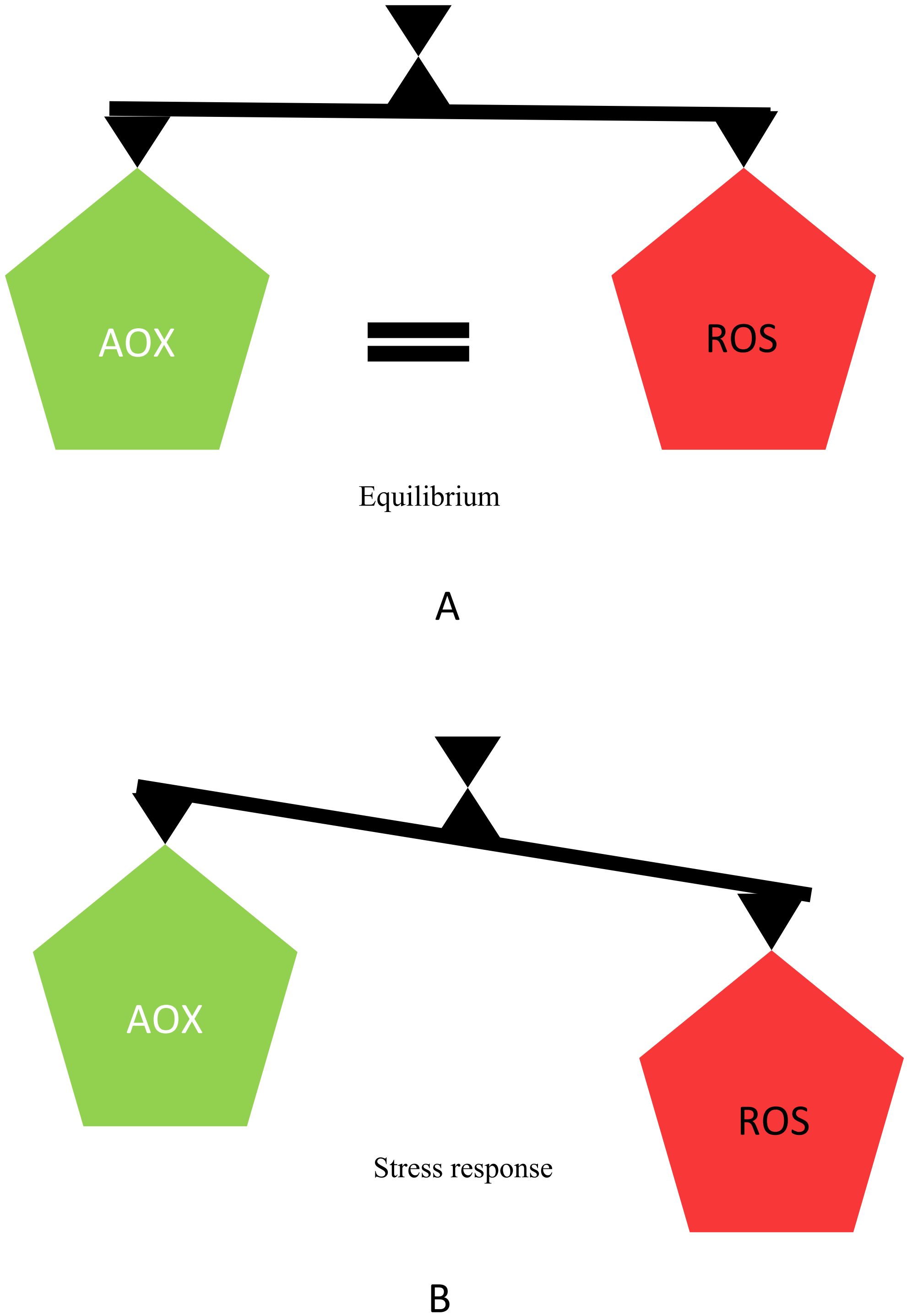

Fig 2

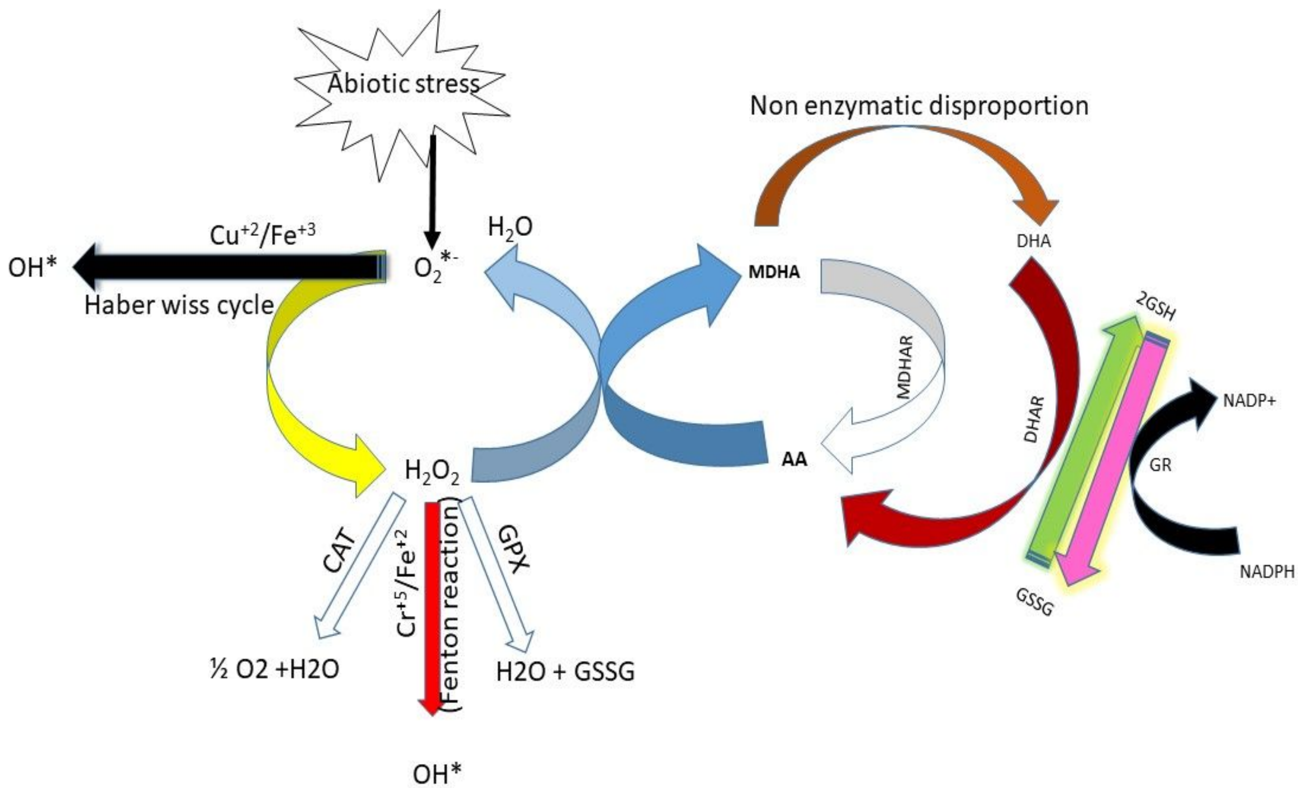
Abiotic stress
Non enzymatic disproportion
Cu^{+2}/Fe^{+3}
OH*
Haber wiss cycle
O_2^{*-}
H_2O
MDHA
DHA
2GSH
MDHAR
DHAR
NADP+
GR
H_2O_2
AA
CAT
Cr^{+5}/Fe^{+2}
(Fenton reaction)
GPX
GSSG
NADPH
½ O2 +H2O
H2O + GSSG
OH*

