J. Crop Prot. 2015, 4 (3): 291-300

Research Article

Evaluation of antifungal activity of silver nanoparticles against some phytopathogenic fungi and *Trichoderma harzianum*

Valiollah Mahdizadeh[1], Naser Safaie[1*] and Fatemeh Khelghatibana[2]

1. Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.
2. Seed and Plant Certification and Registration Institute, Karaj, Iran.

Abstract: *In vitro* antifungal activity of silver nanoparticles, at concentrations of 6, 8, 10, 12, 14 and 16 ppm, was studied on five phytopathogenic fungi, and a biocontrol agent. Then effect of silver nanoparticle at 6 ppm (optimum concentration) was evaluated on *Macrophomina phaseolina* in greenhouse. For *in vitro* experiment, the fungal isolates were grown on potato dextrose agar medium amended with silver nanoparticles. Radial fungal growth was recorded after 1, 2, 3, 5 and 10 days and mycelial growth inhibition rates were calculated. The most sensitive fungus to nanoparticles was *Pythium aphanidermatum*, since all tested concentrations showed 100% inhibition during the 10 days of observation.The second most sensitive fungus was *Sclerotinia sclerotiorum*, since it was able to grow only at concentration of 6 ppm and *M. phaseolina* was the third in sensitivity since its growth was inhibited in all concentrations after three days. In greenhouse experiments, five treatments including no nanosilver-no pathogen (Negative control), no nanosilver +pathogen (Positive control), 6 ppm nanosilver– no pathogen, 6 ppm nanosilver +pathogen, Carboxin-Thiram (0.15%) +pathogen were compared. Four characters viz shoot and root fresh and dry weights were measured. Based on the greenhouse experimental results, treatments with nanosilver and fungicide gave higher yields than the positive control. The chemical control treatment had the highest measured parameters, while 6 ppm nanosilver +pathogen treatment had the same parameters as negative control. It may therefore be suggested to use nanosilver as a safer alternative to chemical fungicides for control of *M. phaseolina*.

Keywords: *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Pythium aphanidermatum*

Introduction

In recent years, resistance to commercially available fungicides by phytopathogenic fungi has been increasing and has become a serious problem (Brent and Hollomon, 1995; Brent *et al.*, 1998; Dekker and Georgopoulos, 1982; Goffeau, 2008), So, the search for new fungicides and alternatives is of paramount importance to combat newly emerging resistant strains of fungal pathogens (Kanhed *et al.*, 2014). One solution would be nanotechnology which enhances antimicrobial activity of materials by converting them to nanoparticles. The improved antimicrobial activity of nanoparticles compared to their salts is due to their unique properties i.e. large surface area to volume ratio (Kanhed *et al.*, 2014).

It has been known that silver and its compounds are effective antimicrobial agents (Klasen, 2000; Silver, 2003). Since nano-Ag (silver) is highly toxic to most microorganisms, it may be a good

Handling Editor: Dr. Vahe Minassian

[*] **Corresponding author**, e-mail: nsafaie@modares.ac.ir
Received: 3 October 2014, Accepted: 23 February 2015
Published online: 20 April 2015

alternative for antimicrobial compounds (Herrera *et al.*, 2000; Zhao and Stevens, 1998) and in fact it has received special attention as a possible antimicrobial agent (Baker *et al.*, 2005; Melaiye *et al.*, 2005; Sondi and Salopek-Sondi, 2004). Recently the antifungal effects of nano-Ag have been studied by different researchers Mohebali *et al.*, 2009; Panáček *et al.*, 2009; Prabahu *et al.*, 2010; Qian *et al.*, 2013; Roy *et al.*, 2013; Singh *et al.*, 2013; Xu *et al.*, 2013). However, few studies are available on the effects of nano-Ag on phytopathogenic fungi (Jo *et al.*, 2009; Kasprowicz *et al.*, 2010; Lee *et al.*, 2013; Park *et al.*, 2006; Velmurugan *et al.*, 2009). The aim of this research is to evaluate the antifungal activities of AgNPs on some phytopathogenic fungi, and a fungal biocontrol agent *in vitro* and its effectiveness on charcoal rot of melon in greenhouse condition.

Materials and Methods

Preparation of medium amended with silver nanoparticles

Stock Nano-silver solution at 4000 ppm concentration was obtained from Nanocid Company. Potato dextrose agar (PDA) containing final concentrations of 6, 8, 10, 12, 14 and 16 ppm nanoparticles were prepared.

Fungal isolates

The experimental fungal isolates including *Rhizoctonia solani* AG1, *R. solani* AG4, *Macrophomina phaseolina*, *Sclerotinia sclerotiorum*, *Trichoderma harzianum* and *Pythium aphanidermatum* were grown on PDA incubated at 25 °C for three days.

