

Review Article

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Management of Plant Pathogens using Silver Nanoparticles (AgNPs)

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ABSTRACT

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Agricultural production is continuously reduced worldwide every year due to biotic stress including nematodes. Therefore millions of dollars have been invested in efforts to control the menace caused by the biotic stress. Various natural and artificial methods of control are being practiced for protecting plants from the damage caused by insects, plant disease causing organisms and phytonematodes. Among different methods used for plant pathogens control the use of pesticides is the most prevalent and common practice everywhere. In recent years, environmental hazards caused by excessive use of pesticides have been widely discussed. Therefore, the agricultural fields are searching for alternative measures against pesticides.

Introduction

Changes in agricultural technology have been a major factor shaping modern agriculture. Consequently, innovative technologies are being introduced in modern agriculture to minimize losses caused by the above biotic stress. Among such technological innovations, nanotechnology is gathering noteworthy considerations due to its robust applications in many fields including agriculture because of its unique physical, chemical and biological properties (Nair *et*

al., 2010; Ghormade *et al.*, 2011 and Bakshi *et al.*, 2014) Nowadays, the exploitation of potentiality of metal nanoparticles is still largely unexplored for crop protection and presently there has been a growing interest to utilize their antimicrobial property for the management of plant disease causing organisms and phytonematodes.

Although nanotechnology has wider application in agriculture very meagre work has been carried out in plant protection. The work carried out so far for the management of

pest and disease problem indicated the possession of antifungal and antibacterial properties by nanoparticles (Carmen *et al.*, 2003). However, almost no information is available about their antinematic properties. In this context Joseph and Morrison (2006) opined that in the near future nanostructured catalysts play an important role to increase the efficacy of commercially available pesticides and reduce their level of dosage required to combat pest problem in agricultural and horticultural crops.

Plant pathogens

Considerable work has been carried out on the effect of nanoparticles against disease causing plant pathogens like fungi and bacteria compared to insects and phytonematodes. The information available in the literature on the effects of NPs is summarized below.

Fungal plant pathogens

In an experiment ZnO and MgO NPs were evaluated against *Alternaria alternata*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Mucor plumbeus* at different concentrations. The study revealed that higher concentrations of NPs were most effective than lower concentrations to inhibit the germination of spores of above fungi (Wani and Shah, 2012). Similarly Wani and Shah (2012) also reported that higher concentrations of NPs with magnesium iron and zinc *in vitro* inhibited spores germination of *Penicillium notatum*, *Aspergillus niger* and *Nigrospora oryzae*. The same trend was also confirmed by Yehia *et al.*, (2013) with ZnO NPs against two pathogenic fungi *viz.*, *Fusarium oxysporum* and *P. expansum*.

Post-harvest pathogens

He *et al.*, (2011) reported that ZnO NPs @ 3 mmol/L significantly inhibited the growth of

two postharvest pathogenic fungi *viz.*, *Botrytis cinerea* and *P. expansum* compared to *B. cinerea*. In this study the pathogen *P. expansum* was found to be more sensitive to the treatment with ZnO NPs. Further the authors reported that the ZnO NPs inhibited the growth of *B. cinerea* by causing deformation of fungal hyphae and by affecting their cellular functions.

In addition the ZnO NPs were also found to prevent the development of conidiophores and conidia of *P. expansum* which eventually led to the death of fungal hyphae. Chowdappa *et al.*, (2014) synthesized AgNPs using chitosan as reducing and stabilizing agent and evaluated against *Colletotrichum gloeosporioides* in mango (cv. Alphonso). In this study the authors proved that postharvest decay in mango can be minimized by chitosan mediated AgNPs and concluded that the technology has commercial value.

Antimicrobial properties of NPs with special reference to AgNPs

Antinematic in free living nematode and plant parasitic nematodes

The free living nematode is the most frequently studied nematode for the influence of AgNPs (Yeon Roh *et al.*, 2009; Lim *et al.*, 2012; Meyer *et al.*, 2010 and Yang *et al.*, 2012). The results of the study indicated that the AgNPs affecting behavior and development of the nematodes was toxic to *C. elegans*.

Meloidogyne incognita

In an experiment conducted *in vitro* and under controlled pot conditions for the effect of AgNPs, SiO₂ and TiO₂ against *M. incognita*. It is observed that there was cent percent immobility and mortality of J₂ of root knot nematode by the above NPs with 800, 400

and 200 mg ml/lit except SiO₂. However under pot experimentations AgNPs and TiO₂ were found to reduce the shoot height and root length of tomato compared to untreated control. Hence the authors opined that NPs are causing toxic effect on tomato (Ardakani, 2013). Cromwell *et al.*, (2014) observed that more than 99 per cent of J₂ of *M. incognita* became inactive on exposure to AgNPs in water at 30 to 150µg/ml under laboratory conditions.

