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REVIEW

Nanopesticides: Opportunities in Crop Protection and Associated Environmental Risks

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Abstract The last decade has witnessed significant progress in nanotechnology and its application in diverse fields. This growing interest has also been intensified in agriculture sector, which is evident from the publication of interesting scientific articles and lodging of patent applications, showing the relevance of nanotechnology in sustainable crop production. Crop losses due to plant pathogens, insects and weeds are a considerable challenge that the current agricultural production system faces worldwide. This article depicts the use of nanoparticles that paves the way for developing novel crop protection products in near future. Owing to the fact that nanoparticles have been used in many economically important applications, it is highly desirable to ascertain the possible deleterious effects as well. Moreover, intentional application of nanoparticle-based pesticides and fertilizers is an issue of great concern, as it may pose serious hazards. From this perspective, the authors discussed the recent research aimed at defining effects of nanoparticles on various environments and human health.

Keywords Nanopesticides · Nanoparticles · Nanotechnology · Crop protection

Introduction

Attempts to boost food production with ever increasing population growth, limited availability of land and water resources, climate change, high incidence of diseases and insect pests, and bioaccumulation of agrochemicals are the biggest global challenges. To overcome these massive threats, different technological innovations like high yielding varieties, fertilizers, synthetic pesticides, hybrid seeds and transgenic crops have been developed that have contributed to enhancing global agricultural sustainability. Unfortunately, extreme dependency on these innovations has contributed many ill effects. Micronutrient imbalance, nitrate pollution and eutrophication are the serious consequences of excessively or improperly applied fertilizers. Similarly, heavy pesticide use poses greatest risk to non-target organisms including humans, beneficial microorganisms, beneficial insects, birds, earthworms and aquatic life forms. Furthermore, bio-magnification, development of pesticide resistance and resurgence of pest populations are the serious problems associated with pesticide use. The sensitivity of high yielding crops to diseases, insects and abiotic factors intensified the use of fertilizers and pesticides. Genetically modified (GM) crops are one of the greatest attempts to minimize chemical treatments. Unfortunately, pests may also develop resistance against GM crops, as they have already developed resistance to many pesticides. For instance, field-evolved resistance against Bt crops for some populations of 5 of 13 major pest species has recently reviewed, compared with resistant development in only one pest species in 2005 [1]. Moreover, novel genes may trigger risk of horizontal gene transfer with more severe, long-lasting ecological and economic consequences. Except new strains of plants, each of these modern agriculture innovations is totally reliant on

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the energy resources, especially petroleum. Global petroleum production is predicted to arrive at a maximum in the coming decades and to decline thereafter, a phenomenon known as peak petroleum [2]. Moreover, the world's population is projected to reach 9.7 billion by 2050, placing an unprecedented pressure on global food security [3]. This alarming situation calls the production of an additional 1 billion tonnes of cereals and 200 million tonnes of meat annually [4]. Additionally, the growing demand for meat will put enormous burden on agricultural land and farmers need to grow crops to produce animal feed. Unfortunately, insect pests, plant pathogens, and weeds account for 14, 13 and 13 % crop losses respectively, causing an annual loss of $2000 billion worldwide [5]. Worldwide, about 3 million metric tons of pesticides, costing around $40 billion are applied annually [5]. This situation calls an alarming condition of more than 26 million cases of non-fatal pesticide poisonings [6]. In addition, deleterious effect on non-target beneficial microorganisms, risk to humans and other life forms, ground and surface water contamination and development of pesticide resistance are some major risks associated with heavy pesticide use. Moreover, climate change will affect the occurrence and distribution of insect pests and diseases that may cause unpredictable negative impacts on agricultural production worldwide [7]. This inevitable risk to food security and agriculture will invite more troubles in developing countries. For instance, a recent study by The Associated Chambers of Commerce and Industry of India said that annual crop losses due to pest and disease infestation results in crop losses worth Rs. 50,000 crore ($500 billion) that is significant in a country like India where at least 200 million people go to bed on an empty stomach each night [8]. Given the background, nanopesticides or nano-plant protection products represent a hopeful scientific development that offer a variety of benefits including increased effectiveness, durability, and a reduction in the amounts of active ingredients (AIs) that is being used in protecting crops against diseases, insects and weeds [9]. The last decade has witnessed the development of a variety of nanoformulation types including nanoemulsions, microemulsion, nanocapsules, nanospheres, solid lipid nanoparticles, products containing metals and metal oxides nanoparticles, porous hollow silica nanoparticles, layered double hydroxides and nanoclays. Excellent reviews were published that provide knowledge on these latest developments in nanopesticide research [9–14]. More than 3000 patent applications have been lodged in the last decade for nanopesticides alone [15]. Polymer-based formulations, inorganic metal/metal oxide, and nanoemulsions have received the greatest attention [15]. Increased efficacy of the nanoformulations have not yet been fully characterized perhaps, increase in the apparent solubility of weakly soluble AIs, slow or targeted release of AIs and protection of AIs from premature degradation makes nanoformulations superior in comparison to commercial formulations [9, 13]. Moreover, nanoformulations are more efficacious and required in lower doses than the conventional formulations that result in lower environmental burdens. The value of nanoformulations in crop protection is therefore huge to enhance the crop production. But, there is still no consensus among researchers, where the formulation and material scientists are in agreement with this emerging field, environmental scientists communicate on the notion of possible hazards [16]. These issues through different sets of eyes can make large differences in overall framing of nanopesticides among the general public. The aim of this review is to summarize nanopesticide research in crop protection that has been made over the last decade. Moreover, the benefits and possible hazards associated with nanoparticles are discussed. The information gathered herein identifies important directions for future research.

Definitions

International organization for standardization (ISO), the world's largest developer of standards, has defined nanomaterial as a material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale, where length range from approximately 1–100 nm is considered as nanoscale [17]. Materials with one, two or three external dimensions in the nanoscale are nano-objects, and nanoparticles are those nano-objects in which all three external dimensions lie in the nanoscale range where the lengths of the longest and the shortest axes of the nano-object do not differ significantly [17]. Several countries and international organizations including Organization for Economic Cooperation and Development (OECD), Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR), European Commission (EU), United States Food and Drug Administration (US FDA), United States Environmental Protection Agency (US EPA), Health Canada, National industrial Chemical Notification and Assessment Scheme (NICNAS), Danish Ministry of the Environment etc. have presented their own definitions but there is no universal agreement regarding the definition of nanomaterials [15, 18]. Unfortunately, the proposed definitions vary considerably in their breadth of understanding nanomaterials that may lead to conflicting classification and assessment of nanomaterials, which might have unpleasant impacts on commerce and public perceptions of nanotechnology [18]. Given the background, the products that should be considered as nanopesticides under one definition may not be considered as nanopesticide in another. Further, at this stage, it is difficult to answer that nanopesticides are already in the market or not, until one arrives at a universally agreed definition [14, 16]. Researchers from the Department of Environmental Geosciences of the University of Vienna discussed nanopesticides with a broader definition, where all plant-protection products that (1) intensionally include entities covering a size range up to 1000 nm (2) designated with nano-prefix (3) claimed to exhibit novel properties associated with their small size, are considered as nanopesticides [9, 14]. For this review, the authors have used the same broader definition, in order to offer a summary of the nano-plant protection products discussed in peer-reviewed papers and reports. Further, the role of nanofertilizers for balanced crop nutrition has also been discussed.

Plant Disease Management

Nanoparticles of metals and metal oxides attracted a great scientific attention in plant disease management, and were found promising against serious plant pathogens that are responsible for huge economic losses. Moreover, recent studies also indicate the great potential of polymers and Ag-based nanocomposites as alternatives to commercial fungicides. The potential benefits of nanoparticles in plant disease management and better crop protection are summarized in Fig. 1.

Siver Nanoparticles (AgNPs)

AgNPs are the most commonly used anti-bacterial agents in health industry, food storage, textile industry and a number of environmental applications. Studies conducted during the last decade have also demonstrated the efficacy of AgNPs against major plant pathogens causing huge losses worldwide. Nanosized silica-silver (1–5 nm) prepared by combining $AgNO_3$, sodium silicate and water soluble polymer, successfully controlled powdery mildews of pumpkin in both field and greenhouse conditions at 0.3 ppm [19]. Moreover, at 3.0 ppm, the developed product significantly inhibited a variety of plant pathogenic fungi in vitro, and found better than 20 nm AgNPs and 100 nm AgNPs. Interestingly, higher concentration (10 ppm) did not cause any adverse effect on a number of beneficial bacteria or plant pathogenic bacteria but complete inhibition was observed at 100 ppm [19]. *Colletotrichum gloeosporioides* induces anthracnose in a wide range of fruits, such as apple, avocado, mango and papaya, is controlled primarily by the application of synthetic fungicides during the postharvest period. Unfortunately, the pathogen has established cross resistance against benzimidazole fungicides. Although, prochloraz showed strong toxicity to *C. gloeosporioides*, but a recent study indicates the residue levels of this fungicide found above the maximum residue limit [20], which may represent serious harm to human health. AgNPs (5–24 nm) at a minimum concentration (56 µg Ag/mL PDA) showed 90 % growth inhibition *of C. gloeosporioides* [21]. Although the results are encouraging but the study was done only in Petri dishes where AgNPs exhibited a dose-dependent fungistatic activity. AgNPs synthesized using a novel bacterial strain BHU-S4 (*Serratia* sp.), designated as bsAgNPs (10–20 nm), showed complete inhibition of conidial germination of *Bipolaris sorokiniana* [22], the cause of one of the most dreadful diseases as spot blotch of wheat. Detached leaf assays indicated the obstruction in conidial germination. Interestingly, glass house experiments proved the beneficial effect of bsAgNPs as indicated by significant increase in root length, shoot length and shoot dry weight to withstand pathogen harmful effects. It is important to note here that histochemical staining revealed maximum lignifications of vascular bundles, which plays a key role in plant development as well as disease resistance, and SDS-PAGE profile revealed no protein damage in bsAgNPs treated plants [22].