Evaluation of antifungal activity

Plugs (4 mm in diameter) of 3-day-old fungal cultures were transferred to plates amended with different concentrations of silver nanoparticles. Control treatment for each fungus was prepared using culture medium without nanoparticles. All plates were incubated at 27 °C for 10 days. Radial growth of fungal colonies was recorded after 1, 2, 3, 5 and 10 days.

Growth inhibition percent was calculated using the radial growth of mycelium according to the following equation.

$$\text{Growth Inhibition (\%)} = \frac{\text{Control Radial Growth} - \text{Treatment Radial Growth}}{\text{Control Radial Growth}} \times 100$$

Greenhouse experiments

In greenhouse tests, the effects of nanosilver and carboxin-thiram fungicide (1.5 per 1000) were evaluated on melon growth parameters including fresh and dry weights of shoots and roots. Plastic pots were filled with pasteurized field soil, perlite and peat moss (1:1:1 ratio). Field soil was sterilized by autoclaving two times. Inoculum of fungal pathogen was prepared by growing them on millet grains in 100 ml flasks as follow: the grains were soaked overnight in distilled water, autoclaved twice (121 °C for 45 min) and then inoculated with three agar discs (5-mm-diameter) of 5-day-old fungal culture. Flasks were incubated at 28 °C in dark for 21 days. The fungal inocula were then mixed with soil (10 g per 1 kg of soil) (Etebarian *et al*. 2000). Four melon seeds were planted in each pot and the pots were kept at 25-33 °C, watered every other day until seedling emergence and daily thereafter for 60 days. Nanosilver was applied at rate of 6 ppm and the pots were watered with 100 ml of this solution at sowing time and one week later. There were five groups of treatments with three replications for each treatment, arranged in a randomized complete block design. Treatments included: 1)no nanosilver-no pathogen (negative control), 2)no nanosilver+pathogen (positive control), 3)6 ppm nanosilver-no pathogen, 4)6 ppm nanosilver+pathogen, 5)Carboxin-Thiram (1.5ml/l) + pathogen.

The experiment was carried out in a glasshouse at day temperature 32 °C and night temperature 25 °C with natural day light. Plants were harvested two months after sowing and five characters; the plant height, root and shoot fresh weights and root and shoot dry weights were measured.

Statistical analysis

Experiments were conducted in a completely randomized design and complete randomized block design for in vitro and in vivo tests, respectively, with three replications. The analysis of variance was performed by the general linear model using SAS software

version 9.1. Mean comparisons were performed using Duncan's Multiple Range Test.

Results

Effect concentrations of nanosilver on fungi

As shown in Fig. 1A there were significant differences between different concentrations of silver nanoparticles on growth inhibition of *R. solani* AG1while there was no growth inhibition in the negative control. Nanoparticles at concentrations of 6 and 8 ppm significantly inhibited the fungal growth by 75% and 80%, respectively. Meanwhile at concentrations of 10, 12 and 14 ppm nanosilver the fungal growth was inhibited by 90% and at16 ppm there was 100% inhibition. Various concentrations of silver nanoparticles were significantly different in growth inhibition of *R. solani* AG4. As presented in Figs. 1A-D all tested concentrations (up to 10 ppm) showed up to 90% or more growth inhibition and for concentrations of 12, 14 and 16 ppm, a 100% inhibition was observed. *M. phaseolina* growth was completely inhibited in all concentrations.

As depicted Figs. 1 B-D, growth of *S. sclerotiorum* was completely inhibited in all concentrations higher than 6 ppm. Various concentrations of nanoparticles had significantly different effects on *Trichoderma harzianum*. Concentrations of 6, 8 and 10 ppm showed 80%, 84% and 90% growth inhibition, respectively. Concentrations at 12, 14, and 16 ppm caused 100% growth inhibition. All applied concentrations of silver nanoparticles on *P. aphanidermatum* culture showed complete inhibition of its growth.