M. graminis

In turfgrass AgNPs reduced *M. graminis* population in soil by 92 and 82 per cent after 4 and 2 days of exposure under field conditions. Similarly the biweekly application of 90.4 mg/m² of AgNPs caused reduction in gall formation in root and improved quality of turfgrass for two years without phytotoxicity (Cromwell *et al.*, 2014).

Antifungal properties of AgNPs under *in vitro* conditions

Gajbhiye *et al.*, (2009) evaluated the effect of AgNPs along with fluconazole for the antifungal activity against *Phoma glomerata*, *P. herbarum*, *F. semitectum*, *Trichoderma* sp. and *Candida albicans*. In this study the authors observed that the antifungal activity of fluconazole enhanced against the test fungi in the presence of AgNPs. The fluconazole in combination with AgNPs showed the maximum inhibitory effect against *C. albicans* followed by *P. glomerata* and *Trichoderma* sp. whereas no significant enhancement in the inhibitory effect was noticed against *P. herbarum* and *F. semitectum*.

Various forms of silver ions and nanoparticles were tested for their antifungal activity against plant pathogenic fungi *viz.*, *Bipolaris sorokiniana* and *Magnaporthe grisea*. The

study conducted by Jo and Kim (2009) indicated that both forms inhibited colony formation of above pathogens. In this assay the effective concentrations (EC₅₀) of the silver compounds to inhibit the colony formation was found to be higher for *B. sorokiniana* than for *M. grisea*.

The fungal hyphal growth of sclerotium forming species *viz.*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *S. minor* inhibited remarkably by AgNPs in a dose dependent manner. The antimicrobial property of the AgNPs was differing among the fungi and it was in the order of *R. solani* > *S. sclerotiorum* > *S. minor* (Min *et al.*, 2009).

A study on fungal sclerotial germination revealed that the nanoparticles had significant inhibitory effect. Further it is stated that the sclerotial germination of *S. sclerotiorum* was most effectively inhibited at lower concentrations of AgNPs. In addition it is observed that the hyphae of *S. sclerotiorum* damaged severely by the AgNPs and resulting in the separation of layers of hyphal wall and collapsing of hyphae.

Hence the authors suggested the possibility of using AgNPs as an alternative to pesticides for the management of sclerotium forming phytopathogenic fungi. Kim *et al.*, (2012) treated silver colloidal solution at the concentrations of 10, 25, 50, and 100 ppm against eighteen different plants pathogenic fungi on potato dextrose agar, malt extract agar and corn meal agar plates.

The results indicated that AgNPs possess antifungal properties against these plant pathogens at 100 ppm. Green synthesized AgNPs using *Acalypha indica* leaf extracts were tested on fungal plant pathogens *viz.*, *Alternaria alternata*, *S. sclerotiorum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Botrytis cinerea* and *Curvularia*

lunata. The results showed that 15 mg concentration of AgNPs showed excellent inhibitory activity against all the tested pathogens (Krishnaraj *et al.*, 2012). Sahayaraj *et al.*, (2012) reported that plant based AgNPs inhibited the growth of *Fusarium oxysporum* f.sp. *vasinfectum* affecting cotton.

Elumalai and Vinothkumar (2013) demonstrated the effectiveness of biogenic synthesized AgNPs using aqueous extract of shade dried leaves of *Conyza ambigua* against *Aspergillus niger*, *A. flavus* and *S. rolfsii*. Banadkouki *et al.*, (2013) found that AgNPs was effective for the management of *F. solani* causing dry rot of potato under storage conditions and also suggested the opt time of application of AgNPs for the management of the fungus.

Bholay *et al.*, (2013) documented that there is a synergistic effect when fluconazole combined with AgNPs in the management of *A. alternata*, *F. oxysporum* and *Cladosporium herbarum*. Pulit *et al.*, (2013) proved that nanosilver at 50 ppm inhibited the growth of *C. cladosporoides* and *A. niger* by 90 and 70 per cent respectively. Narayanan and Park (2014) evaluated the green synthesized NPs against wood degrading fungal pathogens viz., *Gloeophyllum abietinum*, *G. trabeum*, *Chaetomium globosum* and *Phanerochaete sordida* and proved its effectiveness.