Sclerotium-forming phytopathogenic fungi are responsible for many economically important diseases worldwide. For instance, *Rhizoctonia solani*, the causal organism of sheath blight of rice (*Oryza sativa* L.), is responsible for significant yield losses in all rice growing countries. Application of fungicides (azoxystrobin and flutolanil) appeared to be less efficient in increasing yields of moderately susceptible rice cultivars than susceptible and very susceptible cultivars. Moreover, fungicides are suggested only when the disease outbreaks cause significant losses, because azoxystrobin and flutolanil cost approximately $118 and $102 per ha, respectively [23]. Similarly, basal drop caused by *Sclerotinia sclerotiorum* is one of the most destructive diseases of horticultural crops worldwide. In a lab based study, AgNPs (4–8 nm) significantly inhibited the hyphal growth of *R. solani, S. sclerotiorum* and *S. minor* in a dose-dependent manner where hyphae exposed to AgNPs were severely damaged that ultimately resulted in plasmolysis and collapse of hyphae [24]. Moreover, AgNPs (7 ppm) were found to completely inhibit the sclerotial germination of *S. sclerotiorum*, while that of *R. solani* and *S. minor* were suppressed by more than 75 % [24]. Although the results are laboratory based but have significant practical implications because sclerotia play a key role in disease cycle; contributes to an increase in inoculum density and resists unfavorable abiotic factors such as heat, drought, and fungicides. Interestingly, in addition to *R. solani* and *S. sclerotiorum*, AgNPs (10–50 nm) prepared using *Acalypha indica* leaf extract also showed tremendous inhibitory action against *Alternaria alternata*, *Macrophomina phaseolina*, *Botrytis cinerea* and *Curvularia lunata* at a concentration of 15 mg/10 µL [25]. AgNPs (7–21 nm, 8 µg/mL) synthesized using cell free culture supernatant of a *Bacillus* strain (GP-

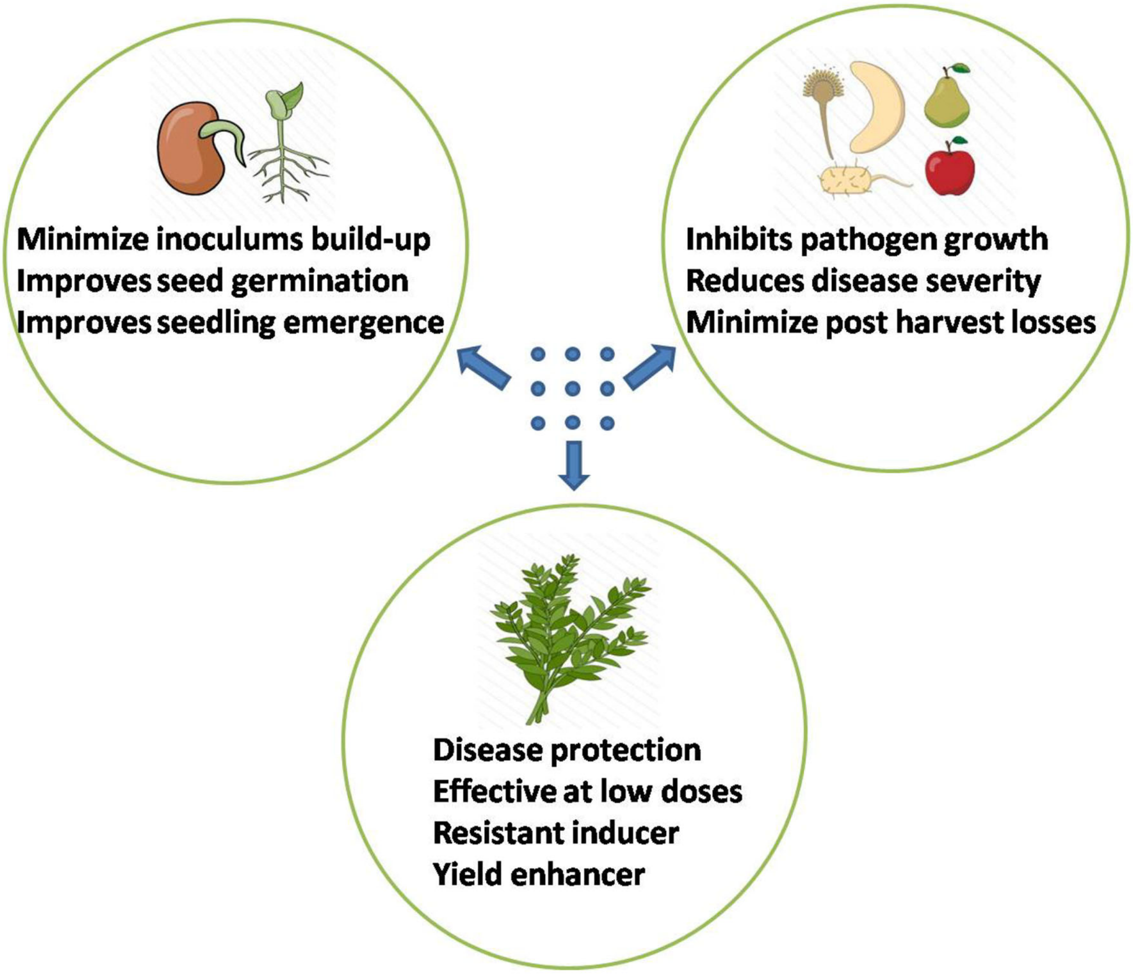

Fig. 1 The potential advantages of nanoparticles in crop protection

23), showed inhibitory activity against *Fusarium oxysporum* by fragmenting and distorting the hyphae and disrupting the spores [26].

Seed-borne diseases are primarily controlled by fungicides, hot water, or chlorine treatment. Since, certain level of risk is associated with all the fungicides, therefore treated seeds should be handled cautiously and regulated in an effective way to avoid their use for human consumption or livestock feed. Bakanae disease of rice, caused by *Gibberella fujikuroi*, is one of the most important seed-borne fungal diseases causing significant yield losses worldwide. A recent study demonstrated the potential of AgNPs to manage this menace effectively. AgNPs (7.5 nm), at a concentrations ranging from 0.015 to 1.5 µg/mL reduced the conidial viability of *G. fujikuroi* by 50 %, when exposed for 1–20 min [27]. Further, significant reduction in colony-forming units (CFUs) of the pathogen was observed by the treatment of infested rice seeds with 150 µg/mL of AgNPs for 10 min and up to 24 h. Interestingly, seed treatment with 150 µg/mL AgNPs for 12 or 24 h significantly improved seedling emergence and plant height, without showing any adverse effects on germination rate and seedling growth even up to 48 h exposure [27]. This study is of great interest to develop novel seed disinfection methods that not only minimize inoculum build-up on seeds but also contributes to improve seedling emergence and plant height. Two conidia-producing fungi that are economically important pathogens of grasses are *B. sorokiniana*, which causes seedling blight, root rot, crown rot, and spot blotch on various gramineous species, and *Magnaporthe oryzae*, the cause of blast on rice and gray leaf spot on turfgrass. Silver (Ag) ions and AgNPs (20–30 nm) were demonstrated to produce a significant effect on the colony formation of *B. sorokiniana* and *M. oryzae*. Moreover, both ionic and nanoparticle Ag significantly reduced disease severity when applied 3 h before spore inoculation on perennial ryegrass (*Lolium perenne*) [28]. Surprisingly, their effectiveness significantly reduced when applied at 24 h after inoculation. The pathogens reproduce by means of asexual conidia, infection is initiated at high humidity (∼100 % relative humidity) and warm temperature (25 °C) and germ tubes penetrate plant surfaces within 24 h [29]. Reduction in antifungal activity of Ag after 24 h of inoculation indicates that direct contact of Ag with spores or germ tubes is critical in disease inhibition [28].

Fusarium culmorum is an ubiquitous soil-borne plant pathogen which infects different small-grain cereals, in particular wheat and barley, and is the culprit behind foot rot, root rot and head blight. Significant yield reductions and deterioration of the quality by contamination of the grains with the fungal mycotoxins is a matter of concern. Relative to the control, a significant reduction in mycelial growth, in the number of germinating fragments and sprout length was observed for fungal spores incubated for 24 h with 2.5 ppm solution of AgNPs (5–65 nm) [30]. The results are only lab based but signify the importance of AgNPs to hinder spore germination that play a key role in pathogenesis. White rot caused by *Sclerotium cepivorum* is a serious disease of onion (*Allium cepa* L.) and other *Allium* spp., causing significant yield reductions prior to harvest or causing rot in storage. Laboratory and field testing of three different types of nanosilver liquid (WA-CV-WA13B, WA-AT-WB13R, and WA-PR-WB13R) having average particle size of 7–25 nm, found very promising to tackle this menace and provided 90 % inhibition at a concentration of 7 ppm, increased the biomass and dry weights, without affecting the soil microbial population [31]. In a follow-up study, these nanosilver formulations were further tested against eighteen fungal pathogens that cause economically important diseases on crops, vegetables and fruits. Significant inhibition of fungal pathogens was observed at 100 ppm and maximum inhibition of most fungi was recorded with WA-CV-WB13R [32]. AgNPs synthesized using the crude extracts of two brown seaweeds (*Spatoglossum asperum* and *Hedophyllum sessile*) showed extremely high antimicrobial activity against *Xanthomonas axonopodis* pv. *citri, X. oryzae* pv. *oryzae* and *Ustilaginoidea virens* [33]. Moreover, antimicrobial activity of AgNPs has also been reported against an important tree pathogen [34]. AgNPs (4–8 nm) were reported to cause significant growth reduction inhibiting conidial germination of *Raffaelea* sp., that has become a destructive pathogen in Korea, causing oak wilt and damaging both forest and landscape oaks [34]. All these studies suggest the possibility of AgNPs as an alternative to pesticides for the management of a variety of phytopathogenic fungi.