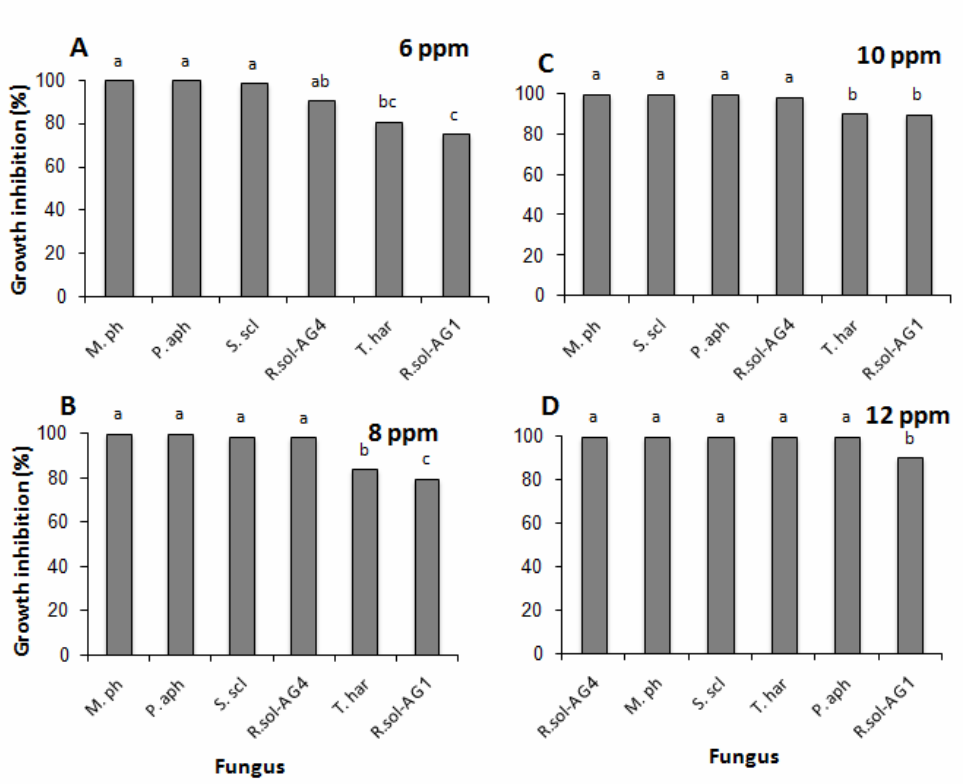

Figure 1 Growth inhibition of *Rhizoctonia solani* AG1 (R.sol.AG1), *Rhizoctonia solani* AG4 (R.sol.AG4), *Macrophomina phaseolina* (M.ph), *Sclerotinia sclerotiorum* (S.scl.), *Pythium aphanidermatum* (P.aph), *Trichoderma harzianum* (T.har) at different concentrations of nanosilver as percent of negative control.

Growth inhibition of silver nanoparticles in different fungi

The inhibitory effect of silver nanoparticles on different fungal species at 6 ppm concentration is presented in Figure 1. The first and second highest growth inhibitions were 100% and 98% for *M. phaseolina*, *P. aphanidermatum* and *S. sclerotiorum*, respectively. *R. solani* AG1 and *Trichoderma harzianum*, showed the lowest growth inhibitions by 75% and 81%, respectively. *R. solani* AG4, *M. phaseolina*, *S. sclerotiorum* and *P. aphanidermatum* were the most sensitive to 8 ppm and showed more than 98% inhibition. The lowest growth inhibition (80%) was found for *R. solani* AG1 (Fig. 1B). At 10 ppm the fungal isolates were divided into two significantly different groups. While four fungal species, *R. solani* AG4, *M. phaseolina*, *S. sclerotiorum* and *P. aphanidermatum* showed 100% growth inhibition, *R. solani* AG1 and *Trichoderma harzianum* causing 85% growth inhibition were less sensitive to nanoparticles (Fig. 1C). As shown in Fig. 1D nanoparticles at 12 ppm caused complete inhibition on *R. solani* AG4, *M. phaseolina*, *S. sclerotiorum*, *Trichoderma harzianum* and *P. aphanidermatum* and the growth rate of *R. solani* AG1was also inhibited about 90% (Fig. 1D). Concentration of 12 ppm caused complete growth inhibition of *R. solani* AG4, *M. phaseolina*, *S. sclerotiorum*, *Trichoderma harzianum* and *P. aphanidermatum* and the growth inhibition for *R. solani* AG1was 90% (data not shown). Moreover, a concentration of 16 ppm lead to complete inhibition of all examined fungal isolates (data not shown).

Time course study

To study the growth inhibition over time, the radial growth of fungal isolates was measured after 1, 2, 3, 5 and 10 days. Results are presented in Figure 2. *R. solani* AG1 at 16 ppm had no growth until 2 days. At lower concentrations it had a relatively slow growth (Fig. 2A). Concentration of 10 ppm of silver nanoparticles completely inhibited growth of *R. solani* AG4. At 16 ppm, its growth increased after 5 to 10 days. This situation may assign to creation of resistant sectors to silver nanoparticles by the fungus (Fig. 2B). *M. phaseolina* was completely inhibited by all concentrations after 1, 2 and 3 days of incubation. However, at 6 and 8 ppm, fungal growth was observed after 5 days. At 10 ppm and higher concentration, fungal growth was observed 10 days after incubation (Fig. 2C). In case of *S. sclerotiorum* no growth was observed at 8, 10, 12, 14 and 16 ppm up to 10 days of incubation, except at 6 ppm when fungal growth was observed up to 10 days (Fig.2D). *T. harzianum* did not grow at 10, 12, 14 and 16 ppm one day after inoculation. However at 10 ppm slight growth was shown two days after incubation. After 3 day at 12 and 14 ppm, the fungus began growing slightly. At a concentration of 16 ppm fungal growth inhibition was observed up to 5 days after inoculation (Fig. 2E). In case of *P. aphanidermatum,* all concentrations resulted in its complete inhibition of growth (Fig. 2F). General downward trend in growth with increasing concentration of silver nanoparticles can be observed, but this trend was not observed at 12 to 14 ppm after 5 or 10 days. As noted in other cases, it could be a possible adaptation at these concentrations.

