

Inorganic Nanoparticles to Promote Crop Health and Stimulate Growth



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Abstract As global food needs grow to keep pace with an ever-growing population, increased stress will be placed upon agricultural output. Current agriculture practices are wasteful and inefficient, especially with regard to fertilizer application. Inefficiency in plant uptake of nutrients leads to repeated over application, which in turn causes increased runoff of NPK into the environment. Recent developments in nanotechnology can enable more efficient delivery and uptake of vital nutrients to plants when they are needed. Published research has shown that targeted delivery of micro and/or macronutrients at critical development stages can boost plant growth, improve crop yields, increase nutritional content, or aid in disease suppression. Although further work is necessary to completely understand the mechanisms and implications of their use, the application of nanoparticles in agriculture can provide the changes needed to keep the world fed.

Keywords Biostimulation · Agriculture · Sustainability · Plant growth

The exposure of agricultural crops to nanoparticles (NPs) has long had a stigma of potential negative effects, and this is highlighted by the initial studies into the phytotoxicity of nanoparticle exposure to plants of agricultural interest. Results demonstrating reduced seed germination, diminished root and shoot growth, increased oxidative stress activity, or even complete yield losses were common—as were

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excessively high application doses. However, as engineered nanoparticles are developed and the phytotoxicity studies evaluating their effects have matured, the research has begun to show several beneficial aspects associated with metal and nonmetal-based nanoparticles. Studies spanning the entire life cycle of plants, grown in soil-based media or in field plots, and with exposure to environmentally relevant conditions have demonstrated the ability of NPs to stimulate crop growth and photosynthetic output, improve plant health, and bolster defenses against pest and disease—often outperforming bulk or conventional material equivalents. Consequently, the use of nanoparticles is gaining momentum for applications in agricultural settings such as nanoscale fertilizers or other crop amendments, and recent studies have demonstrated their efficacy for enhancing disease suppression, modifying nutrient accumulation and distribution, and increasing crop yield. As the global population is expected to surpass 9.5 billion by 2050 and food demand will nearly double (Zhao et al., 2020), conventional approaches to agriculture will most certainly prove inadequate. In fact, the lack of sustainability of many of the current agricultural practices is contributing to environmental problems that will limit arable land in the near future. The projected global demand for NPK fertilizers in 2020 was estimated to be over 200 million tons (FOA, 2017). It is also widely known that certain applied agrichemicals are persistent in soil, often affecting vital soil functions such as nutrient content, pH, and soil microbiota health (Mandal et al., 2020; Meena et al., 2020; Prashar & Shah, 2016). The adoption of nanotechnology in agricultural practices can help alleviate this burden. Herein, we describe some of the sustainable and practical uses where different nanoparticles can positively and sustainably impact agriculturally relevant crops (Fig. 1).

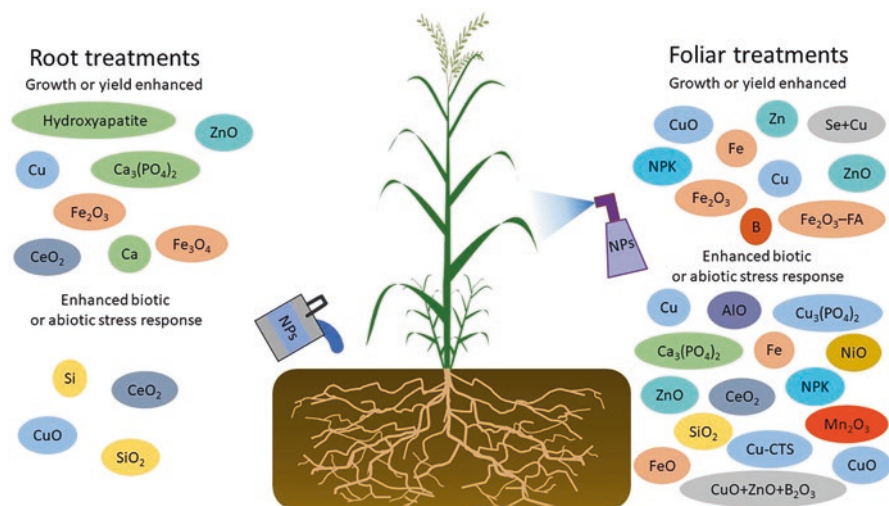


Fig. 1 Inorganic-based nanoparticles have been shown to enhance crop performance. Exposure has increased crop performance by modulating nutrient accumulation or triggering the activation of certain plant defense mechanisms. This has led to increased growth, better yields, and/or enhanced disease tolerance

1 Nanoparticles to Enhance Plant Growth and Increase Yield

One desirable outcome from the application of NPs to agricultural crops is increased growth and improved yield, although this clearly depends on the type of NPs utilized and the crop, as well as the environmental conditions (e.g., biotic and abiotic stressors). Nutrient deficiencies in agricultural soils are common due to soil erosion, over-production, and monoculture cultivation; so, a starting point could be with the application of nanoscale or nano-fertilizers when mineral fertilizers would otherwise be employed. Among the nutrients that plants require in order to maintain healthy functions, nitrogen (N), phosphorous (P), and potassium (K) are applied in the greatest amounts. However, the use efficiency of applied N, P, and K fertilizers is less than 50%, 10%, and 40%, respectively, with the vast majority of nutrients remaining in the soil to be washed away or volatilize (Baligar et al., 2001). Therefore, overfertilizing is a common and non-sustainable agricultural practice. For example, the excessive use of N might be necessary to meet production goals, but N amendments lead to water pollution from runoff and nitrous oxide (N₂O) emissions to the air, a greenhouse gas more potent at trapping heat than CO₂ (Woodbury & Wightman, 2017). Alternatives to reduce the amounts of agrochemicals include optimization of irrigation practices (e.g., drip irrigation to avoid runoff), improved fertilization regimes (delivery rate and times), and cultivation of nutrient-efficient plants. An approach under investigation is the use of nanofertilizers which often show enhanced efficacy at lower amounts than typical salts. Foliar application of nano NPK to cucumber plants improved yield by over 50%, compared to conventional mineral NPK fertilizer (Merghany et al., 2019). The treatment (foliar) of wheat with nano NPK, combined with conventional NPK, was effective at boosting plant growth and enhancing grain yield (Abdelsalam et al., 2019). Treating bean with foliar nano NPK at the flowering stage of growth significantly increased yield 133%, while treating at any growth stage improved yield 61% (Mohsen et al., 2020). Meanwhile, nano NPK foliar application to chickpea plants significantly enhanced seed weight (12%), seed yield (25%), and biological yield (14%), compared to non-fertilized controls (Drostkar et al., 2016). Potato foliar-treated with chitosan-coated NPK (CTS-NPK) showed growth increases of 18.5–36.5%, 17% greater yield, and improved nutrient content over conventional mineral NPK (Elshamy et al., 2019). Spraying chitosan-coated nano NPK enhanced the growth of bean and coffee and augmented the mineral nutrient content of coffee (Ha et al., 2019; Hasaneen et al., 2016).