Recently, Ag-based nanocomposites (Ag@dsDNA@GO) were developed using dsDNA as a template for growing AgNPs on graphene oxide (GO), and tested against *X. perfonans*, the cause of one of the most dreadful diseases of tomatoes that leads to reduction in production by 10–50 % [35]. Excellent antibacterial activity was achieved with 20 ppm Ag (18 nm)@dsDNA@GO and 16 ppm Ag (5 nm)@dsDNA@GO composites in vitro after an incubation of only 1 h. More importantly, in a greenhouse experiment, Ag (18 nm) @dsDNA@GO at 100 ppm significantly reduced the disease severity as compared to untreated control and provided similar results to those of the current grower standard treatment (copper + mancozeb), without showing any phytotoxicity [35]. Further, within 15 min of exposure in vitro, Ag-dsDNA-GO (10 µg/mL) killed both copper tolerant and sensitive *X. perforans* strains in suspensions containing approximately 10^3 CFU/mL, whereas copper alone (10, 25, 50 µg/mL) did not reduce bacterial population significantly, as compared to the untreated control even after 24 h of exposure [36]. Interestingly, Ag-dsDNA-GO also showed antibacterial activity against copper-tolerant *X. vesicatoria*, *X. euvesicatoria*, and *X. gardneri* strains. More importantly, significant reduction in disease severity was observed when the plants were treated with Ag-dsDNA-GO (75 or 100 µg/mL) prior to artificial inoculation, as compared to copper-mancozeb and negative controls [36]. Both the studies highlight the possibility of Ag-dsDNA-GO as an efficient alternative to copper in tomato production.

Titanium Dioxide Nanoparticles (TiO_2NPs)

Besides Ag-based nanocomposites [35], TiO_2NPs were also found effective against *X. perfonans* [37]. A significant reduction in the CFUs was observed with nanoscale TiO_2(<8 nm) doped with either Zn or Ag, whereas undoped TiO_2NPs were not found effective within 10 min of light exposure [37]. Moreover, a preliminary greenhouse study herein showed a significant reduction in bacterial spot lesions with all the nanoformulations as compared to control. Interestingly, significant reduction in bacterial spot lesions with nanoscale TiO_2/Ag as compared to TiO_2NPs indicate that doping with Ag improved the bactericidal activity of nanosized TIO_2 [37]. Nanoscale TiO_2/Ag was numerically better than nanoscale TiO_2/Zn but both are statistically similar. It is important to mention here that further testing of nanoscale TiO_2/Zn in greenhouse and growth-chamber experiments significantly reduced disease severity as compared to the control, and no significant difference was observed between control and industry standard copper + mancozeb treatment [37]. Moreover, nanoscale TiO_2/Zn (500–800 ppm) significantly reduced bacterial spot disease incidence as compared with copper, copper + mancozeb and the untreated control in field trials. More importantly, nanoparticle treatments did not cause any undesirable effects on tomato yield in any of the field trials [37]. Results of this study has significant practical implications as the Environmental Protection Agency (EPA) classifies zinc as a minimum-risk pesticide, and application of zinc doped nanoparticles may offer a more practical and reliable choice than Ag [37]. Laboratory and field testing of TIO_2NPs/Zn (7 nm) was also found successful against a new *Xanthomonas* sp., causing bacterial leaf spot of rose [38].

Zinc Oxide Nanoparticles (ZnONPs)

The effect and mode of action of ZnONPs on the growth of plant pathogenic fungi is in the preliminary stage. *B. cinerea,* the causal agent of grey mold, severely affects stored table grapes causing heavy loss while *Penicillium expansum* is the main culprit behind the rotting of stored apples and pears. Unfortunately, both the pathogens are accountable for heavy postharvest fruit loss, even after the application of most superior postharvest technologies [39]. More alarmingly, *B. cinerea* has also developed multiple fungicide resistance due to repeated and incorrect use of fungicides [40]. ZnONPs with size of 70 ± 15 nm significantly suppressed the growth of *B. cinerea* by disturbing cellular functions and causing hyphal deformity, while inhibited conidiophores and conidia development of *P. expansum* that ultimately resulted in the death of fungal hyphae [41]. This is the first study which demonstrated the antifungal nature of ZnONPs against two major post harvest pathogens and suggests the potential role of ZnONPs to minimize postharvest losses, ensuring food safety. Research on ZnONPs has also been focused on remediation, where the use of nanoparticles not only inhibit the target pathogen but also contribute in the breakdown of pesticides. For instance, ZnO-thiram composite antifungal system (0.25 g/L of 20 nm ZnONPs with 0.01 g/L thiram) was demonstrated to inhibit *Phytophthora capsici* growth in a synergistic mode, where thiram enhanced the ZnO-induced oxidative damage [42]. Interestingly, 0.25 g/L ZnONPs completely degraded 0.01 g/L thiram under simulated sunlight irradiation within 6 h [42]. Thiram, a widely used dithiocarbamate fungicide, is known to cause necrotic type death of human skin fibroblasts cells and recently demonstrated to have adverse effect on reproduction and immune functions of birds [43]. In view of this, these kinds of residue-free green synergistic antifungal nanotechnology developments have the potential to manage plant disease economically; more significantly and greatly benefit the human society by minimizing health and environmental burdens.

Copper Nanoparticles (CuNPs)

Cost is one of the most important factors in the selection of protection measures, and copper based fungicides remain inexpensive and efficient relative to modern strobilurin fungicides, viz., azoxystrobin and fenbuconazole, as strobirulins have potential for resistance development [44]. Rotation of these compounds frequently leads to the inclusion of copper in disease management programs. The problems associated with copper fungicides are the adverse effects on entomopathogenic fungi, phytotoxicity, allergic reactions, itching, and eczema. Studies on the effect of CuNPs highlight their potential in managing some important diseases at a relatively low dose. For instance, bacterial blight of pomegranate (*Punica granatum*), caused by *X. axonopodis* pv. *punicae* (*Xap*), has been reported to cause huge economic losses in India. CuNPs was found to suppress *Xap* growth only at 0.2 ppm that is 10,000 times lower than that of commercial copper-oxychloride [45]. In addition, nanocopper treated bacterial cell walls were degraded, unable to colonize the plant tissues and did not produce intense water soaking [45]. Recently, CuNPs (3–10 nm) were found superior than the commercially available fungicide bavistin and demonstrated to produce a significant antifungal effect against *Phoma destructiva, C. lunata, A. alternata* and *F. oxysporum* [46].

Nano Sulphur

Sulfur alone and in combination with lime has incontestably long been the most popular and certainly one of the oldest substances having fungicidal, miticidal and insecticidal properties. But, lime sulfur is not necessarily the best choice in all situations, especially for controlling apple scab because of its high phytotoxicity values, and had a tendency to reduce leaf size and fruit quality [47]. Moreover, as an acaricide, its application resulted in the death of the natural enemies, besides showing severe phytotoxicity symptoms in physic nut [48]. A nanosulphur formulation (IARI nano-S, 50–90 nm) significantly inhibited the conidial germination of *Erysiphe cichoracearum* (Powdery mildew fungi) at 1000 ppm and found superior than commercial sulphur products, viz., sulphur 80WP, merck sulphur and canadian nano-S [49]. Moreover, IARI nano-S was found to cause the shredding of the myceloid appendages and the affected cleistothecia were failed to release ascospores [49]. These results are of great importance as appendages help the cleistothecia to adhere with the host plant and the ascospores are responsible to cause new infections. Similarly, as compared to commercial sulphur, significantly higher bioactivity of nanosulphur (1–100 nm) was reported against *E. cichoracearum* and *Tetranychus urticae* (Red spider mite) [50].

Chitosan Nanoparticles (CNPs)

Chitosan, a natural polymer, is reported to induce systemic resistance in plants, acts as a yield enhancer, and has potential role in induction of cell death and stomatal closing [51]. Bulk chitosan has drawn less attention as an antifungal agent due to its insolubility in aqueous media and lower antifungal activity [52]. However, in recent times, use of chitosan in nanoform has also paved the way for its entry as a successful plant protection agent. Copper–CNPs (196.4 ± 2.2 nm) at 0.1 % concentration has been demonstrated to inhibit the growth of *A. alternata*, *M. phaseolina* and *R. solani* by 89.5, 63.0 and 60.1 %, respectively [52]. In a follow up study, a significant increase in tomato seed germination percentage, seedling length, fresh weight and dry weight was observed with Copper–CNPs (374.3 ± 8.2 nm) as compared to control [53]. Moreover, a significant reduction in mycelial growth and spore germination of *A. solani* and *F. oxysporum* was observed at 0.10 and 0.12 % of Copper–CSNPs. In pot experiments, Copper–CNPs treated plants appeared healthy without showing any Fusarium wilt symptom and showed significantly lower Alternaria blight disease severity that was significantly superior to 0.2 % mancozeb and control [53]. More importantly, a recent study first time demonstrated the relatively high effectiveness of CNPs (90 ± 5 nm) in triggering innate immune plant responses by inducing defense related enzyme, up regulating the genes involved in plant defense including that of several antioxidant enzymes as well as elevating the levels of total phenolics and nitric oxide, even at almost ten times low dose regimens, as compared to that required for natural chitosan, therefore introducing CNPs as a potential candidate for next generation organic cultivation [54].

Insect Pest Management

The development of nanoformulations against insect pests is particularly aimed at minimizing the quantity of AIs, controlled release and protection from degradation (Fig. 2). To this end, a variety of formulations have been proposed for insecticidal compounds. Results of some of the studies are encouraging and may have significant positive impact to fight notorious insect pests.