Greenhouse Experiment

Statistical differences were identified among treatments based on the shoot fresh weight, shoot dry weight, root fresh weight and root dry weight at the 1%. The positive control (0 ppm nanosilver + pathogen) had the lowest yield and was significantly different from all other treatments in all of the growth parameters. According to shoot fresh weight, based on their statistical analysis, treatments fell into four groups. The positive control (0 ppm nanosilver + pathogen) had the lowest weight while the treatment Carboxin-Thiram +pathogen had the highest weight (Fig. 3). According to Root Fresh Weight, treatments were grouped with significant differences in three groups. Positive control (0 ppm nanosilver + pathogen) had lowest weight. Treatments 1, 3 and 5 had the highest weight.

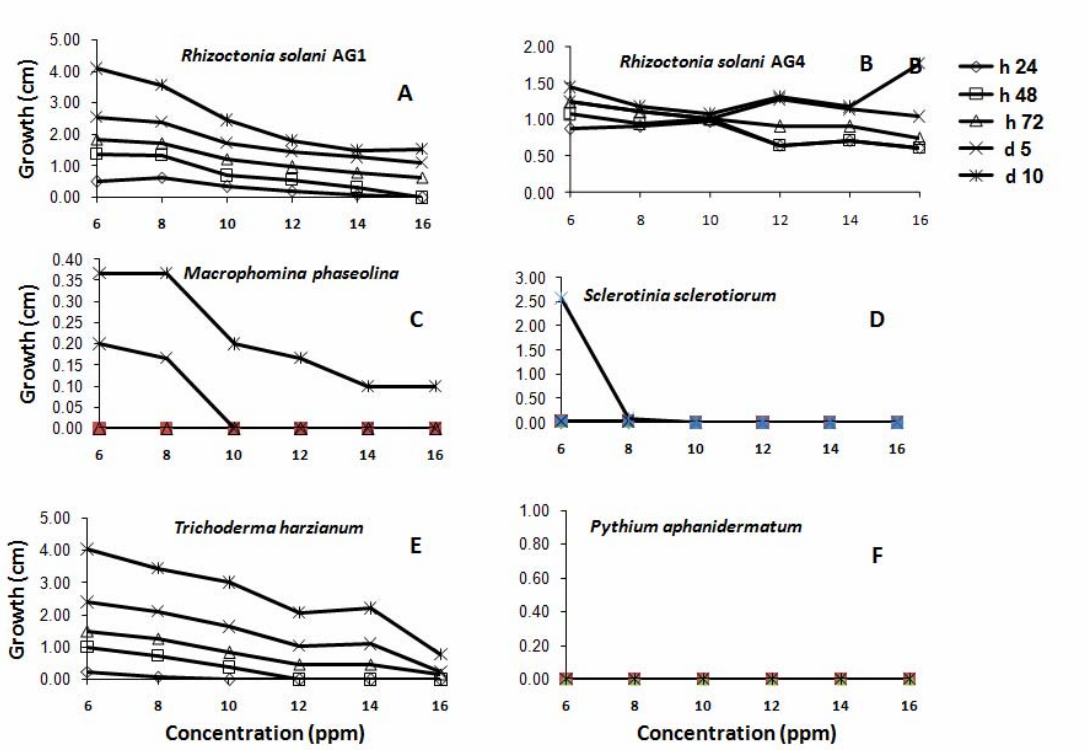

Figure 2 The growth of five phytopathogenic and one biocontrol fungi at different concentrations of silver nanoparticles over time.