As nanotechnology advances, researchers are developing smart or tunable engineered nanomaterials or pairing NP with conventional treatments. For example, the application of ZnO NPs applied with mineral NPK fertilizer increased sorghum grain yield and modified NPK accumulation versus the use of NPK fertilization alone (Dimkpa et al., 2017b). Combined NPK-Zn treatments led to increased grain yield with higher levels of K and Zn in the plants, regardless of the form (nanoscale or salt). Additionally, NPs applied as mixtures (ZnO, CuO, and/or B₂O₃) increased

soybean branching, number of flowers, shoot dry weight, and N uptake. However, this is a distinct composite effect; when B_2O_3 NPs were omitted, only increased shoot growth was noted (Dimkpa et al., 2019). Likewise, soybean treated with ZnO, CuO, or B NPs separately showed only increased biomass (Pérez et al., 2020). This suggests that combined NPs have different effects than when applied individually. The foliar application of Fe, Zn, and NPK was able to increase the number of branches in chickpea plants; however, when NPK was removed and only Fe and Zn were sprayed, plants achieved the highest seed yield and seed weight of any of the tested treatment combinations (Drostkar et al., 2016). Meanwhile, improvements were reported when both Cu NPs and Se NPs were applied at varying concentrations (Se 1–20 mg L⁻¹, Cu 10–250 mg L⁻¹), including increased vitamin C content, firmer tomato fruit, and 25% heavier fruit (Hernández-Hernández et al., 2019). On the other hand, Elmer et al. (2021) found that a single application of CuO NPs was superior to combinations with Mn_2O_3 , and/or ZnO at increasing the yield of eggplant and suppressing the *Verticillium* wilt disease. Importantly, the benefits of many NPs are, among other significant factors, concentration-dependent. For instance, wheat plants treated with ZnO NPs at low concentrations (~2 mg kg⁻¹) showed increased shoot growth and a non-significant trend toward higher yield (Dimkpa et al., 2020). However, when wheat was separately exposed to treatments of 10–200 mg kg⁻¹, yield was increased by up to 56% (Du et al., 2019). Priming wheat seeds in ZnO NPs before sowing increased plant growth, biomass, and grain weight (Rizwan et al., 2019). Others have demonstrated that ZnO NPs increased the plant height, branching, and seed yield of chickpea (Drostkar et al., 2016); plant height, stem diameter, biomass, yield, and capsaicin content of habanero pepper (García-López et al., 2019); biomass and leaf area of mung bean (Patra et al., 2013); and biomass and photosynthetic activity of coffee (Rossi et al., 2019). These benefits could be attributed to the slow release of Zn ions. For example, application of NPs—either by foliar treatment or seed coating—provides a long-term surface attached source of Zn ions that can enter via roots, the stomata, or ruptures in the leaf surface. Easy access of Zn ions to the leaf promotes improved photosynthesis, as a positive interaction between ZnO NP application, carbon assimilation, and stomatal conductance has been observed (Rossi et al., 2019). Seed priming activates certain metabolic pathways that lead to the promotion of plant growth, such as photosynthetic pathways and reactive oxygen species (ROS) scavenging enzymes (Rizwan et al., 2019). Research into the benefits of seed priming has shown the technique activates vital protective functions, including antioxidant defense, that protect the plant from DNA/RNA damage (Buchman et al., 2019; De La Torre-Roche et al., 2020).

More recently, applying NPs of different forms and at lower doses under different regimes have produced positive effects. In many cases, nano-treatments improve growth parameters in comparison to ordinary amendments and therefore, studies to optimize the use of nano-formulations are also taking place. A study on maize revealed that nano NPK at lower ratios of 12-12-36 was superior than higher percentages (20-20-20) at stimulating growth and increasing yield in maize (Alzreejawi & Al-Juthery, 2020). Maize grown in soil amended with sulfur-enhanced nano NPK

(NPKS) provided better growth promotion than standard nano NPK (Dhramini et al., 2020). The application of a nanoscale NPK, containing two sources of N (nitrate and urea), at a rate of 15 kg N ha⁻¹ allowed for a 40% reduction in the amount of traditional N fertilizer applied without any adverse effects on wheat kernel weight (Ramírez-Rodríguez et al., 2020). Moreover, foliar application of CuO NPs and Cu₃(PO₄)₂ nanosheets increased total chlorophyll content and carotenoids in the leaves of watermelon plants (Borgatta et al., 2018). Metallic Cu NPs have been successful at improving the production of tomato fruit, with foliar applications increasing fruit firmness, lycopene, and vitamin C content (López-Vargas et al., 2018; Pérez-Labrada et al., 2019). Applications of Cu NPs at low concentrations of 30 ppm enhanced the yield of wheat by increasing number of spikes, number of grains per spike, and overall grain weight (Hafeez et al., 2015). Firmer tomato fruit could be the result of increased lignin formation, enhanced by the accumulation of Cu in plant tissues (López-Vargas et al., 2018); increased wheat performance has been attributed to NPs ability to better deliver ions over an extended period of time. Additionally, copper nanowires were successful at improving root and shoot length, and biomass of exposed alfalfa (Cota-Ruiz et al., 2020). Although the application of copper-based NPs provides negligible to slightly beneficial effects when applied to unstressed crops, the opposite effect has been observed in plants experiencing biotic or abiotic stress (refer to the section “Engineered Nanomaterials for Plant Disease Management”).