Nanoemulsions

Nanoemulsions are promising candidates for the delivery of water-insoluble AIs and typically contain 5–10 % of surfactant, as compared to 20 % in microemulsions [14]. An efficient two-step process for the preparation of oil-in-water nanoemulsions at constant temperature (25 °C) was earlier described by Wang et al. [55]. Further, β-cypermethrin (β-CP) loaded nanoemulsions were compared with β-CP microemulsion. It is important to mention here that precipitation of commercial β-CP microemulsion occured in the sprayed solution within 24 h of dilution, whereas excellent stability with no precipitation of sprayed solution diluted from the nanoemulsion was observed [55]. Recently, a promising green oil-in-water nanoemulsion for β-CP delivery was developed using methyl laurate as oil phase, and alkyl polyglycoside (APG) and polyoxyethylene 3-lauryl ether ($C_{12}E_3$) as mixed surfactants [56]. Further, β-CP loaded nanoemulsion was demonstrated to have excellent spreading performance and was found homogeneous after dilution [56]. In both the studies, incorporation of β-CP did not cause any noticeable effect on the size and stability of the nanoemulsions and the solubility; hence the bioavailability of β-CP has increased in these nanoemulsion systems. Besides nanoemulsions, microemulsion of nanosized permethrin (131 ± 9 nm), a pyrethroid pesticide, was found safe to various plant species and soil microbes, as compared to permethrin [57]. It is important to mention here that there is substantial uncertainty about the use of these two terms in scientific literature, but nanoemulsions and microemulsion are distinguished by their thermodynamic stability [58]. Microemulsions are thermodynamically stable, whereas nanoemulsions are thermodynamically unstable [58]. Nanoemulsions are also developed for the efficient delivery of poorly-water soluble/insoluble naturally occurring compounds having insecticidal properties. For instance, nanoemulsion (155.2 ± 3.8 nm) containing apolar fraction from fruits of *Manilkara subsericea* (5 %), octyldodecyl myristate as oil (5 %), sorbitan monooleate/polysorbate 80 as surfactants (5 %) and water (85 %) exhibited significantly higher levels of mortality of cotton pest *Dysdercus peruvianus* [59]. Further, no significant inhibition of acetylcholinesterase (fish origin) and non-toxicity in mice indicated the safe nature of the developed product [59]. Nanoemulsion (43.31 nm) developed using *Simmondsia chinensis* (Jojoba) seed-oil and Tween-20 as a nonionic surfactant and water was found more promising against *Sitophilus oryzae*, as compared to the bulk jojoba oil [60]. Insect mortality bioassays recorded LC_{50} of 0.31 and LC_{90} of 0.66 mL/kg for nanoemulsion, compared to LC_{50} of 3.12 and LC_{90} of 10.16 mL/kg for bulk jojoba oil [60]. The loss of eucalyptus oil by volatilization was stabilized by the addition of aqueous filtrate of de-oiled karanja (*Pongamia glabra*) and jatropha (*Jatropha curcas*) in eucalyptus essential oil nanoemulsion (77 nm) that showed LC_{50} values of 0.1646 mg/L against *Tribolium castaneum*, a secondary pest of stored grains, whereas LC_{50} value of 5.4872 mg/L was recorded without the aqueous filtrate [61]. The sum of all these studies suggest the greater efficacy and better biosafety of nanoemulsions that makes them promising from both environmental and economical points of view.

Polymer-Based Nanoformulations

Recent research is focused exclusively on the controlled release of herbicides and insecticides using polymer-based

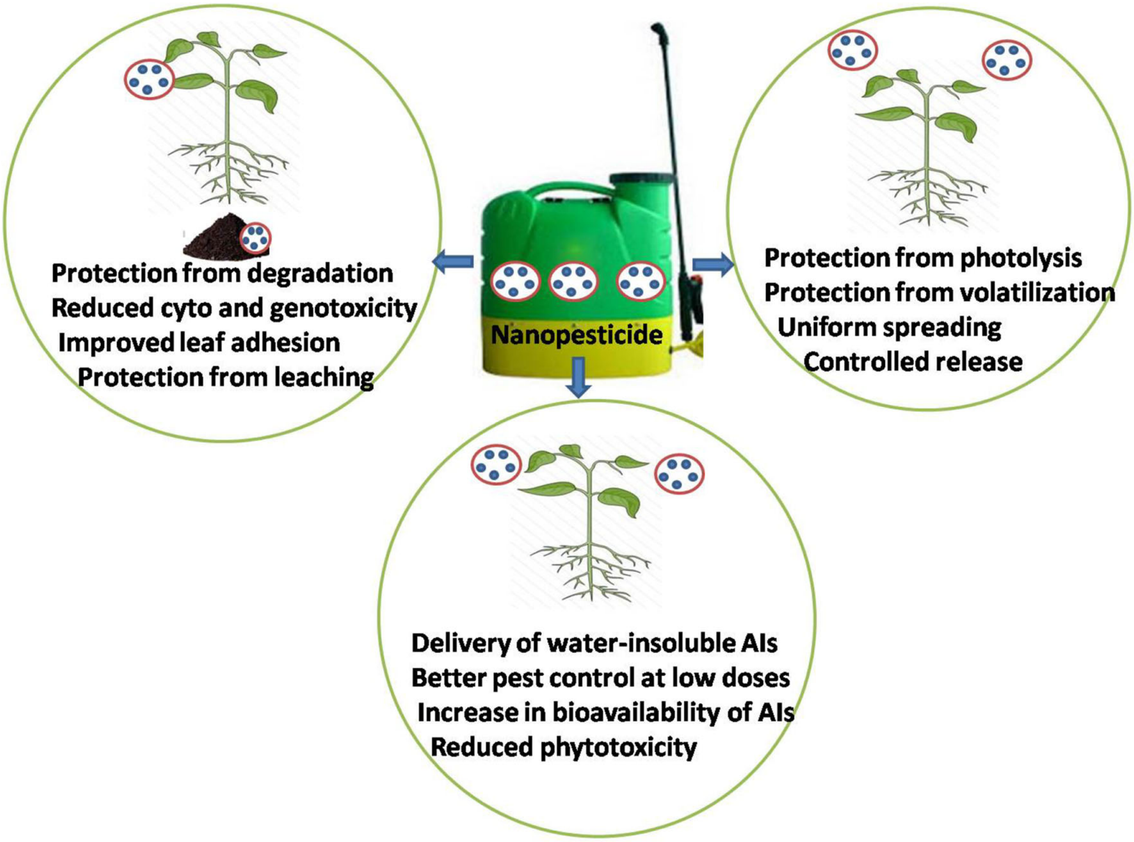

Fig. 2 The potential advantages of nanopesticides in crop protection

nanoformulation. These studies demonstrated the wide range of polymer-based nanosystems for the controlled release and protecting of photo-labile compounds in pest management programmes. In this context, controlled release of azadirachtin-A was achieved by encapsulation in nano-micelles of polyethylene glycol (PEG)-based amphiphilic copolymers and various dimethyl esters [62]. Moreover, an increase in the molecular weight of PEG during polymerization further reduced the release rate of azadirachtin-A, which increased the shelf-life of this photo-liable pesticide [62]. Nano-micelles of PEG-based amphiphilic polymers were also reported for the controlled release of carbofuran, β-cyfluthrin and imidacloprid [63–65]. The developed β-cyfluthrin nanoformulation showed prolonged activity and found more effective against *Callosobruchus maculatus,* as compared to commercial formulation [64]. In all the above studies, the release of AIs in water was significantly slower than the commercial formulations and followed first-order kinetics. In sandy loam soil, encapsulation of thiamethoxam with nano-ranged PEG-based amphiphilic polymers resulted in much slower release than commercial formulation [66]. Higher efficacy and stability of these formulations may be attributed to the high solubilization power and low critical micelle concentration (CMC) of amphiphilic polymers [65]. Nanoacephate (80–120 nm), prepared by encapsulation of acephate in PEG, was found more promising against *Spodoptera litura* and did not induce any cytotoxicity in human fibroblast cell line [67]. Interestingly, PEG-based amphiphilic polymers not only found suitable for the controlled delivery of thiram, a contact fungicide, but also acted as moisture barriers, reduced the seed deterioration rate and checked thiram degradation [68]. These findings can further be explored for the targeted and single application of PEG-based nanoformulations for effective pest control in field conditions.

Chitosan, a biodegradable polymer obtained from the deacetylation of chitin, has attracted considerable interest to achieve effective and controlled release. Nano types of pyrifluquinazon prepared using chitosan as a carrier, showed best lethal efficiency against green peach aphid, *Myzus persicae* at 14 days after treatment, whereas the non-nanoformulation had the best lethal efficiency at 2 days after treatment [69]. Efforts were also made to develop controlled release hybrid nanoformulation using polymer coated liposomes. For instance, controlled release of etofenprox was achieved by encapsulation in chitosan coated lecithin molecules. The developed nanoformulation

(100–800 nm) showed prolonged activity and higher efficacy against *S. litura* than pure etofenprox [70]. Moreover, chitosan-coated beeswax solid lipid nanoparticles (CH-BSLNs) were also found successful in reducing the photolysis of deltamethrin, achieving highest encapsulation efficiency (95 %) and higher payload (approximately 12.5 %) [71]. Further, compared to BSLNs, higher payload (approximately 1.79 times), higher encapsulation efficiency (83.6 %) and higher photo-protection of deltamethrin were achieved by a novel nano-structured lipid carriers (NLC) system with corn oil as liquid lipid [72]. Recently, neem-based nanocapsules prepared using biodegradable polymers, viz., poly-ε-caprolactone (PCL) and poly-β-hydroxybutyrate (PHB), caused higher mortality of *Bemisia tabaci*, a serious pest of many crops [73]. These findings suggest the wide range of novel nanocarrier systems in protecting photo-labile compounds used in crop protection.