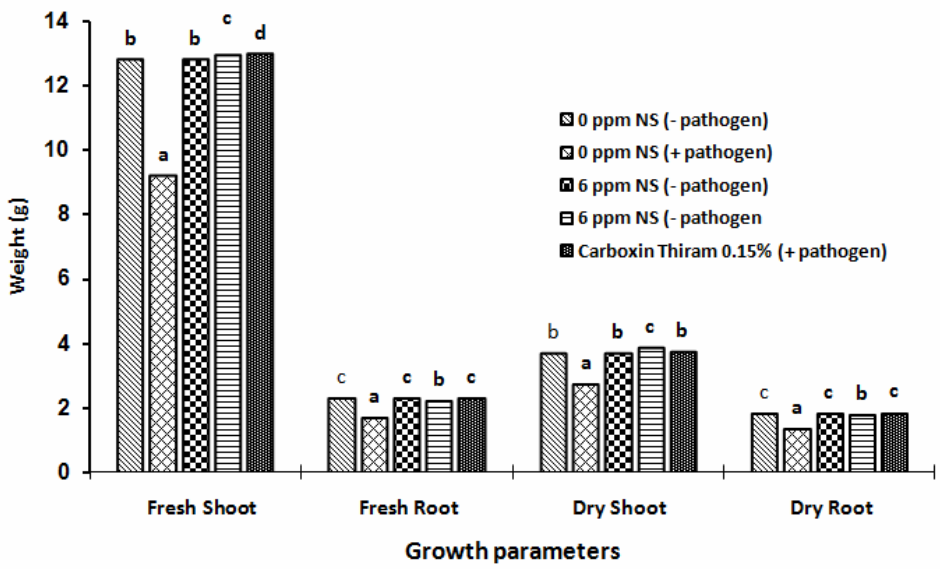

Figure 3 Effects of 6 ppm nanosilver (NS) formulation on control of charcoal rot of melon based on fresh and dry weights of roots and shoots.

According to Shoot Dry Weight, treatments were statistically categorized into three groups. Positive control (0 ppm Nanosilver +Pathogen) treatment had lowest weight. Treatment 6 ppm + pathogen have highest weight. According to Root Dry Weight, treatments were grouped into three groups. Positive control (0 ppm nanosilver + pathogen) had lowest weight. Treatments 1, 3 and 5 had highest weight.

Discussion

Since silver nanoparticles have different modes of action including interfering with fungal cell membrane potential and causing cell death (Clement and Jarret, 1994), its application for control of various plant pathogenic fungi is relatively safer compared to conventional synthetic fungicides (Park *et al.*, 2006). Various studies have been made on the effects of silver nanoparticles to control and combat a variety of microorganisms, but so far there has been limited research on the use of silver nanoparticles on plant pathogenic fungi (Jo *et al.*, 2009; Kasprowicz *et al.*, 2010; Park *et al.*, 2006; Velmurugan *et al.*, 2009). Anti-fungal effect of nano-Ag have been studied by different researchers (Abdel-Aziz *et al.*, 2013; Chwalibog *et al.*, 2010; Gajbhiye *et al.*, 2009; Gupta *et al.*, 2013; Jain and Sharma, 2013; Kim *et al.*, 2008; K.-J. Kim *et al.*, 2009; Kumar and Sudha, 2013; Li *et al.*, 2013). Because of the importance of pathogenic agents and also due to limitations of chemical control of soil-borne diseases, in this study, as a first step, we have used silver nanoparticles to assess in vivo inhibitory effects of these compounds on different fungi. Fungal pathogens were selected based on their importance and the unavailability of appropriate fungicides for their control.

According to our results there are significant differences between the different concentrations of silver nanoparticles on the inhibition *R. solani* AG1. For concentrations of 10, 12, 14 more than 90% and for concentration above 14 ppm, 100% of growth inhibition was observed. There are significant differences between the treatment and control of various concentrations of silver nanoparticles on the inhibition *R. solani* AG4. The examined concentrations up to 10 ppm showed up to 90% and the concentrations of 12, 14 and 16 ppm showed 100% growth inhibition. These results indicated that AG4 is more sensitive to silver nanoparticles than AG1. As AG4 has a wide host range, the above compound is a promising alternative for controlling this pathogen.

All the treatments completely inhibited *M. phaseolina*, *S. sclerotiorum* and *P. aphanidermatum*. These fungi had a higher sensitivity than the *Rhizoctonia* isolates. The highest growth inhibitions by nanosilver at concentration of 6 ppm were 100, 98 and 100 percent in *M. phaseolina, S. sclerotiorum* and *P. aphanidermatum*, respectively. The lowest levels belonged to fungi *R. solani* AG1 and *T. harzianum* with 75 and 81% growth inhibition, respectively. *R. solani* AG4, *M. phaseolina, S. sclerotiorum* and *P. aphanidermatum* were the most sensitive to silver nanoparticles at 8ppm resulting with more than 98 percent growth inhibition. The minimum growth inhibition (80%) belonged to *R. solani* AG1. The most sensitive pathogen to silver nanoparticles was *P. aphanidermatum*, since its growth was inhibited even 10 days after incubation. Since this pathogen is a Stamenopilous fungus-like organism from Kingdom Stramenopila, its high sensitivity (absolute growth inhibition) is very interesting to examine silver nanoparticles to other plant pathogens from this Kingdom. The next sensitive fungus to silver nanoparticles was *S. sclerotiorum*, because fungus was able to growth only in concentration of 6ppm and not at higher concentrations. Next to these two fungi, *M. phaseolina* was most sensitive to silver nanoparticles and in all concentrations after 3 day showed 100% inhibition.