Like other metallic NPs, iron (Fe) NPs have demonstrated the ability to enhance crop growth and promote yield. Specifically, iron-based NPs such as Fe₂O₃, Fe₃O₄, and metallic Fe NPs have been used in agricultural settings with notable success. Fulvic acid coated onto Fe₂O₃ NPs caused a significant increase in soybean N fixation by increasing the weight of root nodules by 120%, leading to a 91% and 49% increase in root and shoot biomass, while the use of bare Fe₂O₃ NPs increased biomass by approximately 60% compared to conventional Fe-EDTA fertilizer (Yang et al., 2020). Iron oxide NPs (Fe₃O₄) used to prime wheat seeds significantly improved plant height (35%) and spike length (49%), and increased the dry weight of roots, shoots, spikes, and grains by an average of 67% (Rizwan et al., 2019). Wheat germination and shoot length were also improved when seeds were primed with Fe₂O₃ (Sundaria et al., 2019). Similarly, metallic Fe NPs have shown promising results in chickpea, significantly increasing plant height and enhancing production by nearly 23%, compared to control (Drostkar et al., 2016). Similar results are achieved when chickpea seeds were primed with FeS₂ prior to sowing. Treated chickpeas produced denser roots with larger nodules, which led to increased yield and nutrient accumulation (Jangir et al., 2020).

A number of other elements have been investigated for similar applications. Equally promising is the application of Si-based NPs to promote crop growth. SiO₂ NPs successfully increased wheat shoot growth and enhanced grain yield and 1000-grain weight (Behboudi et al., 2018). Similarly, Si NPs increased cucumber yield while simultaneously improving nitrogen content, chlorophyll production, and growth (Alsaedi et al., 2019). Other NPs, such as those based on B and Ca, have also shown promise for increasing crop growth. The use of a B nano-fertilizer

significantly increased shoot growth of lettuce by 55% and zucchini by 14% compared to the application of a conventional boron fertilizer, in boron-deficient media (Meier et al., 2020). Calcium-based NPs such as $\text{Ca}_3(\text{PO}_4)_2$ (CaP NPs) and nano-hydroxyapatite (nHA) have also demonstrated the ability to enhance the performance of rice, rye, and tomato (Marchiol et al., 2019; Sun et al., 2018; Upadhyaya et al., 2017). Nanoscale CaP was shown to increase rice growth at low concentrations ($\leq 20 \text{ mg L}^{-1}$), increasing root and shoot length by 5%, and root and shoot biomass by 10%. The application of nHA at rates of 200–2000 mg L^{-1} significantly increased the root elongation of hydroponically grown tomatoes. Collectively, the body of evidence suggests that nanoscale nutrients provide a unique and tunable source of necessary ions to sustainably increase the performance of crucial cellular functions, resulting in greater plant growth. Importantly, these positive impacts are rarely evident with conventional nutrient formulations, highlighting the importance of nanoscale size to the observed benefit.

2 Nanoparticles to Boost Plant Nutrition

Improving nutrient availability to plants can help enhance response to external factors and can also lead to enhancement in the nutritional value of edible tissues. Plants require essential elements such as N, P, K, S, Ca, Mg, Mn, Cu, B, Zn, Fe, Ni, and Mo to activate a complex set of metabolic functions leading to the production of carbohydrates, antioxidants, proteins, and other important biomolecules (Datnoff et al., 2007). Often plants are grown in environments that are lacking in some or many of these crucial nutrients, requiring the soils to be amended exogenously with fertilizers. However, the efficiency of delivery and utilization of conventional fertilizer formulations is often quite low ($<25\%$), resulting in overapplication to maintain growth but that also leads to secondary and potentially significant environmental damage over the long term (Hofmann et al., 2020; Kah et al., 2018; Lowry et al., 2019). The use of nanomaterials has shown significant promise for enhanced delivery efficiency as part of a sustainable agriculture framework, by using less chemicals to increase productivity with fewer impacts on the environment. The application of conventional mineral fertilizers is plagued by poor availability in non-acidic soils and low basipetal translocation with foliar treatments. However, the application of nano-based fertilizers can increase the mobility of nutrients in plant tissues (Elmer & White, 2016; Pérez et al., 2020). Increased availability of nutrients could allow for a reduction in agrichemicals, wasting fewer resources from production, saving the grower money, reducing environmental impact, and lowering the risk of exposure to farmworkers.

With respect to potentially fortifying edible tissues, the application of ZnO NPs has been shown to significantly increase Zn content in edible tissues of species such as wheat, soybean, and sorghum (Dimkpa et al., 2020; Dimkpa et al., 2017a; Dimkpa et al., 2017b). The technique of seed priming is a promising method for fortifying crops in the field. The use of ZnO or Zn-chitosan NPs significantly increased Zn

content in the edible parts of rice, maize, and pinto beans (Choudhary et al., 2019; Mahdih et al., 2018; Rameshraddy et al., 2017). Seed treatment of wheat with Fe_2O_3 NPs increased Fe content in the grains by 45.7% in a high-iron genotype (IITR26) and by 26.8% in a low-iron genotype (WL711) (Sundaria et al., 2019). This technique has also been successful at increasing the concentration of the applied element in other plant tissues, thereby promoting overall crop health. Peanut seeds coated with ZnO NPs had increased Zn content in leaves and kernels by 100 and 84%, compared to control, and 42 and 24%, compared to ZnSO_4 (Prasad et al., 2012). Similarly, coating seeds of maize, soybean, pigeon pea, and ladies finger with ZnO NPs increased Zn in shoots to a greater extent than ZnSO_4 or the bulk equivalent (Adhikari et al., 2016). Additionally, chickpea seeds primed with FeS_2 increased the content of Fe in the more densely produced roots (Jangir et al., 2020).