Papaiah *et al.*, (2014) considered that metallic AgNPs as an alternative to synthetic chemicals for the management of stem rot causing *S. rolfsii*, dry root rot causing *R. bataticola* and collar rot causing *A. niger*. Ouda (2014) reported that AgNPs and CuNPs were effective against *A. alternata* and *B. cinerea*.

In this study it is observed that 15 mg/lit exhibited maximum inhibitory effect on the growth of fungal hyphae through damaging its hyphae and conidia.

Combined effect of AgNPs with other metal NPs

Almost no work has been carried out on the combined effect of AgNPs with other metal NPs against plant pathogenic fungi. However Ouda (2014) observed higher effectiveness of AgNPs against *A. alternata* and *B. cinerea* when compared to AgNPs in combination of CuNPs. The AgNPs at 100 ppm caused maximum inhibitory effect on the growth of fungal hyphae as well as conidial germination of anthracnose *Colletotrichum* sp. in pepper and fungal pathogens responsible for causing powdery mildew on cucumber and pumpkin (Lamsal *et al.*, 2011).

Under greenhouse conditions

Nanosilver at 7 ppm exhibited inhibitory effect on *S. cepivorum* by more than 90 per cent and increased biomass and dry weight of onion (Jung *et al.*, 2010). The spraying of AgNPs on rice seedlings was highly effective against *M. grisea* according to Elamawi *et al.*, (2013). In this study the SEM image revealed the AgNPs caused detrimental effect on the growth of fungal mycelia. Sandhya *et al.*, (2014) noticed complete inhibition of conidial germination of *B. sorokiniana* causing spot blotch disease of wheat by AgNPs.

In this experiment the histochemical studies revealed deposition of lignin in vascular bundles due to AgNPs. The nanosized silica silver consisting of nanosilver and silica molecules at 3 ppm exhibited antifungal activity against many phytopathogenic fungi viz., *B. cinerea*, *R. solani*, *C. gloeosporioides*, *M. grisea* and *Pythium ultimum*. In contrast, a number of beneficial bacteria or plant pathogenic bacteria viz., *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas syringae* pv. *syringae*, *Xanthomonas campestris* pv. *vesicatoria*, *Azotobacter chroococcum* and

Rhizobium tropici were found to be affected at higher concentration of 100 ppm of nanosized silica silver (Park *et al.*, 2006). However the authors concluded that the effectiveness of nanosized silica silver was higher in respect of pathogen causing powdery mildews of pumpkin.

In plant growth chamber

Both ionic and AgNPs significantly checked the fungal pathogens *viz.*, *B. sorokiniana* and *M. grisea* on perennial ryegrass. In plant growth chambers both silver ions and AgNPs influenced spore colonization and progression of disease. The study concluded that the efficacy of silver ions and AgNPs was greater. Therefore, it can be used as preventive measure since it promotes the direct contact with spores and germ tubes and affect their viability (Jo and Kim, 2009).

In vivo conditions

It is observed that AgNPs inhibited *Colletotrichum* sp. in a field experiment conducted by Lamsal *et al.*, (2011). On observations through SEM it is understood that AgNPs causing detrimental effect on mycelial growth of the fungus. Park *et al.*, (2006) evaluated nanosized silica silver (0.3 ppm) against pathogen causing powdery mildew of pumpkin and found to be effective under field conditions.

Antibacterial properties of nanoparticles under in vitro conditions

The green synthesized AgNPs with *Ulva fasciata* inhibited the growth of *X. campestris* pv. *malvacearum* with zone of inhibition of 14.00 ± 0.58 mm. In this study the minimum inhibitory concentration was fixed as $40.00 \pm 5.77 \mu\text{g/mL}$ (Rajesh *et al.*, 2012). Similarly another plant based AgNPs was reported to inhibit the growth of same

bacterium with 10.33 ± 0.33 mm zone of inhibition by Sahayaraj *et al.*, (2012). The green synthesized AgNPs using leaf and fruit extracts of oak (*Quercus infectoria*) showed antibacterial activity against the plant pathogenic bacteria *viz.*, *Pectobacterium carotovorum*, *Ralstonia solanacearum*, *Erwinia amylovora* and *X. citri* (Mahmood Chahardooli *et al.*, 2014).

Under glasshouse conditions

The DNA-directed AgNPs developed by Ismail Ocsoy *et al.*, (2013) effectively decreased the disease incidence of bacterial spot caused by *X. perforans* in tomato. In this study even low concentration of 16 ppm of AgNPs had significant advantages of improved stability and stronger adsorption properties with excellent antibacterial effect.

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