Perspective

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Metal nanoparticles against fungicide resistance: alternatives or partners?

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Abstract

Chemical control suffers from the loss of available conventional active ingredients due to strict environmental safety regulations which, combined with the loss of fungicide efficacy due to resistance development, constitute major problems of contemporary crop protection. Metal-containing nanoparticles (MNPs) appear to have all the credentials to be next-generation, eco-compatible fungicide alternatives and a valuable anti-resistance management tool. Could the introduction of MNPs as nano-fungicides be the answer to both reducing the environmental footprint of xenobiotics and dealing with fungicide resistance? The potential of MNPs to be utilized as nano-fungicides, both as alternatives to conventional fungicides or/and as partners in combating fungicide resistance, is discussed in terms of effectiveness, potential antimicrobial mechanisms as well as synergy profiles with conventional fungicides. However, their "golden" potential to be used both as alternatives and partners of conventional fungicides to combat resistance and reduce environmental pollution is challenged by undesirable effects towards non-target organisms such as phytotoxicity, toxicity to humans and environmental ecotoxicity, constituting risks that should be considered before their commercial introduction as nano-pesticides at a large scale.

Keywords: nano-fungicides; Ag-NPs; Cu-NPs; ZnO-NPs; synergy; fungicide resistance

1 INTRODUCTION

Of all the available plant disease control methods considered, chemical control via the use of synthetic fungicides remains the most cost-efficient, food sustaining and safety-ensuring disease management strategy to date.[1,2] This great asset of the plant protection arsenal is seriously challenged by a number of inherent shortcomings of fungicides: high environmental footprints, side effects in non-target organisms, increasing research and development costs and the registration of new active ingredients and loss of effectiveness due to the development of resistance. Issues of environmental pollution, especially water contamination, have resulted in the withdrawal of an unprecedented number of older fungicide active ingredients, enforced by the implementation of European Union regulations and a halt in the development of new ones.[3] The limited number of available fungicides has rendered resistance even more difficult to manage, highlighting the need for alternative disease management agents capable of controlling both sensitive and fungicide-resistant pathogens and, at the same time, minimizing environmental risks.

Nanotechnology has made a dramatic entrance into modern agriculture, providing novel means for improving crop production and protection introducing nanoparticles (NPs) as nano-fertilizers, nano-pesticides or as carriers for relevant active ingredients. Metal nanoparticles (MNPs), taking advantage of their unique physicochemical properties, have shown significant effectiveness in controlling numerous plant pathogens, both sensitive and drug-resistant, requiring lower doses compared with their bulk/ionic protective counterparts.[3] The lower doses required for antimicrobial action and the potential to synthesize MNPs by green methods utilizing plants or microorganisms (or their metabolites) make them eco-compatible alternatives to synthetic fungicides.[4] The question that arises is: should these potent plant protection agents be used instead of or as partners to fungicides?

2 METAL-NANOFUNGICIDES

Nanomaterials with the potential to play an important role in plant disease management include organic or inorganic NPs (typically 10–100 nm in size) acting directly as nano-pesticides or as formulating agents. Their unique physicochemical properties enable them to maximize pathogen control at lower doses, achieve optimized drug delivery or increase residual action by controlled-release at slower rates, making NPs ideal, environmentally compatible fungicide alternatives.[1,5–8] Nano-pesticides containing metals such as zinc, silver or copper have demonstrated significant antibacterial, antifungal or even antiviral activity, and are increasingly scrutinized by scientists for their application potential against several plant pathogens.[6] The effectiveness of MNPs in controlling fungal plant pathogens including *Monilinia*