Fig 3

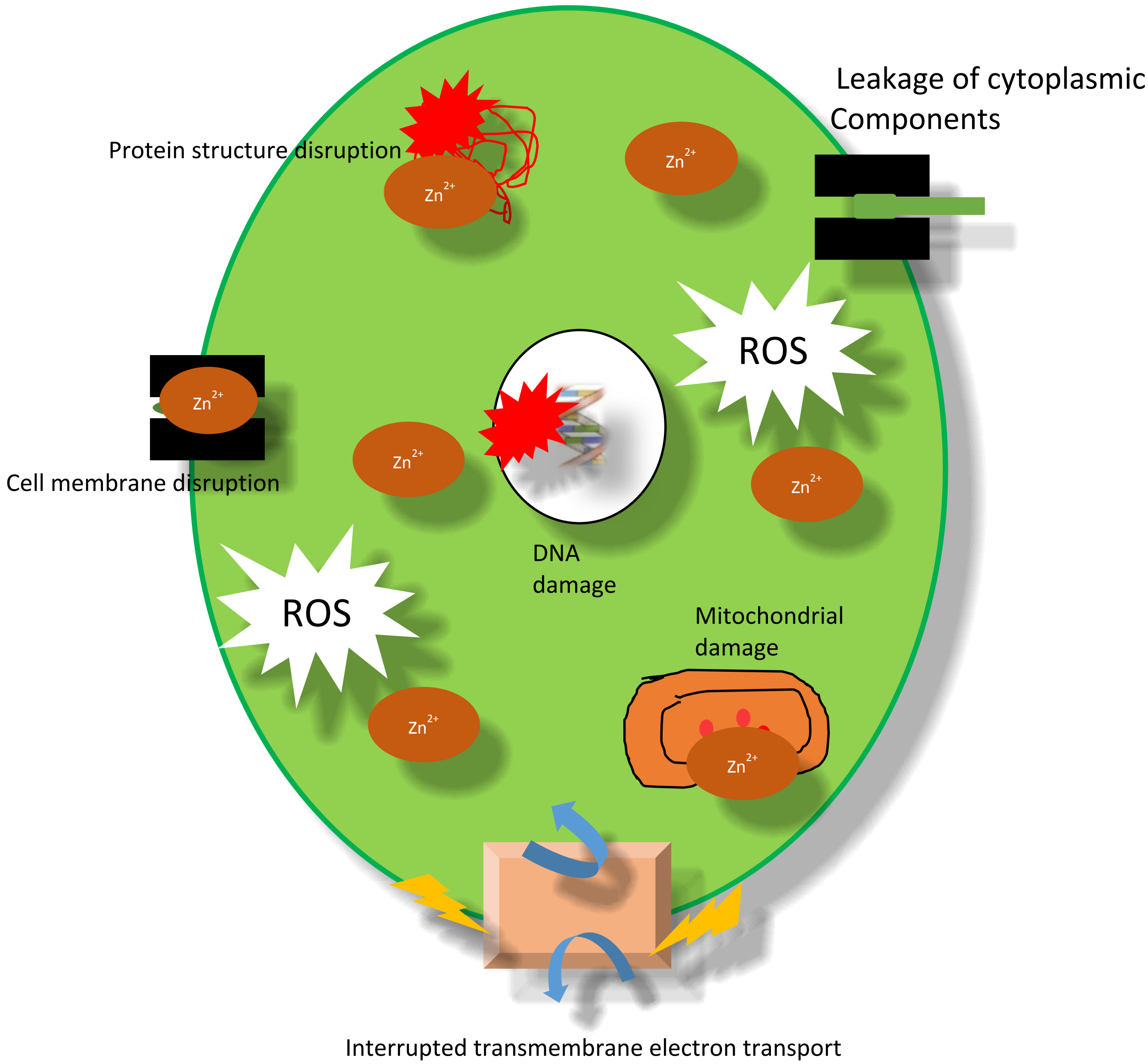
Leakage of cytoplasmic
Components
Protein structure disruption
Zn2+
ROS
Cell membrane disruption
DNA
damage
Mitochondrial
damage
Interrupted transmembrane electron transport