Differences between fungi in response to nanoparticles have been reported (Clement and Jarret, 1994). It is because of different mode of action of silver nanoparticles, as well as different resistance mechanisms in different fungi (Clement and Jarret, 1994). Jo *et al.* (2009) showed effective concentrations for inhibition of colony formation by silver compounds on *Bipolaris sorokiniana* was greater than that on *Magnaporthe grisea*.

Prabahu *et al.* (2010) showed potential activity of nanoparticles against clinical isolates

(*Trichophyton mentagrophytes*, *T. rubrum*, *Microsporum canis*, *M. persicolor* and *Candida albicans* (IC_{80}, 1-6 µg/ml). The activity of the nanoparticles was comparable with that of Amphotericin B (1-5 µg/ml) and Fluconazole (10-30 µg/ml). Their results showed that nanoparticles exerted antifungal activity on the pathogenic fungal mycelia. This study highlights that nanoparticles may have considerable antifungal activity deserving further investigation for clinical applications. Kim *et al.* (2009) studied antifungal activity in three different forms of silver nanoparticles against *Raffaelea* sp., the causal agent of death in the large number of oak trees in the world. Fungal growth in the presence of silver nanoparticles was significantly inhibited at different concentrations. They also examined the efficacy of a combination of various forms of nanoparticles. Microscopic observations showed that the silver nanoparticles have devastating effects not only on mycelia but also on conidial germination.

Xu *et al.* (2013) showed that the activity of nano-silver against *Aspergillus* spp. is two times greater than that of amphotericin B. Nano-silver's antifungal activity was superior to those of amphotericin B against pulmonary pathogenic fungi in vitro. Lee *et al.* (2013) showed that the synthesized nanoparticles (2 mM) can significantly inhibit (87.1%, 86.5%, and 83.5%) the growth of phytopathogens *Colletotrichum coccodes*, *Monilinia* sp., and *Pyricularia* sp.

Our results are similar to results of Park *et al.* (2006) according to the concentration range. Park *et al.* (2006) studied the effect of silica-silver nanoparticles on control of plant pathogenic microorganisms. They reported that among plant pathogenic fungi investigated, silica-silver nanoparticles effectively controlled squash powdery mildew at a concentration of 3ppm in both field and greenhouse tests. Pathogen on infected leaves, three days after spraying of plants with this substance, disappeared and the leaves remained healthy thereafter. Number of beneficial bacteria and a number of plant pathogenic bacteria was not significantly affected by the concentration of 10 ppm; however, at a concentration of 100ppm the growth was completely inhibited. Results indicated that this material is effective to control various fungal pathogens.

Our results differ with those of Petica *et al.* (2008) in inhibition concentration. They showed that a stable colloidal solution containing 35 ppm silver nanoparticles has effective antifungal properties against *Aspergillus*, *Penicillium* and *Trichoderma*. The cause of this difference can be attributed to nano-silver type and the strain of fungus. This study was carried out to compare the effects of silver nanoparticles on some plant pathogenic fungi, and determine the best killing dose and the optimum dose that stops the growth of the fungus. Our results showed that the sensitivity of the studied fungal species was as follows *P. aphanidermatum* > *S. sclerotiorum* > *M. phaseolina* > *R. solani* AG4 > *R. solani* AG1 > T. harzianum. Silver nanoparticles had minimal effect on *Trichoderma*. Therefore using proper concentration of nanoAg can control pathogenic species and have little impact on the rate of fungal biocontrol as the results of *in vitro* tests showed. However *in vivo* tests to confirm this in vitro finding is necessary.

In greenhouse experiment, our data showed that all treatments without pathogen have higher weight as compared to positive control. Chemical control had the highest yield while 6ppm nanosilver + pathogen has the same weight with negative control, it is therefore reasonable to use nanosilver instead of fungicide to control *M. phaseolina*.

Our findings indicate that silver nanoparticles have the potential to control plant fungal pathogens and could be applicable in the field.

References

Abdel-Aziz, M. S., Shaheen, M. S., El-Nekeety, A. A. and Abdel-Wahhab, M. A. 2013. Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using *Chenopodium murale* leaf extract. Journal of Saudi Chemical Society 18: 356-363.

Baker, C., Pradhan, A., Pakstis, L., Pochan, D. J. and Shah, S. I. 2005. Synthesis and Antibacterial Properties of Silver

Nanoparticles. Journal Nanoscience and Nanotechnology, 5: 244-249.

Brent, K. J. and Hollomon, D. W. 1995. Fungicide Resistance in Crop Pathogens: How can it be managed? FRAC Monograph No.1: Fungicide Resistance Action Committee, Bristol, UK. 56 pp.