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fructicola, *Fusarium* sp., *Sclerotinia homoeocarpa*, *Botrytis cinerea*, *Aspergillus niger*, *Penicillium expansum* and *Rhizopus stolonifer* has been evaluated in a number of studies both in vitro and in vivo.[2,9–13] Positively charged MNPs adhere to the microbial cell membrane because of electrostatic attraction with the negatively charged cell membrane of microbes, damaging membrane integrity and eventually leading to cell death. Other modes of antimicrobial action of MNPs against plant pathogens include: protein/enzyme deactivation, production of reactive oxygen species and antioxidant depletion, disruption of ion homeostasis and DNA damage.[14] These mechanisms result from ion release or are NP-specific and are often interlinked, resulting in a multitargeted action ideal for effectively controlling plant pathogens.[4,15] Although MNPs share this multisite mode of action with their bulk/ionic metal counterparts, in the majority of cases MNPs exceed the efficacy of their counterparts against pathogens.[3] Differences in the level of toxicity between MNPs and their ionic counterparts could be due to either the way they are distributed around the fungal cell or an additional mechanism dependent on the properties of NPs. Whereas metal ions are uniformly distributed around fungal cells without specific localization, larger MNPs are unevenly distributed creating focal sources of continuously unleased ions. This large NP-generated ion concentration induces ion penetration and impairs ion homeostatic mechanisms, which cannot cope with the ion release rates produced, leading to a more profound toxic effect than individual metal ions.[11,16,17] Besides ion release, NPs can cause additional cell mechanical damage directly (because of their large surface to volume ratio or physical defects in their nanostructure) or by producing H_2O_2 that is able to penetrate the membrane and cause internal damage.[17] This could explain the superior fungitoxic effect of MNPs against fungal pathogens compared with their ionic counterparts, an advantage that makes them preferable to ionic forms in terms of both effectiveness and eco-compatibility because smaller xenobiotic quantities produce a lower environmental footprint.

3 ANTI-RESISTANCE AGENTS

Their multisite antimicrobial properties make MNPs ideal anti-resistance management tools to be used in rotation with or in mixtures with conventional drugs, a fact initially demonstrated in studies involving human bacterial pathogens resistant to one or more antibiotic drugs. This is especially important in the case of life-threatening multidrug-resistant pathogens with a growing list of success stories regarding the use of silver or other metal-containing NPs in the control of hard to kill, highly adaptive, drug-resistant bacterial or fungal human pathogens.[18,19] In the case of fungicide-resistant plant pathogens, reports on the potential of MNPs to counter resistance are scarce, but quite promising. NPs containing copper (Cu-NPs) or silver (Ag-NPs) have been shown to provide effective control against both sensitive and benzimidazole-resistant *B. cinerea* and *Monilia fructicola* isolates.[3,9] Ag-NPs have been shown to effectively inhibit mycelial growth in *Fusarium graminearum* isolates resistant to carbendazim, tebuconazole, prochloraz, fludioxonil, phenamaril and pyriflumetofen in vitro.[20] This was also the case for ZnO-NPs that exhibited excellent activity against both sensitive and boscalid-resistant *Alternaria alternata* isolates in vitro and in vivo.[10]

Fungal pathogens achieve fungicide resistance via a number of biochemical mechanisms that help them escape inhibition. The most important mechanism involves target site modification/overexpression, which reduces the affinity of the fungicide with/or modifies abundance of its target molecule. The remainder of the biochemical resistance mechanisms either prevent the active form of the active ingredient from reaching its target (reduced influx, increased efflux, detoxification), usually resulting in non-specific/multidrug resistance, or utilize alternative biochemical pathways (for example, alternative oxidase in the case of quinone outside inhibitors (QoIs)) to circumvent inhibition by the active ingredient.[21] Multiple targeting of both metal ions and MNPs make them low resistance risk antimicrobial agents, at least as far as the target site modification mechanism is concerned. Resistance to metal ions may occur via mutations that result in the disruption of the cellular ion homeostasis mechanisms that regulate ion influx/efflux and sequestration of excess ion loads that are toxic to the cell. Because the mode of action of MNPs extends beyond mere ion release, their risk for resistance development should be even lower than that of the respective metal-containing protective fungicides. Their ability to control bacterial pathogens is also very important because antibiotics are not allowed in agriculture and alternatives are rare (mostly consisting of copper-containing inorganic/organic compounds). Differences between NPs and their bulk counterparts also mean that they could be used in the control of copper-resistant bacteria – which has already been reported.[22] The above make MNPs ideal tools for any effective resistance management strategy against plant pathogens.