Pheromones are highly specific chemical compounds that are used to disrupt the mating and trapping of targeted insect pests. Volatile pheromones are extremely unstable due to their chemical structure; and it is highly desirable to protect them from decomposition and the formulation must ensure a controlled release [74]. Nanofiber webs obtained by incorporating pheromones in Polyamide 6 as well as cellulose acetate polymer carriers via electrospinning was demonstrated to release pheromones in a nearly linear fashion over several weeks [75]. Therefore, nanofiber webs can further be explored to reduce the frequency of pheromone recharging in field conditions.

Nanogels and Nanohybrids

The problems associated with the use of pheromone hydrogels are swelling and shrinking with the changes in temperature and humidity, limits their use under adverse environmental conditions [76]. Pheromone nanogels having the fiber diameters ranging from 500 nm to 1 um was developed by immobilizing methyl eugenol (ME) into nanosized low-molecular mass gelators (LMMGs), i.e. all-*trans* tri (*p*-phenylenevinylene) bis-aldoxime, showed high pheromone retention capacity and enhanced the shelf-life of ME by protection from evaporation. More importantly, under adverse rainy season, this controlled release system showed excellent efficacy in a fruit orchard against *Bactrocera dorsalis*, one of the harmful pests affecting a number of fruits including guava [76]. Recently, zinc-layered hydroxide (ZLH) nanohybrid intercalated with insect pheromone hexenoic acid (HE) was developed using a simple co-precipitation technique [77]. It is important to note here that the controlled released of HE was successfully achieved and the developed formulation did not cause any adverse affect on the germination of wheat and green bean seeds. Interestingly, higher percentage of seed germination and higher radical seed growth was observed with this nanohybrid, as compared to HE alone [77]. These findings are of strong practical significance and can be best utilized against insect pests of various crops.

Nanoherbicides

Weed control becomes one of the biggest challenges in the agriculture sector. Single herbicide use or lasting exposure of weeds with diverse herbicides results in the development of herbicide resistance. In addition, longer persistence time of toxic herbicidal residues can create germination problem for the next crop. The most important aspect in controlling the multiplication of any weed is the destruction of seed bank in soil, to restrict their germination. Nanotechnology provides a solution by developing target specific herbicides, which may enter the roots and get transported in all plant parts. Their interaction with metabolic pathways restricts the glycolysis, thus ultimately causing the death of the target due to starvation [78]. Moreover, due to their tiny size, nanoherbicides are being able to mix easily in soil and eradicate the target species without leaving any contradictory effects [79]. Nanoencapsulation ensures better herbicide application by avoiding phytotoxicity, provides improved penetration through cuticle and tissue, and allows slow and constant release of the active ingredients (Fig. 2) [11]. For instance, alginate/chitosan and chitosan/tripolyphosphate (CS/TPP) nanoparticles (400 nm) as carriers for the combined herbicides (imazapic and imazapyr) showed efficiencies exceeding 60 %, improved their mode of action and reduced their toxicity [80]. Moreover, after 300 min, free imazapic and imazapyr showed release percentages of 55 and 97 %, respectively, while a 30 and 20 % decrease was observed for the nanopreparation of imazapic and imazapyr [80]. An earlier study also demonstrated good encapsulation efficiency (62 %) of CS/TPP nanoparticles (300 nm) for paraquat that was found to be stable for at least 60 days [81]. Moreover, MTT test revealed reduction in the cytotoxicity of encapsulated herbicide on CHO cells, and nanoparticle-bound paraquat showed less chromosome damage on *A. cepa*, as compared to the free herbicide [81]. Similarly, encapsulation percentages of 64–84 % were achieved with nanocapsules of poly (epsilon-caprolactone) containing herbicide atrazine, which offers efficient delivery, as well as improved adhesion of herbicides to the target plants [82]. Additionally, this system was found to be effective for the control of the target species (*Brassica* sp.) where the use of poly (epsilon-caprolactone) nanoparticles reduced the mobility of atrazine in soil, and reduced the genotoxicity that is evident from *A. cepa* chromosome aberration assay [83]. It is also important to note that encapsulation of ametryn and atrazine with poly (epsilon-caprolactone) nanocapsules lower the toxicity to both microalgae *Pseudokirchneriella subcapitata* and human lymphocyte cultures, as compared to the herbicides alone [84]. Moreover, the association efficiencies of ametryn, atrazine, and simazine in poly (epsilon-caprolactone) nanocapsules were better than 84 % and the controlled release was governed mainly by relaxation of the polymer chains [85]. Similarly, solid lipid nanoparticles (SLN) showed good physicochemical stability and high encapsulation efficiencies for atrazine and simazine and reduced their toxicity [86]. These reports are in general agreement that the nanoencapsulation of herbicides provides a valuable means of weed control in agriculture while minimizing human and environment burdens. Investigations aimed to determine the environmental fate of nanoherbicides is still scare. To determine the fate of atrazine nanoformulation in soil, pioneering study by applying regulatory protocols defined in the OECD guidelines indicates quick discharge of atrazine from the polymer nanocarriers relative to the degradation kinetics, and atrazine coupled with the nanocarriers was subject to biotic or abiotic degradation [87].

Nanofertilizers

Fertilizers play foremost role to boost the production across the spectrum of crops. The nutrient use efficiencies (NUE) of nitrogen (N), phosphorus (P), and potassium (K) had remained constant for the past several decades and hardly exceed 30–35, 18–20 and 35–40 %, respectively [88]. Improvement of NUE is must required to enhance the crop production in marginal lands with low nutrient availability. But, little success has been achieved to increase the NUE in conventional fertilizer formulations so far [89]. Fortunately, the emerging nanofertilizer based approaches are predicted to be more proficient than even polymer-coated conventional slow-release fertilizers, due to their high surface area to volume ratio [90]. Nanofertilizers are expected to improve the NUE by preventing the nutrient ions from either getting fixed or lost in the environment [91]. These smart delivery systems of encapsulated nanofertilizers restrict the interaction of nutrients with soil, water and microorganisms, and release nutrients after their internalization in the plant system [90].

Nitrogen fertilizers are one of the most important crop boosters, when judged with regard to the energy required for their synthesis, tonnage used and monetary value. However, as compared with amounts of N applied to soil, the nitrogen use efficiency by crops is very low [90]. Urea is one of the commercially available, fast-release N fertilizers, which is easy to use in agricultural fields. Global urea demand is anticipated to reach 120 Mt by the year 2018–2019. Efforts to reduce the release rate of urea can increase its efficiency of use and further prevent groundwater contamination. Given the context, a novel urea slow-release nanocomposite, based on urea intercalation into montmorillonite clay showed slow-release behavior for urea dissolution, even in low montmorillonite amounts (20 % in weight) [92]. Similarly, urea blended with nanoporous zeolite (1:1) and conventional zeolite (1:1) was shown to release N up to 48 and 34 days respectively, while N release from conventional urea was found to cease within 4 days under ambient conditions [93]. In a follow up study using two different soil types (inceptisol and alfisols) and maize as a model crop, the grain N content of nanozeolite + urea on both the soils were found to be consistently higher than conventional urea but the response was more pronounced in alfisol than inceptisol [94]. An early burst followed by a slow-release even on the 60th day was observed in a urea-modified hydroxyapatite nanoformulation encapsulated under pressure into cavities of the soft wood of *Gliricidia sepium*, while commercial fertilizer showed a heavily early burst and subsequent release of low and non-uniform quantities until around day 30 [89]. The major advantages of using slow-release nanoformulation of fertilizer is improved NUE efficiency, higher crop yield, less environmental burdens from leaching of N, as compared to the conventional water-soluble fertilizers [89].

Surface water eutrophication is the major problem associated with phosphate salts that are heavily used in agriculture. Synthetic apatite nanoparticles (15.8 ± 7.4) developed using a one-step wet chemical method, demonstrated to increase the growth rate and seed yield of soybean by 32.6 and 20.4 %, respectively, as compared to ($Ca(H_2PO_4)_2$), a regular P fertilizer [95]. In addition, above ground and below ground biomass production was enhanced by 18.2 and 41.2 %, respectively. Apatite nanoparticles may act as a novel class of P fertilizer that can potentially enhance crops' yields and biomass production, while minimizing risks of water eutrophication [95]. Recent research also highlighted the importance of the nanofertilizers in improving yield. Cucumber leaves sprayed with nanofertilizers (nanonat and ferbanat) significantly improved the yield as compared to control [96]. Similarly, iron (Fe), P and K nanofertilizers were demonstrated to significantly increase yield, flower number, fresh stigma weight, dry stigma weight, stigma length, fresh flower weight and dry flower weight of saffron [97]. Application of Fe nano-oxide solution as a foliar spray in wheat was found to be promising, as highest values of spike weight, 1000 grain weight, biologic yield, grain yield and protein content were achieved [98]. Iron deficiency is a widespread problem, mainly in high pH and calcareous soils; therefore, combining Fe compounds with nanotechnology may be a solution to combat Fe deficiency. Moreover, besides enhancing growth parameters, foliar applications of nanofertilizers (ZnO and Fe_3O_4 NPs-containing Hoagland solution) alleviate salt stress in *Moringa peregrina* [99]. Similarly, a significant increase in rice grain yield and nitrogen use efficiency was observed after applying slow-released fertilizer added nanosized carbon. For instance, in comparison to Jingzhengda (JSCU), a slow-released fertilizer having 42 % N, the total N concentration in surface water of paddy soil under JSCU+ nanocarbon was declined by 31.0 %, and the period of N runoff loss due to rainfall was shortened by 2.2 days. Similarly, treatment with Stanley slow-released compound fertilizer (SSRF, N-P_2O_5-K_2O = 20:9:11) + nanocarbon, the average total N concentration was decreased by 29.8 % and the time of N runoff loss was shortened by 1.8 day [100]. Recently, nanocomposites based on polyacrylamide (PAAm), methyl cellulose (MC), and calcic montmorillonite (MMt) hydrogels showed excellent controlled desorption of urea and demonstrated to release higher amounts of nutrient that is almost 200 times slower than pure urea [101]. Moreover, nitrous oxide (N_2O) emissions in the field were reduced substantially for urea nanocomposites associated with an exfoliated clay mineral prepared using PAAm hydrogel or polycaprolactone [102]. Comparison in the release profiles of a urea based controlled release fertilizer indicated that 100 % release of urea takes place in the first 5 h, 100 % of urea was released in 97 h, and 87 % was released in 150 h from pure urea, urea intercalated kaolinite nanocomposite, and the encapsulated nanocomposite, respectively [103]. Moreover, swelling and release of urea was dependent on the type and concentration of salt solution added to the medium, pH levels of the solutions, and temperature, where the release of urea was predominated by a Case II release mechanism with skeleton erosion [104]. A novel nanocomposite based on urea–formaldehyde in the presence of MMt may act as a new controlled release system for N fertilizers, where nanocomposites offered excellent mechanical resistance and urea release was evidently controlled by the extent of polymerization [105]. These results are of strong practical significance as showing the efficacy of novel nanosomposites for slow-release of fertilizers that increases the nutrient use efficiency and yield, whereas reduces pollution.