However, data on the effect of NPs treatments on the accumulation and distribution of other macro- and micronutrients is fragmented and less clear. The foliar application of CeO_2 NPs on tomato increased fruit concentrations of K, P, and S by an average of 27%; and Ca content by 261% (Adisa et al., 2020). When citric acid-coated CeO_2 NPs were applied via soil, Al accumulation in roots and leaves of tomato increased by 175% (Barrios et al., 2016). Seed priming has also been successful in providing a significant increase in the uptake of additional nutrients, as illustrated with chickpea primed with FeS_2 ; mature plants contained increased root concentrations of Mo, Mg, P, K, Mn, and Ca—ranging from 80 to 415%—compared to no priming (Jangir et al., 2020). Seed priming chickpea with FeS_2 also increased leaf concentrations of Mo by 300%, Mg by 98%, and doubled Ca content. The application of copper-based NPs has led to mixed results. Foliar application of CuO NPs increased leaf content of P, Ca, and Mn; however, it decreased Na, Fe, and Zn concentrations (Pérez-Labrada et al., 2019). In some cases, particle morphology and composition affect the accumulation of nutrients. For example, the application of CuO NPs (round edges, ~30 nm) to watermelon decreased uptake of Si, Mn, Mg, and Fe; while the application of $\text{Cu}_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}$ in the form of nanosheets (flat sides, sharp edges, ~151 nm) increased the amounts of these elements (Borgatta et al., 2018). These accumulation patterns are also a function of plant species; Zn, P, and Mn were decreased with CuO NPs in watermelon but increased in tomato (Ma et al., 2019). Several types of NPs have been shown to increase the uptake of either N or K, with subsequent growth promotion (Alsaeedi et al., 2019; Dimkpa et al., 2017a; Dimkpa et al., 2017b; López-Vargas et al., 2018; Pérez et al., 2020; Yang et al., 2020). Additionally, there are instances where the application of NPs does not significantly alter nutritional content in the edible tissues (Elmer et al., 2021; Elmer & White, 2016), but provides plant protection (see Plant Disease Management). Last, for some NP applications, it is difficult to assess their impact on the plant nutritional profile because the overall elemental analysis is lacking in some studies that report only the element present in the treatment. For example, calcium NPs increased the content of tomato crude protein, crude fiber, and crude fat while SiO_2 NPs increased wheat protein content (Azeez et al., 2020; Behboudi et al., 2018). However, in general, these types of nutrient analyses are lacking in many studies. In addition, given that elemental analyses are destructive, the pattern of nutrient

accumulation over the course of the plant life cycle is rarely known; an understanding of that process could inform the design of optimized materials and treatment regimens. The elucidation of this could be accomplished through techniques like portable X-ray fluorescence (pXRF), which would allow for real-time monitoring of key nutrients (Montanha et al., 2020).

3 Engineered Nanomaterials for Plant Disease Management

Fungi, bacteria, viruses, and parasites are pathogens that threaten plant health (Elmer & White, 2018; Worrall et al., 2018). Although management options do exist for most pathogens, strategies are plagued by a range of shortcomings such as low overall efficacy, high cost, lack of sustainability, and induced pest resistance. Interest in the use of nano-enabled strategies in crop disease management has increased significantly in the last 5 years (Table 1). Nanomaterials can be engineered as sensors to detect disease, as agrichemical delivery carriers to inhibit or mitigate infection, as nano-enabled pesticides that directly inhibit the pathogen, or as nanoscale micronutrients that indirectly protect the host by stimulating host defense (Elmer & White, 2018). Metal oxide micronutrient nanoparticles have consistently provided positive results on a variety of crops infected with several soil-borne diseases, such as *Fusarium* wilts, *Verticillium* wilt, and Black Scurf disease. Convincing evidence has been published on the efficacy of foliar-applied CuO NPs, used alone or in combination with other NPs, to suppress soil-based pathogens. Foliar treatments carrying only a few milligrams of Cu in 2–3 mL of solution consistently provide effective long-term protection. The spatial separation between treatment (foliar) and infection (root) and the fact that conventional forms of Cu are ineffective highlights the importance of unique nanoscale properties in this strategy. Foliar application of CuO NPs has been shown to decrease the severity of *Fusarium* diseases on watermelon, tomato, and soybean; *Verticillium* wilt on eggplant; and Black Scurf Disease on potato (Borgatta et al., 2018; El-Shewy, 2019; Elmer et al., 2018, 2021; Elmer & White, 2016; Ma et al., 2019, 2020; Pérez et al., 2020). More specifically, foliar treatment with CuO NPs at 500 mg L⁻¹ in watermelon suppressed *Fusarium* wilt and restored fruit yield to levels recorded in healthy controls; root and/or foliar-dip treatment with 1000 mg L⁻¹ (of which less than 3 mL are retained in the tissues, resulting in a low dose) reduced area under the disease progress curve (AUDPC)—a metric for monitoring disease progress—by 53% (Borgatta et al., 2018). In a greenhouse study on the foliar application of Al₂O₃, CuO, Fe₂O₃, MnO, NiO, and ZnO NPs against *Fusarium* wilt, CuO, MnO, and ZnO were found to be the most effective in treating tomatoes (Elmer & White, 2016). CuO NPs provided the greatest *Fusarium* wilt suppression, with AUDPC being reduced 34%, while MnO and ZnO NPs reduced disease by 28% (Elmer & White, 2016). Foliar treatment of eggplant with CuO NPs reduced disease by 69% and ZnO NPs reduced AUDPC by 36%. The treatment of eggplants with MnO NPs had no impact on disease progression. Foliar application of Al₂O₃, Fe₂O₃, and NiO also reduced AUDPC in tomato, but did not

Table 1 Selected treatment outcomes of inorganic nanoparticles applied to agricultural crops to enhance disease tolerance