Brent, K. J., Hollomon, D. W. and Federation, G. C. P. 1998. Fungicide Resistance: The Assessment of Risk: Global Crop Protection Federation Brussels, Belgium.52 pp.

Chwalibog, A., Sawosz, E., Hotowy, A., Szeliga, J., Mitura, S., Mitura, K., Grodzik, M., Orlowski, P. and Sokolowska, A. 2010. Visualization of interaction between inorganic nanoparticles and bacteria or fungi. International Journal of Nanomedicine, 5: 1085.

Clement, J. L. and Jarret, P. S. 1994. Antimicrobial silver. Metal-Based Drugs, 1: 467-482.

Dekker, J. and Georgopoulos, S. 1982. Fungicide Resistance in Crop Protection: Centre for Agricultural Publishing and Documentation (Pudoc). Wageningen, Netherlands.

Gajbhiye, M., Kesharwani, J., Ingle, A., Gade, A. and Rai, M. 2009. Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole. Nanomedicine: Nanotechnology, Biology and Medicine, 5: 382-386.

Goffeau, A. 2008. Drug resistance: The fight against fungi. Nature, 452: 541-542.

Gupta, A., Bonde, S. R., Gaikwad, S., Ingle, A., Gade, A. K. and Rai, M. 2013. Lawsonia inermis-mediated synthesis of silver nanoparticles: activity against human pathogenic fungi and bacteria with special reference to formulation of an antimicrobial nanogel. Institution of Engineering and Technology 8: 172-178.

Herrera, M., Carrion, P., Baca, P., Liebana, J. and Castillo, A. 2000. In vitro antibacterial activity of glass-ionomer cements. Microbios, 104: 141-148.

Ishida, K., Cipriano, T. F., Rocha, G. M., Weissmuller, G., Gomes, F., Miranda, K. and Rozental, S. 2013. Silver nanoparticle production by the fungus *Fusarium oxysporum*: nanoparticle characterisation and analysis of antifungal activity against pathogenic yeasts. Memorias do Instituto Oswaldo Cruz, 109: 220-228.

Jain, P. and Sharma, A. 2013. Antimicrobial activity of silver nanoparticles synthesized from Aspergillus species against common oral pathogens. Journal of Chemical & Pharmaceutical Research, 5: 14-17.

Jo, Y. K., Kim, B. H. and Jung, G. H. 2009. Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. Plant Disease, 93: 1037-1043.

Kanhed, P., Birla, S., Gaikwad, S., Gade, A., Seabra, A. B., Rubilar, O., Duran, N. and Rai, M. 2014. In vitro antifungal efficacy of copper nanoparticles against selected crop pathogenic fungi. Materials Letters, 115: 13-17.

Kasprowicz, M. J., Koziol, M. and Gorczyca, A. 2010. The effect of silver nanoparticles on phytopathogenic spores of *Fusarium culmorum*. Canadian Journal of Microbiology, 56: 247-253.

Kim, K.-J., Sung, W. S., Moon, S.-K., Choi, J.-S., Kim, J. G. and Lee, D. G. 2008. Antifungal effect of silver nanoparticles on dermatophytes. Journal of Microbiology and Biotechnology, 18: 1482-1484.

Kim, K.-J., Sung, W. S., Suh, B. K., Moon, S.-K., Choi, J.-S., Kim, J. G. and Lee, D. G. 2009. Antifungal activity and mode of action of silver nano-particles on Candida albicans. Biometals, 22: 235-242.

Kim, S. W., Kim, K. S., Lamsal, K., Kim, Y. J., Kim, S. B., Jung, M., Sim, S. J., Kim, H. S., Chang, S. J., Kim, J. K. and Lee, Y. S. 2009. An In Vitro Study of the Antifungal Effect of Silver Nanoparticles on Oak Wilt Pathogen *Raffaelea sp*. Journal of Microbiology and Biotechnology, 19: 760-764.

Klasen, H. J. 2000. A historical review of the use of silver in the treatment of burns. II. Renewed interest for silver. Burns, 26: 131-138.

Kumar, P. S. and Sudha, S. 2013. Biosynthesis of silver nanoparticles from *Dictyota bartayresiana* extract and their antifungal activity. Nano Biomedicine & Engineering, 5: 72-75.

Lee, K.-J., Park, S.-H., Govarthanan, M., Hwang, P.-H., Seo, Y.-S., Cho, M., Lee, W.-

H., Lee, J.-Y., Kamala-Kannan, S. and Oh, B.-T. 2013. Synthesis of silver nanoparticles using cow milk and their antifungal activity against phytopathogens. Materials Letters, 105: 128-131.

Li, C., Wang, X., Chen, F., Zhang, C., Zhi, X., Wang, K. and Cui, D. 2013. The antifungal activity of graphene oxide–silver nanocomposites. Biomaterials, 34:3882-90.