Risks of Nanoparticles

Nanoparticles have raised concern of environmental risks due to their widespread applications. Despite growing scientific facts of possible hazards, hundreds of thousands of tones of nanomaterials are already being released into the environment. The most reliable data on environmental contamination indicates that 260,000–309,000 metric tonnes of global engineered nanomaterials (ENMs) produced during 2010, ended up in landfills (63–91 %), soils (8–28 %), water bodies (0.4–7 %), and the atmosphere (0.1–1.5 %) [106]. Ecotoxicology and environmental safety issues focused on the interactions of nanoparticles in different environmental systems, including their bioavailability, assimilation in beneficial organisms, damage mechanisms, biological responses of non-target organisms and subsequent fate is a serious issue to be addressed. The following section will shed light on the recent studies in determining the effect of nanoparticles in different systems.

Effects on Beneficial Soil Microorganisms

Microbial population in the rhizosphere may play a key role in plant reaction to nanoparticles, thus must be taken into consideration in effect assessment. For instance, a significant increase in the biomass of maize plants was observed with the application of 100 mg/kg of AgNPs (20 nm), which could be correlated with a significant alteration in rhizosphere associated bacterial community that significantly altered carbon use and community composition profiles [107]. Comparatively, fungal communities were less susceptible, and the bulk and AgNPs treated rhizospheric soil were significantly different with respect to the observed microbial changes [107]. By contrast, application of 350 and 790 mg/kg AgNPs (20 nm) in soil significantly reduced lateral and fine root development and shoot biomass of pine after 4 months [108]. Further, at highest concentration, no ectomycorrhizal fungi (EMF) was found to be associated with AgNPs contaminated roots, whereas 5 EMF genera were observed in control [108]. Similarly, application of 10 nm AgNPs (1 and 3 mg Ag/L) in sand was found toxic to beneficial soil bacterium, *Pseudomonas chlororaphis* O6, as evident from the loss in bacterial culturability, whereas no cell death was observed in loam soil. Interestingly, addition of humic acid in AgNPs amended sand maintained the culturability [109]. The high toxicity of AgNPs was also demonstrated on arctic soil microbial community, where *Bradyrhizobium canariense*, a community-identified plant-associating bacterium showed noticeable sensitivity to AgNPs [110]. Moreover, mixtures of nanoparticles (Ag, copper, and silica) even at very small concentrations can disrupt microbial communities [111] and some AgNPs can act as a continuous source of bioaccessible Ag fraction over time [112]. The toxicity of nanoparticles depends on the morphology and surface properties of nanoparticles. For example, the first comparative study on the bactericidal properties of AgNPs on *Escherichia coli* indicates shape dependent biocidal action as truncated triangular Ag nanoparticles with a {111} lattice plane as the basal plane were found to be more biocidal, compared to spherical and rod-shaped nanoparticles [113]. Moreover, the toxicity of four AgNPs representing various surface charging scenarios suggest that surface charge is one of the most important factors that govern the toxicity [114]. Thus, risk evaluation for each nanosilver product should consider the physical, chemical, and possibly toxic characteristics unique to that product [115]. Therefore, there is an urgent need to evaluate Ag-based nanomaterials on a case-by-case basis. Bar-coded pyrosequencing, to explore the responses of diverse bacterial taxa to TIO_2NPs (15–20 nm) and ZnONPs (20–30 nm) in incubated soil microcosms indicates the susceptibility of symbiotic nitrogen-fixing bacteria under the order Rhizobiales, the family Bradyrhizobiaceae, and the genus *Bradyrhizobium* [116]. Moreover, members of the family Methylobacteriaceae, which contain methanotrophs, that metabolize methane as their only source of carbon and energy, declined by both the nanoparticles [116]. Therefore, application of these nanoparticles as agrochemicals may have serious consequences by hampering symbiotic nitrogen-fixation in major legume crops and preventing methane emissions from soil. In addition, maximum toxicity of ZnONPs on soil microorganisms was observed in acidic soil, followed by the neutral soil. Relatively, the toxicity of ZnONPs was less in alkaline soil [117]. Nanoparticles of Ag, CuO and ZnO showed toxicity to *P. putida* KT2440, a beneficial environmental microbe, suggesting that the NP ($\leq$1 mg Ag/L, $\approx$10 mg Cu, Zn/L) application may put negative impact on soil microbial processes [118]. Effects of CuONPs and AgNPs were accompanied by cell death whereas ZnONPs were bacteriostatic. Further, bulk materials of these nanoparticles lacked inhibitory activity suggesting the aggregation of nanoparticles into larger particles may reduce their non-target effects [118]. Therefore, studies on the soil environment that can promote aggregation of nanoparticles may lessen their toxic activity. In contrast to the above mentioned studies showing the harmful effect of metal based nanoparticles on soil beneficial organisms, nanoformulations of commercial fungicide hexaconazole showed no adverse effect. For instance, nitrogen-fixation, nitrogen-assimilation, indole acetic acid (IAA) production and phosphate solubilization of nitrogen-fixing blue green algae (BGA) and bacteria was found to be significantly inhibited in the presence of commercial hexaconazole, whereas stimulatory to slightly inhibitory effect on the growth measurable parameters of the organisms were observed with nanohexaconazole [119]. Nanohexaconazole was also found to be more stable, causing no adverse significant effect on soil nitrifiers and was found superior in comparison to conventional hexaconazole [120].

All these findings suggest a complex interaction of nanoparticles with soil organisms where different soil types, soil factors and nanoparticle size and type may play significant roles in determining the environmental fate of nanoparticles. Beneficial microbial populations are one of the most important key factors that influence soil ecosystem processes, especially by decomposing soil organic matter, nutrient recycling, disease suppression and growth enhancement etc. Therefore, any factor that induces significant negative effects on soil microbial populations would certainly deteriorate soil quality and sustainability. Thus, intentional application of nanoparticles, particularly in crop protection is a prime concern and identification of safe nanoparticles, procedures for their safe application, follow up mitigation and remediation strategies, if found hazardous, should be a priority. Estimation of beneficial microbial communities in soil must be considered as a crucial factor while investigating the impacts of nanoparticle exposure in soil. Evidences also support the inhibitory effect of AgNPs on soil exoenzyme activities that are crucial to support the soil biochemical processes [121].

Effects on Terrestrial Ecosystem

Sophisticated exposure models and ecotoxicological literature explain that AgNPs concentration below the current and future predicted environmental concentrations (PECs) in diverse ecological compartments can affect prokaryotes, invertebrates and fish, is an indicative of noteworthy environmental hazards [122]. In terrestrial ecosystems, earthworms are considered as soil engineers, dominating invertebrate biomass, and good indicators of soil health. The first ecotoxicological life history trait data on earthworms using the limit-test design for Ag, Cu, Ni, Al_2O_3, SiO_2, TiO_2 and ZnO_2 nanoparticles and their corresponding metal salt confirmed the toxicity of AgNPs, CuNPs and TiO_2NPs, and total reproductive failure was observed with both the Ag treatments [123]. The first evidence on the capacity of earthworms (*Eisenia fetida*) to sense and consistently avoid soils containing AgNPs suggests that nanoparticle-organism interactions may be unpredictable and may result in ecologically significant effects on the behavior at environmentally relevant concentrations [124]. Moreover, significant decrease in the reproduction of *E. fetida* was observed with $AgNO_3$ (94.21 mg/kg), AgNPs coated with oleic acid (50.60 + 1.02, 727.6 mg/kg), and AgNPs coated with polyvinylpyrrolidone (56.35 +1.16 nm, 773.3 mg/kg), where more Ag accumulating was observed with $AgNO_3$ as compared to earthworms exposed to equivalent concentrations of AgNPs [125]. Toxicity mechanisms are still poorly characterized, apparently in some cases nanoscale specific properties may cause significant bio-uptake and toxicity than that caused by Ag ions [122]. For instance, reduced bioavailability and toxicity of ionic Ag with time, whereas increase in toxicity of AgNPs (50–80 nm) with time suggests that long term soil aging experiments are required to properly assess the environmental risk of