Disease	Crop	NP	Outcome	References	
Fusarium wilt <i>Fusarium oxysporum</i> f. sp. <i>Lycopersici</i>	Tomato <i>Solanum lycopersicum</i>	Al ₂ O ₃ , Fe ₂ O ₃ , NiO	Foliar treatment significantly reduced disease progression.	Elmer and White (2016)	
		CuO	Foliar spray led to a 30% drop in disease and a 33% increase in yield.		
		MnO, ZnO	Foliar spray caused a 30% reduction in disease severity.		
		CeO ₂	Up to 57% reduction in disease.		Adisa et al. (2018)
		CuO	Foliar dipping caused a 31% reduction in disease.		Ma et al. (2019)
		Cu ₃ (PO ₄) ₂ • 3H ₂ O	Foliar dipping caused a 31% reduction in disease, resulting in a 50% increase in biomass.		
	Eggplant <i>Solanum melongena</i>	CuO	Foliar treatment resulted in a 69% reduction in disease, a 64% increase in biomass, and a 34% and 73% increase in yield.	Elmer and White (2016)	
		ZnO	Foliar treatment caused a 36% reduction in disease.		
Fusarium wilt <i>Fusarium oxysporum</i> f. sp. <i>niveum</i>	Watermelon <i>Citrullus lanatus</i>	CuO	53% reduction in disease when treated with a foliar “dip” or via root treatments. There was a 23% reduction in disease with a foliar spray treatment and a 40% increase in biomass.	Borgatta et al. (2018)	
		Cu ₃ (PO ₄) ₂ • 3H ₂ O	Foliar dipping caused a 58% reduction in disease, leading to a 261% increase in biomass. Foliar spray reduced disease by 25% and increased biomass by 40%.		
		CTS-MSN, MSN	Foliar dipping led to a 27 and 40% reduction in disease.		Buchman et al. (2019)
		SiO ₂	Supplied silicic acid as NPs dissolve mitigating disease and enhancing fruit yield ~80%.		Kang et al. (2021)
		B, CuO, MnO, SiO ₂ , TiO ₂ , ZnO	CuO outperformed the other NPs overall giving higher yields. CuO in the presence of fusarium upregulated PPO gene expression and activation of PPO enzyme.		Elmer and White (2018)

(continued)

Table 1 (continued)

Disease	Crop	NP	Outcome	References
Verticillium wilt <i>Verticillium dahliae</i>	Eggplant <i>Solanum melongena</i>	CuO	In greenhouse studies, foliar dipping resulted in a 40% reduction in disease and a 47% increase in biomass. In field experiments, treatments caused a 28% reduction in disease and a 33% increase in yield.	Elmer et al. (2021)
		ZnO, Mn ₂ O ₃	Foliar treatment had no effect on disease, but increased yield 21 and 17%.	
		CuO + ZnO	Combined foliar treatment of CuO and ZnO (greenhouse) led to a 47% reduction in disease and a 52% increase in biomass.	
Black scurf disease <i>Rhizoctonia solani</i>	Potato <i>Solanum tuberosum</i>	Ca ₃ (PO ₄) ₂ , CuO, SiO ₂	Soaking tuber followed soil drenching mature plants. All NPs at 150 ul/L mitigated disease like a commercial fungicide. NPs activated defense-related enzymes.	El-Shewy (2019)
Sudden death syndrome <i>Fusarium virguliforme</i>	Soybean <i>Glycine max</i>	CuO, ZnO, Mn ₂ O ₃	Foliar dipping resulted in 18.9, 24.7, and 17.1% less root rot.	Peréz et al. (2020)
		CuO (NP or NS), Cu ₃ (PO ₄) ₂	Foliar dipping caused an increase in biomass of up to 50%. Disease severity was reduced due to the activation of plant defense mechanisms upon treatment.	Ma et al. (2020)
Curvularia leaf spot <i>Curvularia lunata</i>	Maize <i>Zea mays</i>	CTS-Cu	Foliar treatment significantly reduced disease up to 25%.	Choudhary et al. (2017)
Cucumber mosaic virus	Cowpea <i>Vigna unguiculata</i>	Mg-Al layered double hydroxides (MgAl-LDHs)	Nanosheets as carriers of CMV-dsRNA, reduced the number of infected plants.	Mitter et al. (2017)
Pepper mild mottle virus	Tobacco <i>Nicotiana tabacum</i>	MgAl-LDHs	Nanosheets supplied dsRNA from PMMoV, protected tobacco plants for 20 d from viral infection.	Mitter et al. (2017)
Phytophthora/ bacteria	Tobacco <i>Nicotiana tabacum</i>	Ag	Biosynthesized Ag NPs mitigated disease without causing apparent toxicity in tobacco seedlings.	Ali et al. (2015)

(continued)

Table 1 (continued)

Disease	Crop	NP	Outcome	References
<i>Xanthomonas perforans</i> (bacteria)	Tomato <i>Solanum lycopersicum</i>	Cu/Zn hybrids	Nanohybrids reduced disease by 80% in a Cu-tolerant species where traditional treatments are inefficient.	Carvalho et al. (2019)
		MgO	MgO suppressed disease without being toxic to the plants.	Liao et al. (2019)

perform as well as CuO, MnO, or ZnO NPs. In field trials, none of the tested NPs reduced disease occurrence; however, CuO NPs increased eggplant yield 40% and 73%, compared to untreated control, for two consecutive seasons.

In a separate study, greenhouse-grown eggplant infected with the *Verticillium* wilt fungus had AUDPC values reduced 40%, and biomass increased 47%, when plants were foliar-treated with CuO NPs at 500 mg L⁻¹ (Elmer et al., 2021). Interestingly, when this treatment was combined with ZnO NPs at 500 mg L⁻¹, there was no added benefit. Similarly, when studies were performed in the field across multiple seasons, CuO NPs lowered disease ~27%, and increased biomass and fruit mass. In addition, CuO NPs increased eggplant yield 17 and 33% during two growing seasons. Once again, the combined treatment of CuO and ZnO NPs had no effect on disease progression, but did increase yield by 15%, which, to a grower, could be a considerable increase of marketable produce from disease-impacted plants.