4 SYNERGY WITH CONVENTIONAL DRUGS

The suitability of MNPs for use as alternatives to or partners of conventional fungicides is evident, both theoretically due to their multisite mode of action and practically, as shown by their effectiveness against fungicide-resistant plant pathogens. What is not self-evident, is the "hidden" property of MNPs to exhibit a synergistic effect when combined with conventional drugs. In a large number of human disease cases, NPs containing Ag, Fe or Zn increased the antimicrobial activity of antibiotic drugs against both sensitive and drug-resistant bacteria such as *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis*.[19,23–25] Recently, a number of studies have confirmed a similar pattern concerning the combination of MNPs with conventional fungicides. Ag-NPs and ZnO-NPs combined with the fungicides carbendazim, mancozeb and thiram exhibited enhanced toxicity against *B. cinerea*, *A. alternata*, *Fusarium oxysporum, Aspergillus niger* and *Penicillium expansum*.[26] A similar synergistic effect was reported for ZnO-NPs when applied with the fungicide thiram in *Phytophthora capsici*.[27] To make things even more interesting, Ag-NPs, Cu-NPs and ZnO-NPs have been shown to "neutralize" fungicide resistance when applied against benzimidazole or boscalid-resistant isolates of *B. cinerea*, *M. fructicola* or *A. alternata* respectively; a profound synergistic effect was found when combining MNPs with fungicides that were otherwise ineffective due to target site resistance.[3,9,10]

The exact mechanisms by which MNPs can achieve such synergy with fungicides and bypass fungicide resistance are largely unknown, even though certain suggestions exist based on the mode of action of MNPs and their interaction with fungicides. MNPs can act as chemosensitizing agents, complementing fungicide action, or synergists that neutralize fungicide resistance mechanisms. Synergy between MNPs and fungicides may result from: (i) enhanced membrane perturbation, (ii) disruption of ion homeostasis, (iii) inhibition of efflux pumps, (iv) inhibition of detoxification enzymes and (v) a "capping" effect resulting in the formation of NP–fungicide conjugates (Figure 1).[10,18,28,29] Most of the above mechanisms are associated with the