AgNPs [126]. Similarly, AuNPs (20 nm) were more bioavailable and demonstrated to be taken up by *E. fetida* from soil and distributed among tissues that may cause undesirable effects on reproduction and have the potential to enter terrestrial food webs [127]. Moreover, trophic transfer of AuNPs from soil along a simulated terrestrial food chain using earthworms (*E. fetida*) and juvenile bullfrogs (*Rana catesbeiana*) indicates the efficient transfer and more bioavailability of nanoparticles to higher order consumers through trophic exposure, as compared to direct exposure [128]. Study conducted on the effect of CuNPs on *E. fetida* suggests that oxidized CuNPs may enter the food chain but earthworms are adversely affected only at relatively high concentrations (>65 mg Cu/kg soil) [129]. Study on the exposure of earthworms (*E. andrei* and *E. fetida*) to TIO_2NPs showed the safe nature of TIO_2NPs [130]. Juvenile survival and growth, adult survival, cocoon production, cocoon viability, or total number of juveniles hatched from cocoons was not significantly affected in field and artificial soil containing between 200 and 10,000 mg TIO_2NPs per kilogram of dry soil. However, earthworms avoided nano-TiO_2 amended artificial soils indicate the capability of earthworms to detect TIO_2NPs in soil [130]. Following a 14 day exposure on filter paper test, ZnONPs showed acute toxicity to *E. fetida*, while TIO_2NPs was found safe [131]. In contrast, both metal oxide nanoparticles were found safe without showing any acute toxicity in sand. In particular, ZnONPs showed greater toxicity than TIO_2NPs in *E. fetida* [131]. In contrast to the safe nature of TIO_2NPs [131], ^1H NMR-based metabolomics provides evidences of significant changes in the metabolic profile, and oxidative stress in *E. fetida*, when exposed to TiO_2 nanomaterial (5 nm) [132]. Hundred percent mortality after 96 h exposure to the highest concentration (1000 mg ZnONPs/kg agar) and highest mortality at the lowest exposure concentration (50 mg ZnO/L) were observed in earthworms exposed in agar and filter paper assay, respectively. Interestingly, significant reduction in the toxicity of ZnONPs on filter paper by applying soil extracts instead of deionized water (DW) to simulate a realistic exposure system, makes filter paper toxicity tests a good choice for the environmental risk assessment of nanoparticles [133]. Reproduction of *E. veneta* was found to be declined by 50 % when exposed to ZnONPs (<100 nm), but was almost totally inhibited by $ZnCl_2$ [134]. Further, ZnONPs did not cause any adverse effect on immune activity, but that was suppressed by 20 % when exposed to $ZnCl_2$ [134]. Based on the above mentioned studies, it is obvious that risk assessment approaches to test individual nanoparticles are required to separate the beneficial from that of hazardous. Moreover, targeted assays that can precisely sense damage at cellular or molecular level are required.

Phytotoxicity

Environmental impacts of nanoparticles can be understood, taking into consideration their toxic effects in different plant systems. The phytotoxicity of TiO_2NPs (27 nm) and AgNPs (10–15 nm) was compared. A significant decrease in the root elongation was observed with AgNPs even at the lowest (50 mg/L) concentrations, while TiO_2NPs showed no phytotoxicity on tomatoes (*Lycopersicon esculentum*) [135]. Both nanoforms were taken up by the plants and deposited in the stems, leaves and fruits. Moreover, lower chlorophyll contents, higher superoxide dismutase activity and less fruit productivity was observed with AgNPs, whereas TiO_2NPs only resulted in higher superoxide dismutase activity at the highest concentration (5000 mg/kg) in green house trials [135]. By contrast, rice seed germination and seedlings growth decreased with an increase in size and concentrations of AgNPs. Smaller AgNPs (20 nm) was taken in higher amounts, showed less negative effects and only trapped in the roots, while deformity in the leaf cells was observed when seeds were treated with 150 nm AgNPs at the concentration of 10 or 100 mg/L [136]. Similarly, AgNPs treatments up to 30 µg/mL accelerated root growth but 60 µg/mL restricted the root growth in rice [137]. Transmission electron microscopy (TEM) revealed the penetration of AgNPs through plant cell wall and damaging effect on cell morphology and its structural features. Moreover, AgNPs caused a significant decline in total soluble carbohydrates in rice roots and damaged rhizosphere associated bacteria by the leakage of reducing sugars and protein through the bacterial membrane [137]. In contrast to the non-phytotoxic effect of TiO_2NPs (27 nm) in tomatoes [135], TiO_2NPs (30 nm) inhibited root hydraulic conductivity, leaf growth and transpiration in maize seedlings [138]. Interestingly, TiO_2NPs (42.8 nm) did not produce any significant effect on plant growth and nutrient content in maize and soybean [139]. Although, arbuscular mycorrhizal fungal (AMF) communities were affected but the composition of rhizosphere associated bacterial communities was not influenced [139]. Fascinatingly, TiO_2NPs (29 ± 9 nm), multi-walled carbon nano tubes (MWCNTs, 20–30 nm) and CeO_2 NPs (>50 nm) did not produce any negative effect on red clover plant biomass and AMF root colonization [140]. Further, as compared to control, 8 % increase in the nitrogen-fixation levels and decrease in the number of flowers was observed with 3000 and 3 mg/kg MWCNTs treatments, respectively [140]. By contrast, soil supplemented with MWCNTs (125–250 nm) induced 2 times more flowers and fruit in tomato, as compared to plant grown in normal soil [141]. Further, Raman spectroscopy confirmed the presence of MWCNTs in flowers, and comparative metagenomic analysis of microbial communities revealed an increase in the relative abundance of *Bacteroidetes* and *Firmicutes*, whereas a sharp decline in *Proteobacteria* and *Verrucomicrobia* communities was observed with increasing concentration of MWCNTs [141]. As compared to control, ZnONPs (10 nm) did not cause any noticeable effect in the stem but significantly increased root elongation in peas (*Pisum sativum* L.). Interestingly, at a concentration of 500 mg/kg, H_2O_2 in the leaves increased by 61 % with a twofold lipid peroxidation, wheras bulk treatments showed no effect in H_2O_2 and lipid peroxidation [142]. By contrast, root elongation was significantly inhibited by CuONPs (95.73 % for maize and 97.28 % for rice) and ZnONPs (50.45 % for maize and 66.75 % for rice) at a concentration of 2000 mg/L [143]. Further, higher toxicity in both maize and rice was observed with ZnONPs (<50 nm), as compared to ZnO microparticles (<5 µm). No negative effects were observed in the corresponding solutions (Cu^{++} and Zn^{++}), suggesting the phytotoxic nature of nanoparticles alone [143]. Similarly, CuONPs exposure to *Brassica juncea* L. resulted in shoot-growth suppression, reduction in total chlorophyll, carotenoids contents and shortening of primary and lateral roots [144]. Application of foliar spray of Fe nanoparticles was not found to induce any toxic effect on *Physcomitrella patens* (bryophyte) as reactive oxygen species (ROS) production, malondialdehyde (MDA) production and glutathione regulation were not disturbed significantly [145]. Similarly, no significant phytotoxicity was observed in macrophytes, viz., *Lepidium sativum*, *Sinapis alba* and *Sorghum saccharatum* for nanosized zerovalent iron (nZVI) tested at 2340 and 33,560 mg/L [146]. Interestingly, seedling length and biomass production was increased at the highest concentrations tested [146]. By contrast, no visible toxic effects were observed on rice seedlings growth with freshly added nZVI (250 mg/kg), whereas noticeable toxic symptoms were observed at the highest concentration (1000 mg/kg) tested [147]. Surprisingly, nZVI toxicity was reduced after ageing with 2 and 4 weeks in soils as compared to fresh nZVI [147]. It is therefore clear that both positive and negative effects have been reported about the same metal/metal oxide nanoparticles in different plant species. Further, some studies differed in their findings about the effect of same nanoparticles on similar plant species. These contradictory findings may be due to the differences in the concentration, size and methods of preparation of nanoparticles used by different workers. Further, different plant species may also differ in their interactions with nanoparticles. For instance, 4–10 times lower Cu uptake was observed in two salt marsh plants, viz., *Halimione portulacoides* and *Phragmites australis*, after exposure of CuNPs. Interestingly, *P. australis* was able to translocate ionic Cu as well as CuNPs but *H. portulacoides* failed to translocate CuNPs [148]. Similarly, a high and extended exposure of TiO_2NPs (5 mg/L for 14 days), did not cause any adverse effect on the growth rate and chlorophyll a content of *Lemna minor*, even TiO_2NPs attached on the cell walls, but no cellular uptake was observed [149].These findings are of great importance and shed light on the effective utilization of appropriate plant species for phytoremediation purposes.