Fusarium wilt in watermelon has also been successfully treated with CuO NPs, where diseased plants showed significantly less disease severity than untreated controls (Elmer et al., 2018). Equivalent doses of MnO and TiO₂ were less effective at treating wilt disease. Additionally, watermelon plants treated with CuO NPs were significantly larger than both control plants or plants that received MnO, SiO₂, TiO₂, or ZnO NPs. However, when the greenhouse-based experiment was repeated, only CuO NPs were effective at treating Fusarium wilt (decreased by 35%). Field trials with CuO, MnO, B, and ZnO NPs were all effective at significantly reducing disease ratings, although CuO NPs were the most effective and increased yield from 35% to 53% compared to no treatment.

Similarly, the use of CuO, SiO₂, or Ca₃(PO₄)₂ NPs at concentrations of 150 and 200 µl L⁻¹ was found to significantly reduce the incidence and severity of Black Scurf disease in potato, with 200 µl L⁻¹ completely eliminated evidence of disease (El-Shewy, 2019). However, amendment with other nutrients may play a role in the efficacy of the treatment, as has been shown with sudden death syndrome in soybean. Plants fertilized with 50 µg N mL⁻¹ and foliar-treated with CuO, Mn₂O₃, or ZnO NPs at 500 µg mL⁻¹ significantly reduced root rot, but when fertilization was increased to 100 µg N mL⁻¹ the treatments were less effective (Pérez et al., 2020). Treatment of Soybean Sudden Death Syndrome was most effective with ZnO or CuO NPs (24.7% and 18.9% less root rot); however, increasing the concentration of CuO NPs to 1000 µg mL⁻¹ reduced the treatment's effectiveness by over 40–60%,

regardless of nitrogen fertilization. Note that strategically, even though these are high concentration treatments, only a small volume of 2–3 mL is transferred to the leaves of young seedlings, resulting overall in low doses (Borgatta et al., 2018; Elmer et al., 2018; Pérez et al., 2020; Shen et al., 2020). These low doses were applied to very young plants, but provided long-term protection, with positive effects observed over the full life cycle of the plants. These results confirm that treatment of plants when in the early stages of growth provides the most effective treatment against soil-borne diseases. The life cycle long protection is a nanoscale-dependent phenomenon; the responses to these specific plant-nanoparticle interactions are under investigation (Pagano et al., 2017).

Several studies have begun to explore how tuning the chemistry of Cu nanomaterials could be used as a strategy to optimize benefit (Fig. 2). For example, work has been done with $\text{Cu}_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}$ nanosheets (NS)—Ma et al. reported that either CuO NPs or $\text{Cu}_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}$ NS reduced Fusarium wilt by an average of 31% in tomatoes (Ma et al., 2019). This study also found that $\text{Cu}_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}$ NS delayed the visual symptoms of Fusarium wilt until day 14, and by the end of the experiment (day 21) signs of disease among infected plants were equivalent to uninfected controls. The application of $\text{Cu}_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}$ NS also increased biomass in diseased plants by nearly 50%, an effect also seen with CuO NPs. Further work with Fusarium-infected soybean showed that $\text{Cu}_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}$ NS, CuO NS, and CuO NPs were all effective treatments, with CuO NS having the greatest impact (Ma et al., 2020). Moreover, analysis of two dozen plant defense and stress-related genes confirmed foliar application of these materials provoked a Cu-induced increase in plant immunity. Conversely, in Fusarium-infected watermelon, $\text{Cu}_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}$ NS reduced AUDPC values to a similar degree as CuO NPs, but at doses that were 100 times lower (Borgatta et al., 2018). Additionally, foliar treatment of tomato with CuO NPs or $\text{Cu}_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}$ NS demonstrated Cu form controls the rate of uptake and internalization; simultaneously, treatments were equally effective as a single dose to seedlings as were multiple doses applied over a period of weeks (Shen et al.,

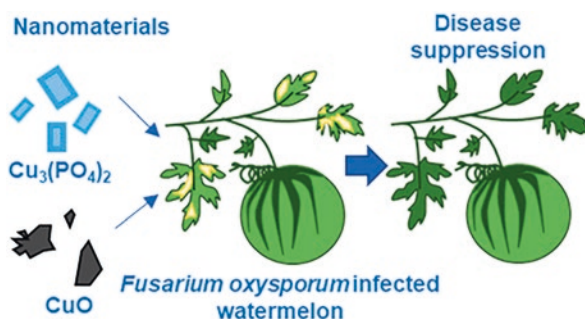


Fig. 2 Foliar treatment of crops infected with Fusarium wilt treated with copper-based nanoparticles showed improved disease tolerance due to treatments enhancing plant defense responses. Figure used with permission from Borgatta et al. (2018)

2020). The mechanism of this action remains unknown, but treatment timing to seedlings is critical to maximize benefit and reduce waste. Chitosan-coated copper NPs (CTS-Cu NPs) have been effective against *Curvularia* leaf spot (CLS) disease in maize. When maize seeds were soaked in suspensions of CTS-Cu NPs at concentrations ranging from 0.04 to 0.16% (w/v), then foliar-treated 35 days later, visual symptoms were delayed twice as long as untreated controls and disease severity was reduced by 24–22% (Choudhary et al., 2017). The mechanism against CLS appears to be 2-pronged: direct, in vitro exposure caused up to 50% inhibition of mycelia growth; and indirect, with Cu amendment stimulating SOD activity—increasing plant defense activity.