Fig 4

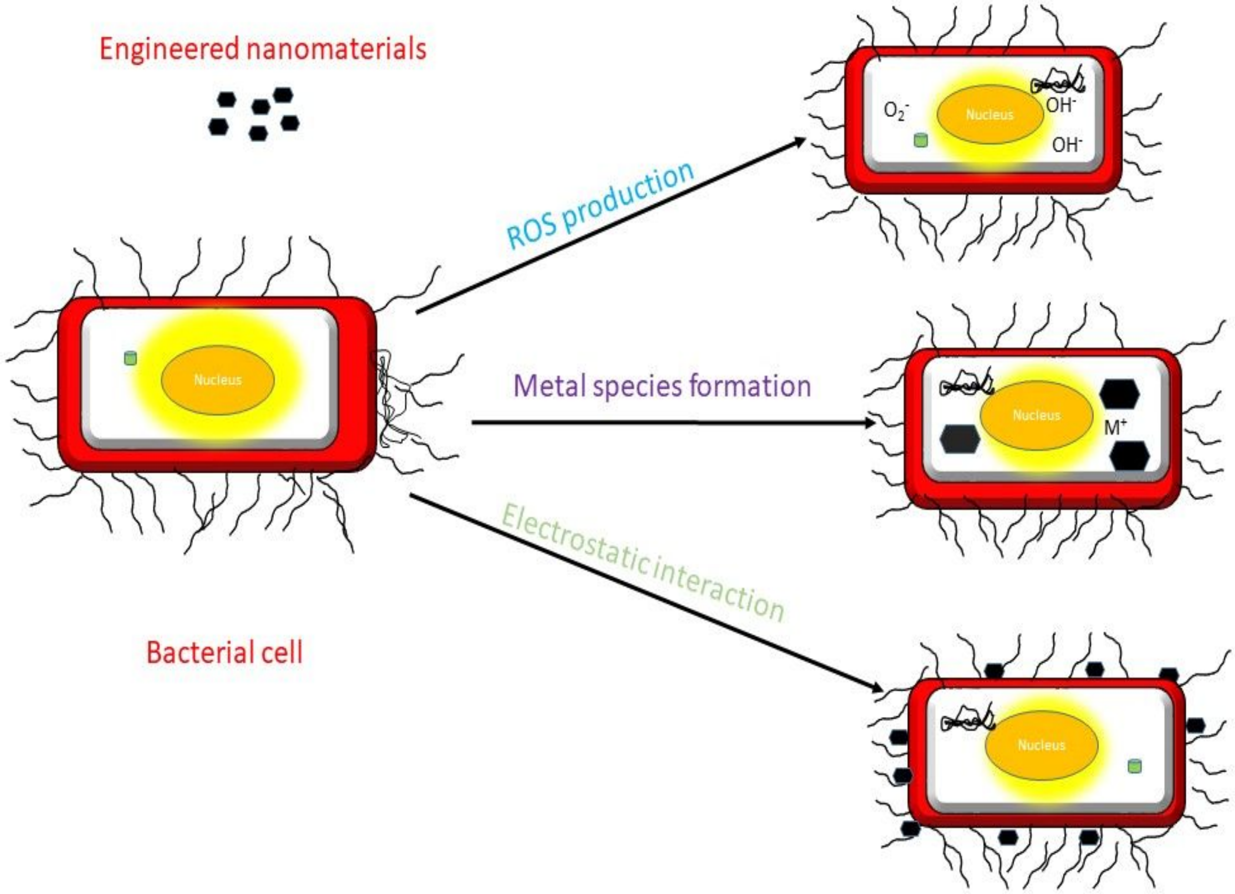
Engineered nanomaterials
ROS production
Metal species formation
Electrostatic interaction
Bacterial cell
Nucleus
O_2^-
OH^-
OH^-
Nucleus
M^+
Nucleus
Nucleus

Fig 5