Human Toxicity

One of the most critical issues for the acceptability of nanopesticides is to ensure that the nano-AIs are tested in the same way humans would be exposed in the real world. The chances of pulmonary and dermal exposure to nanoparticles are more; therefore it is essential to evaluate toxicity in dermal and pulmonary cells. In view of this, two cytotoxic assays (MTT and Neutral Red) performed in two independent laboratories to test the toxicity of 24 nanaoparticles on two human pulmonary cell lines, namely A549 and THP-1. Different copper based nanorticles with size ranging from a minimum of 22.9 nm to a maximum of 94 nm and ZnONPs (53.6 nm) showed most toxicity in MTT assays, which was found to be a reliable and sensitive method to test the toxicity on THP-1 cells [150]. MTT assay was also found reliable to test the toxicity of AgNPs on four different mammalian cell lines. In comparison to Ag ions, the toxicity of AgNPs (70 ± 5 nm) for PC-12 cells and NIH-3T3 cells was 5 and 18 times higher, respectively. Further, electric-cell-substrate-impedance-sensing (ECIS), an automated method to monitor cellular behavior in real-time confirmed that AgNPs confer toxicity with an immediate effect [151]. These findings unveil the sensitivity difference between cell types and cytotoxicity assays that has to be taken very carefully while assessing nanoparticle toxicity. Recent findings on the toxicity of AgNPs on human cell lines revealed size dependent toxicity of AgNPs that could be related to oxidative stress. For instance, testing of AgNPs (47 and 42 nm) and AuNPs (30, 50 and 90 nm) in human pulmonary fibroblasts (HPF) revealed the more toxic nature of 4.7 nm AgNPs than the large AgNPs and AuNPs, whereas similar cytotoxicity was observed for all the AuNPs. Generation of oxidative stress was evident from a significant increase in ROS production, reduction of glutathione level and slight inactivation of superoxide dismutase [152]. Moreover, AgNPs of the same size (4.7 nm) were also found to cause more toxicity than the large AgNPs (42 nm) in human dermal fibroblasts and generated oxidative stress [153]. Interestingly, both AgNPs sizes induced oxidative stress related genotoxicity (DNA damage) in 4 human cell lines, where 4.7 nm AgNPs was more genotoxic and pulmonary fibroblasts showed the highest sensitivity [154]. Inductively coupled plasma mass spectrometry (ICP-MS) revealed that smaller AgNPs (5 nm) enter cells more easily in A549, SGC-7901, HepG2 and MCF-7 cell lines than larger ones, which may be the reason of higher toxicity [155]. Surprisingly, instead of any oxidative stress, AgNPs (20.4 nm) were found to induce mitochondrial injury related cytotoxicity in human liver HepG2 cells, which showed more sensitivity than human colon Caco2 cells [156]. Perhaps, these differences in the toxicity mechanisms may be due to the differential behavior of different cell types that may play an important role in toxicity mechanisms. Besides these variations in cytotoxicity, consensus on the driving force behind the toxicity of AgNPs is still lacking. For instance, toxicity of AgNPs on A549 lung cells was found to be dependent on the Ag ion fraction. AgNPs suspension was found to be more toxic than its supernatant at low Ag ion fraction ($\leq$2.6 %), whereas significant additional toxicity was not observed at high Ag ion fraction ($\geq$5.5 %) [157]. Interestingly, cellular transcriptome analysis of the same cell line revealed alteration in the regulation of more than 1000 genes in response to AgNPs exposure for 24 h, whereas only 133 genes responded to Ag ions [158]. Further, comparison of AgNPs and CuONPs with their metal ions ($CuCl_2$ and $AgNO_3$) revealed the toxicity of CuONPs and $AgNO_3$, whereas AgNPs and $CuCl_2$ did not produce any toxicity on human lung cell lines A549 and BEAS-2B. Non-toxicity of AgNPs was explained due to low release of Ag ions within short time periods [159]. Moreover, exposure of the same cell line (BEAS-2B cells) to different sized (10, 40 and 75 nm) and surface coated AgNPs showed cytotoxicity of only 10 nm particles that is independent of surface coating and associated with the intracellular Ag release rate, an emerging evidence of ‘Trojan horse’ effect [160]. Furthermore, AgNPs (10 nm) had a greater ability to induce cyctotoxic effects in the MC3T3-E1 cells than the larger sized (50 and 100 nm) AgNPs [161]. AgNPs, mainly due to their antimicrobial properties, extensively used in health industry, food storage, textile coatings and a number of environmental applications and manufactured at a rate of 320 tons each year [162]. Moreover, out of 1814 nanoproducts listed in consumer products inventory (CPI), AgNPs are the most popular advertised nanomaterial, present in 438 products (24 %) [163]. At present, AgNPs are emerging as a viable option for the management of many plant pathogens and should be used with caution. Most of the studies demonstrated that AgNPs-induced cytotoxic effects are mainly size-dependent, and thus the particle size needs careful consideration while developing AgNPs based nanopesticides.

TiO_2NPs have increasingly been used in pharmaceuticals and cosmetics due to the unique properties derived from their small size. Interestingly, nanosized TiO_2 and ZnO are used in cosmetics or sunscreens and regarded safe for human [164]. Unfortunately, based on sufficient evidence in experimental animals and inadequate evidence from epidemiological studies, a report by the International Agency for Research on Cancer (IARC) of the World Health Organization declared TiO_2 as a possible Group 2B carcinogenic to humans [165]. Recent studies indicate the dysfunctioning of central nervous system (CNS), ROS production induced genotoxicity and cell damage by TiO_2NPs. For instance, TiO_2NPs (anatase isoform), even at a lower concentration ($\geq$0.1 μg/mL) produced cytotoxicity not only after acute but even after prolonged exposure in two CNS lines, namely human neuronal (SH-SY5Y) and human glial (D384) [166]. The underlying mechanism of strong cytotoxic effect could be related to a constitutive actin reorganization-dependent endocytic uptake and internalization of TiO_2NPs by glial cells [167]. Because cancer is linked with mutation, large genotoxicity data has been generated using standard genotoxicity assays, indicate oxidative stress mediated DNA damage [168]. Although in case of AgNPs, several studies have revealed that nanoparticle size is a critical factor which can influence the toxicity, less and contradictory data is available for TiO_2-NPs. For instance, study on lung adenocarcinoma epithelial cell line (A549) indicates that smaller TiO_2NPs were easily incorporated into the cells and cause phosphorylation of histone H2AX (γ-H2AX) that is regarded as a sensitive marker for genotoxicity [169]. By contrast, a recent study revealed that the genotoxicity is independent of TiO_2NPs size. Two different sized TiO_2NPs (21 and 50 nm) produced statistically significant but similar genotoxic effects in human embryonic kidney cell line (HEK293) only at a higher dose (1000 μg/mL) [170]. Perhaps, this may be due to the difference in sensitivity of different cell types to TiO_2NPs. Fortunately, nearly all tests for measuring the mutagenicity of TiO_2NPs were found negative in different organisms including humans [168]. Moreover, no significant absorption of TiO_2 in human gut was observed after an oral dose, regardless of particle size [171]. Although the toxicity of TiO_2NPs has been demonstrated by in vivo and in vitro studies, but the conclusions are not comparable and even some of them might be conflicting due to differences in the experimental parameters used by different workers [172]. Moreover, little is known about the interaction of TiO_2NPs with CNS yet. TIO_2NPs are used in many commercial products and the potential for human exposure is increasing. Therefore, investigations dealing with the effects of nanoparticles on the CNS with special regard to TIO_2NPs are urgently needed.

There is still insufficient information regarding the particle size related toxicity of ZnONPs, including their transportation in cells and interaction with cell membrane and cell organelles [173]. ZnONPS induces oxidative stress, decreases viability, and increases cell death in Caco2 cells. The cytotoxicity of ZnONPs was found to be time and dose dependent, where 26 nm ZnONPs showed highest toxicity on Caco2 cells, and ZNONPs of 62 nm appeared less toxic than ZnONPS of 90 nm in diameter [173].Similarly, 50 nm ZnONPs showed toxicity in human lung epithelial cells (L-132) at approximately 25 µg/mL concentration, possibly through oxidative stress-induced apoptosis [174]. After 4 h of exposure, ZnO NPs (30 + 5 nm, ≥0.01 µg/mL) significantly inhibited proliferation and induced substantial apoptosis in human pulmonary adenocarcinoma cell line (LTEP-a-2) [175]. Further, an increase in intracellular ROS level up to 30–40 % coincided with approximately 70–80 % depletion of GSH content in LTEP-a-2 cells, indicating that ZnONPs induced apoptosis mainly through increased ROS production [175]. Therefore, with increasing evidences of ZnONPs mediated toxicity, more detailed investigations in the underlying toxicity mechanisms are required.

Conclusion

Global pesticide demand is predicted to increase significantly in the years to come. The application of nanopesticides and nanofertilizers in agriculture aims in particular to reduce pesticide applications and enhance yields through pest suppression and optimized nutrient management. Nanoparticles of ZnO, TiO_2 and especially Ag are intentionally used in many applications, and recent studies also highlighted their potential in plant disease management. Moreover, nanoherbicides and nanofertilizers have been demonstrated to have a great future in the coming years, but a vast knowledge regarding their potential risk is required before products are brought to market. Scientific studies have established toxicity of some nanoparticles to different environments including humans; therefore there should be a strict regulation for the manufacturers to correctly represent the composition and the characteristics of their developed nanopesticide formulation. Current scientific knowledge does not offer a trustworthy measurement to be made of the associated benefits and risk of nanopesticides [14] and lack of satisfactory information and low adequacy of experimental protocols impede inclusive risk assessment. In view of this, quantitative structure–toxicity relationship (QSTR) models for prediction of cytotoxicity of metal oxide nanoparticles are developed by periodic table-based descriptors that can powerfully encode cytotoxicity of metal oxides leading to models with high statistical quality as well as interpretability. Further, the information required for descriptor calculation is independent of nanoparticles size, thus nullifying a noteworthy dilemma that various physical properties of nanoparticles change for different size ranges [176]. Recently, nano quantitative toxicity–toxicity relationship (nano-QTTR) models for interspecies cytotoxicity correlation are developed that can be employed for the extrapolation of the cytotoxicity data of one species to another [177]. Moreover, surface-enhanced Raman spectroscopy (SERS) based on 1, 2-di (4-pyridyl) ethylene (BPE), crystal violet and ferric dimethyl-dithiocarbamate (ferbam) indicator molecules were found promising in rapid detection of AgNPs in environmental and biological samples. It is important to note that ferbam showed the maximum ability to bind AgNPs and was demonstrated to detect a low as 0.1 mg/L AgNPs in genuine surface water and 0.57 mg/L in spinach juice [178]. The knowledge on the behavior of nanomaterials in different environment is rapidly evolving, but is still limited by the lack of robust and integrative research. Collaborative research among different laboratories on the effect of a particular nanoparticle type on different biological systems can make significant impact for developing safer nanopesticides. Each biological system provides a unique setting to examine the fate of nanoparticles, therefore combining the understanding on the behavior of nanoparticles across diverse ecosystem is a critical step in determining the safety of mankind in future.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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