Other successful reported treatments for *Fusarium* wilt include mesoporous silica nanoparticles (MSN), chitosan-coated MSN (CTS-MSN), and CeO₂ NPs. To treat *Fusarium* wilt in watermelon, seeds were vacuum-infused in suspensions of 250 or 500 mg L⁻¹ MSN or CTS-MSN and, subsequent to germination, had their aerial tissues dipped in corresponding solutions of MSN or CTS-MSN at 500 mg L⁻¹; AUDPC was reduced 40% with MSN and 27% with CTS-MSN compared to untreated plants (Buchman et al., 2019). Kang et al. (2021) also showed that MSN synthesis chemistry could be tuned to release silicic acid at a range of desired rates; faster rates were superior to conventional MSN at increasing biomass, yield, and *Fusarium* wilt suppression in watermelon. Interestingly, silicic acid controls had no such benefit. Suppression of *Fusarium* wilt in tomato with NPs CeO₂ was equally effective when applied either through the soil (decrease of 53%) or by foliar spray (decrease of 57%) (Adisa et al., 2018). However, treatment of diseased tomato plants with CeO₂ NPs did impact the fruit (Adisa et al., 2020). Specifically, foliar application of CeO₂ NPs increased fruit dry weight by 67% but decreased total sugars by 50%; while root treatments with NPs CeO₂ increased fruit total sugars by an average of 58%, lycopene by 9%, and the micronutrients Cu and Mn by 51 and 59% respectively.

Mechanistically, it is important to highlight the fact that these foliar amendment strategies are not directly targeting the pathogen but serving to modulate plant nutrition and defense as a strategy to suppress disease damage. For example, in-vitro studies show that the concentration of 500 mg L⁻¹ of CuO NPs does not have a fungicidal effect. Importantly, for the in vivo work, although a dose of 500 mg L⁻¹ may be applied foliarly, only 2–3 ml of the solution is transferred to the plant, yielding an actual dose of only a few mg. Therefore, CuO NPs may act as a more active and available nanoscale supply of Cu for the host plant which uniquely activates an entire range of defense pathways and enzymes (Elmer & White, 2016; Lopez-Lima et al., 2021). The end result is disease management at doses that may be orders of magnitude below conventional treatment options. Conversely, certain metal-based NPs have been shown to be bactericidal (Carvalho et al., 2019; Liao et al., 2019). This is important as a number of strains of bacteria that cause crop diseases have become resistant to conventional copper-based treatments (Lamichhane et al., 2018). In vitro work with *Xanthomonas* spp. demonstrated that hybrid nanoparticles of Cu and Zn were effective at inhibiting bacterial growth and reducing the severity of tomato spot disease by up to 80%, as compared to Kocide 3000, Kocide

3000 + Mancozeb, and untreated controls (Carvalho et al., 2019). In this study, the authors point to a significant reduction in xanthomonadin—a pigment present in the cell membrane—as a possible mechanism for the bactericidal effect. Similarly, MgO NPs were effective in inhibiting the growth (in vitro) of *Xanthomonas* spp. and significantly decreased the severity of tomato spot disease in an in vivo study compared both to controls and conventional treatments (Liao et al., 2019). Other studies with pathogenic bacteria include silver (Ag) nanoparticles (Dimkpa et al., 2011; Ding et al., 2017). Unlike the above studies where the foliar application of low masses of micronutrients to seedlings points to an indirect mechanism of action, Ag NPs act by disrupting the pathogen cell membrane on contact, or through the generation of ROS, either from the nanoparticles themselves or the ions they release, leading their direct bactericidal effect (Levard et al., 2012; You et al., 2012).

Another strategy against disease is the indirect use of nanoscale materials to supply defense agents such as herbicides, hormones, and antimicrobials (Worrall et al., 2018). For example, Xu et al. used an electrospinning approach to coat seeds with nanoscale biopolymer fibers that were pre-loaded with Cu and that provided enhanced germination and growth of lettuce and tomato in the presence of disease (Xu et al., 2020). In addition, NPs are being engineered and tested also as carriers of nucleotides for purposes of gene silencing, which inhibits the replication of viral pathogens. This RNA interference (RNAi) mechanism is inherent to plants, and in order to activate these processes, genetic material of the pathogen needs to enter the plant. The host will detect these nucleic acids and code for destruction instead of replication of the disease agent. However, unprotected nucleotides are prone to degradation before entering the plant or can denature once inside. Therefore, there have been efforts to utilize nanomaterials as nucleotide protectors and carriers to activate RNAi pathways (Elsharkawy & Mousa, 2015; Mitter et al., 2017; Schwartz et al., 2020). Disease management is essential in agriculture in order to mitigate or avoid crop losses; other chapters in this book cover the role of NPs as pesticides (Chap. 6 and Chap. 10), and their performance as nanocarriers (Chap. 11) in greater detail; please refer to those sections for additional details on these important topics.

4 Nanoparticles to Alleviate Environmental Stressors

As the effects of climate change begin to worsen, it is clear many crops will have to be grown under increasingly marginal conditions. For example, drought conditions will become more prevalent across the globe, limiting crop production, and decreasing arable land. The use of NPs to mitigate the detrimental effects of drought stress in crops has shown promise. Wheat treated with SiO₂ NPs, either via soil or through foliar spray, were able to better tolerate drought conditions (Behboudi et al., 2018), having increased chlorophyll (SPAD) content, relative water content (RWC), and greater yield; the soil treatment route provided significantly better 1000-grain weight and yield than foliar treatment. Although treatment with SiO₂ NPs improved some agronomic parameters, SOD activity increased upon treatment under drought

conditions compared to non-drought controls. Under drought conditions, fertilization of soil with ZnO NPs increased the emergence of wheat sprouts by 5 days, and increased chlorophyll and Zn content in the shoots and grain (Dimkpa et al., 2020). Compared to controls, the foliar application of a mixture of NP ZnO, B₂O₃, and CuO to drought-stressed soybean increased yield by 33% and shoot growth by 36%, while also increasing leaf area and leaf number (Dimkpa et al., 2017a). The application of Fe, Cu, or Co NPs improved soybean drought tolerance and increased shoot dry weight under drought conditions when compared to untreated control, while the application of Fe NPs also increased shoot length (Linh et al., 2020). The relative water content was enhanced with the application of either Fe or Cu NPs, and biomass was increased with Fe or Co NPs. Priming maize seeds with Cu NPs have also been reported to increase seed yield and weight of drought-affected plants two-fold (Van Nguyen et al., 2021).