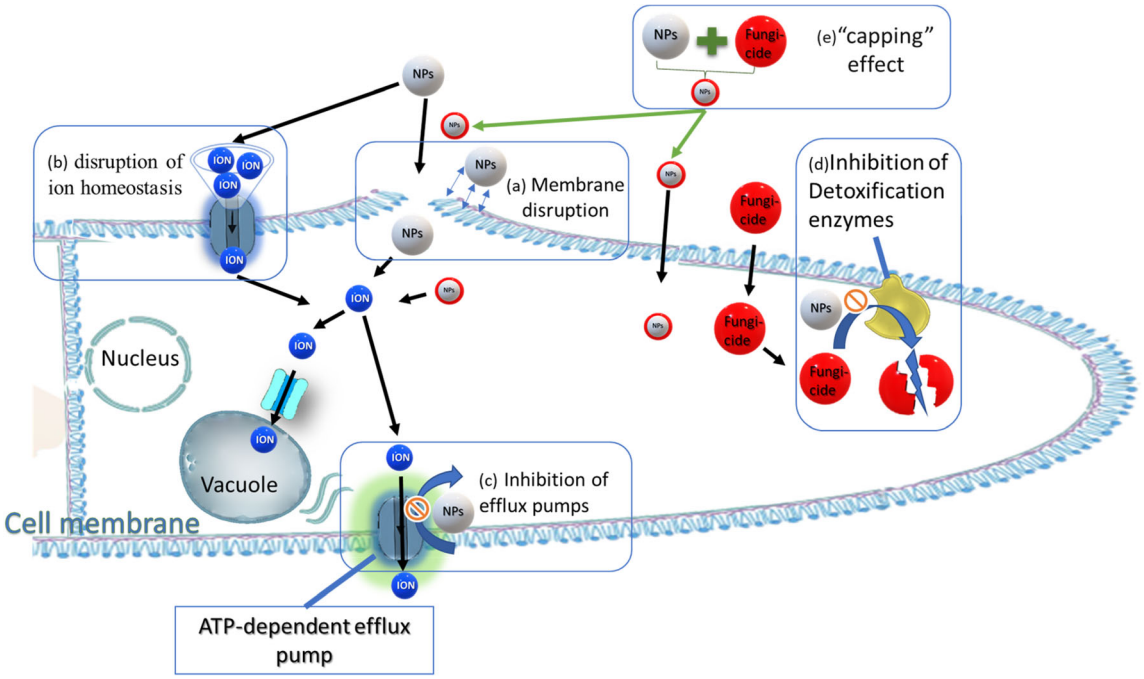

FIGURE 1. Schematic representation of proposed synergy mechanisms between nanoparticles (NPs) and fungicides: (a) enhanced membrane perturbation; (b) disruption of ion homeostasis; (c) inhibition of efflux pumps; (d) inhibition of detoxification enzymes; and (e) "capping" effect resulting in the formation of an NP–fungicide conjugate that deters agglomeration and reduces NP size.

bioavailability of the fungicide inside the fungal cell (the amount of active ingredient that finally reaches its target) facilitated by the action of MNPs. Membrane openings caused by Ag_3PO_4-NPs promoted sodium *O*-phenyl phenolate entrance and enhanced its toxic action against *Phytophthora capsici* and *B. cinerea*.[27] A combination of membrane damage and disruption of a major efflux pump was reported to contribute to the synergy observed between fluconazole and Ag-NPs against drug-resistant *Candida albicans*, whereas depletion of the detoxifying enzyme glutathione caused by thiram resulted in a synergistic toxic effect when combined with ZnO-NPs against *P. capsici*.[18,27] Boscalid acted as a capping agent of ZnO-NPs, reducing NP aggregation and resulting in a reduction in NP size, which probably enhanced the toxic effect of ZnO-NPs against *A. alternata*.[10]

Most of the above-mentioned mechanisms are especially important when we are dealing with multidrug isolates of plant pathogens where decreased influx/increased efflux or detoxification are the main causes of resistance. Nevertheless, MNPs can also suppress target site resistance when the MNP/fungicide combination results in a higher NP bioavailability by, for example, reducing NP size.[10] This highlights that the benefits of the combination of MNPs with conventional fungicides extend beyond the typical use of alternative and/or mixing partners with different modes of action for combating resistance. To exploit the full potential of MNPs as control agents against sensitive and fungicide-resistant pathogens, and broaden the application range of this phenomenon, their synergy profile with fungicides should be further studied and their synergy mechanisms elucidated.

5 SAFETY ISSUES

MNPs can reduce the environmental footprint of plant protection by reducing pesticide doses and, green-synthesized MNPs in particular, could be considered eco-compatible. However, as promising as they are for crop protection, MNPs may pose both known and unknown health and environmental risks. The very same unique properties that enable them to have increased fungitoxic action compared with their bulk counterparts could have undesirable/toxic effects towards non-target organisms. Their ability to penetrate fungal cell membranes, particularly in the case of smaller NPs, could extend to plant or even human cells and cause toxic responses.[30] The ecotoxicity tests typically used for their bulk counterparts may prove insufficient to evaluate NP toxicity because their effect on biological systems is not yet completely understood. Phytotoxicity, human and environmental safety issues should be systematically investigated before the commercial release of MNPs for use as nano-pesticides. The same applies to combinations of MNPs with fungicides, which may result in a special, combined toxicity to non-target organisms.

6 CONCLUSION

In an era of continuously increasing limitations in the availability of active ingredients against plant pathogens, MNPs exhibit great potential for use both as an alternative to conventional fungicides or/and as their partners in combating fungicide resistance. The demonstrated effectiveness of MNPs against a number of sensitive or fungicide-resistant plant pathogenic fungi, alone or combined with conventional fungicides at lower than recommended doses, highlight their "golden" potential to be used both as alternatives and partners of conventional fungicides to combat resistance and reduce the environmental impact of xenobiotics. Targets of NPs' fungitoxic action at a subcellular level could be key for inactivating the resistance mechanisms of pathogens, while their interaction with organic fungicides could facilitate an increase in the bioavailability of both antifungal agents leading to enhanced toxicity. However, because their effect on biological systems is not yet completely understood, MNPs should be

further studied for undesirable effects towards non-target organisms such as phytotoxicity, toxicity to humans and environmental ecotoxicity before their commercial introduction as nanopesticides on a large scale.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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