Agricultural crops can be susceptible to salinity—which can alter vital plant functions, affecting growth, photosynthesis, and yield. Importantly, select nanoscale amendments have been shown to enhance tolerance to or alleviate damage from salinity. Cucumber was grown under salt stress, with varying amounts of additional water stress, and was then treated with Si NPs (Alsaedi et al., 2019). The addition of Si NPs at 200 mg kg⁻¹ improved growth and productivity, regardless of drought status, by increasing the leaf area, total chlorophyll, and plant height, compared to water-stressed controls. Additionally, Si NPs lowered the uptake of Na, while increasing K content in all tissues. This action reduced the Na/K ratio in favor of improved salt tolerance. Moreover, the addition of Si NP—especially at 200 mg kg⁻¹—increased cucumber yield under all watering conditions. Foliar application of nanoscale NPK to hydroponically grown peas exposed to salt stress showed increased growth parameters, including leaf area, leaf number, and shoot length (El-Hefnawy, 2020). Cellular analysis of roots from nanoscale NPK treated plants showed a reduction in chromosomal abnormalities, compared to untreated controls. Tomato plants grown under salt stress suffered reduced growth and a decrease in the yield of up to 50% (Pérez-Labrada et al., 2019). Importantly, foliar application of Cu NPs had no impact on growth under saline conditions; chlorophyll content and fruit yield were unchanged. However, treatment did increase vitamin C content in the tomato fruit. Wheat seeds treated with sulfur (S) NPs and planted in saline conditions experienced improved growth—increasing root and shoot fresh/dry weight, and leaf area—compared to untreated salt-stressed plants (Saad-Allah & Ragab, 2020). Treatment with S NPs alleviated the increased catalase, superoxide dismutase, ascorbic peroxidase, and polyphenol oxidase activities that were elevated due to salt stress. Additionally, leaf pigment concentrations were increased; uptake of N, P, and K was increased; uptake of Na was reduced; and growth was restored to near unstressed control levels. Biochemical indicators of stress, such as malondialdehyde (MDA), H₂O₂, and electrolyte leakage were also reduced—with S NPs being more effective with lower salt concentrations (100 mM NaCl) versus higher (200 mM NaCl) for most endpoints. Given this limited yet promising data, it is clear that select nanoscale treatments have the potential to sustainably increase crop tolerance to environmental stresses anticipated from a changing climate; future

work should focus on uncovering the mechanisms of plant response to these unique nanoscale effects, as well as on the ability to tune material properties to further optimize the observed benefits (An et al., 2020). High-throughput studies have already begun to reveal some mechanisms behind increased photosynthesis upon exposure to metal NPs. Spinach chloroplasts exposure to Mn_3O_4 or Fe NPs for 2 h showed increased photocurrent and electron transport, resulting in a 23 and 43% increase in quantum yield and ATP synthesis (Wang et al., 2020).

5 Future Implications

The use of NPs on agronomic crops plants will be a subject of continuous research, as new materials are introduced, and as current materials are transformed by the environment after their application. Also needed are insights into potential drawbacks arising from the desire to over apply NPs to maximize beneficial results. For example, if a grower decided to increase the dose of an NP to further promote already enhanced growth, they may not receive the desired effect. Exposure of barley to increasing concentrations of CeO_2 NPs resulted in greater biomass accumulation, but the enhancement was all directed at vegetative growth and mature plants failed to produce grains (Rico et al., 2015). Further research will also be needed into the implications of increased NP release into the environment, especially those containing elements not typically found in agricultural soils (e.g., Ce and Ti). Still, there are concerns about the ramifications of prolonged NP application on the food web and how extended ion release could impact the soil and plant microbiomes. Further investigation is also needed on the potential interactions of NPs with the myriad of agrichemicals currently in use and ubiquitous in the environment (Zhao et al., 2019). Great care must be taken with the use of NPs in agriculture, any perceived negative implication may cause rejection by the general public (Hofmann et al., 2020). As with all the outcomes presented, the observed effects are dependent on the type of NP employed and so far, published research has not shown that effects induced by the application of NPs affect the propagation of later generations of crops. In fact, application of ZnO NPs to soil-grown kidney bean produced nearly no residual effects when the subsequent generation was cultivated without amendment (Medina-Velo et al., 2018). Additionally, concerns over the implications on the use of NPs to promote crop performance should not be limited to the effects on the target plants. The use of NPs should be considered on a life-cycle basis, evaluating their total impact on the system in which they are being applied. Consideration must be given to the total environmental impact of scaling up the application of NPs from the laboratory to the field. A recent study evaluated total embodied energy of the manufacture of several NPs, including ZnO, CuO, and CeO_2 , and found that at the currently applied research doses, NPs were not a sustainable alternative to conventional practices as a means of N replacement (Gilbertson et al., 2020). However, as presented above, there are numerous uses for NPs in agriculture other than supplanting the application of N fertilizers, and in some cases, better performance is

obtained from utilizing both. This study did report that both ZnO and CuO NPs used as seed coatings (i.e., seed priming) and ZnO used as a foliar treatment were promising from an embodied resource standpoint. Additionally, some areas will be too remote or lack the infrastructure to mass produce NPs for agricultural applications; it is therefore critical to invest heavily in economical, green synthesis methods. Such approaches utilize more environmentally conscious materials (e.g., plant material, bacteria, or algae) and produce fewer toxic byproducts (Saratale et al., 2018).

6 Conclusion

In conclusion, a rapidly growing body of literature has demonstrated the beneficial qualities of NPs application onto agriculturally relevant plants. This includes a demonstration of how NPs can be utilized to improve disease suppression, drought, and salinity tolerance while improving crop yields. Further, the application of NPs onto non-stressed plants has been shown to provide a performance-enhancing effect. It is clear that current agricultural practices are inadequate to address future predicted food demands and predicted challenges on agriculture from a changing climate. A paradigm shift to more technologically advanced, environmentally sound agricultural practices is needed and has begun. The move to nanoscale-based crop amendment strategies can be critical to sustainably increase food security while reducing our impact on the surrounding environment.

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