Melaiye, A., Sun, Z., Hindi, K., Milsted, A., Ely, D., Reneker, D. H., Tessier, C. A. and Youngs, W. J. 2005. Silver(I)−Imidazole Cyclophane gem-Diol Complexes Encapsulated by Electrospun Tecophilic Nanofibers: Formation of Nanosilver Particles and Antimicrobial Activity. Journal of the American Chemical Society, 127: 2285-2291.

Mohebali, M., Rezayat, M. M., Gilani, K., Sarkar, S., Akhoundi, B., Esmaeili, J., Satvat, T., Elikaee, S., Charehdar, S. and Hooshyar, H. 2009. Nanosilver in the treatment of localized cutaneous leishmaniasis caused by *Leishmania major* (MRHO/IR/75/ER): an *in vitro* and *in vivo* study. Daru-Journal of Faculty of Pharmacy, 17: 285-289.

Panáček, A., Kolář, M., Večeřová, R., Prucek, R., Soukupová, J., Kryštof, V., Hamal, P., Zbořil, R. and Kvítek, L. 2009. Antifungal activity of silver nanoparticles against *Candida spp.* Biomaterials, 30: 6333-6340.

Park, H., Kim, S., Kim, H. and Choi, S. 2006. A new composition of nanosized silica-silver for control of various plant diseases. The Plant Pathology Journal, 22: 295-302.

Petica, A., Gavriliu, S., Lungu, M., Buruntea, N. and Panzaru, C. 2008. Colloidal silver solutions with antimicrobial properties. Materials Science and Engineering: B, 152: 22-27.

Prabahu, N., Ayisha Siddiqua, S., Yamuna Gowri, k. and Divya, T. R. 2010. Antifungal activity of silver fungal nanoparticles - a novel therapeutic approach. Archives of Pharmacal Research, 2: 355-359.

Qian, Y., Yu, H., He, D., Yang, H., Wang, W., Wan, X. and Wang, L. 2013. Biosynthesis of silver nanoparticles by the endophytic fungus Epicoccum nigrum and their activity against pathogenic fungi. Bioprocess and Biosystems Engineering: 48: 1-7.

Roy, S., Mukherjee, T., Chakraborty, S. and Das, T. K. 2013. Biosynthesis, Characterisation & Antifungal Activity of Silver Nanoparticles Synthesized By the Fungus Aspergillus Foetidus Mtcc8876. Digest Journal of Nanomaterials and Biostructures, 8: 197-205.

Silver, S. 2003. Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. FEMS Microbiology Reviews, 27: 341-353.

Singh, M., Kumar, M., Kalaivani, R., Manikandan, S. and Kumaraguru, A. 2013. Metallic silver nanoparticle: a therapeutic agent in combination with antifungal drug against human fungal pathogen. Bioprocess and Biosystems Engineering, 36: 407-415.

Sondi, I. and Salopek-Sondi, B. 2004. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. Journal of Colloid and Interface Science, 275: 177-182.

Velmurugan, N., Kumar, G. G., Han, S. S., Nahm, K. S. and Lee, Y. S. 2009. Synthesis and characterization of potential fungicidal silver nano-sized particles and chitosan membrane containing silver particles. Iranian Polymer Journal, 18: 383-392.

Xu, Y., Gao, C., Li, X., He, Y., Zhou, L., Pang, G. and Sun, S. 2013. In vitro antifungal activity of silver nanoparticles against ocular pathogenic filamentous fungi. Journal of Ocular Pharmacology and Therapeutics, 29: 270-274.

Zhao, G. and Stevens, S. E., Jr. 1998. Multiple parameters for the comprehensive evaluation of the susceptibility of Escherichia coli to the silver ion. Biometals, 11 (1): 27-32.

ارزیابی فعالیت ضدقارچی نانو ذرات نقره در برابر تعدادی قارچ بیمارگر گیاهی و *Trichoderma harzianum*

ولی‌اله مهدیزاده[1]، ناصر صفایی[1] و فاطمه خلقتی‌بنا[2]

۱- گروه بیماری‌شناسی گیاهی، دانشکده کشاورزی دانشگاه تربیت مدرس، تهران، ایران.
۲- مؤسسه ثبت و گواهی بذر و نهال، کرج، ایران.
* پست الکترونیکی نویسنده مسئول مکاتبه: nsafaie@modares.ac.ir
دریافت: ۱۱ مهر ۱۳۹۳؛ پذیرش: ۴ اسفند ۱۳۹۳

چکیده: فعالیت ضدقارچی نانو ذرات نقره علیه پنج قارچ بیمارگر گیاهی و یک عامل بیوکنترل در غلظت‌های ۶، ۸، ۱۰، ۱۲، ۱۴ و ۱۶ پی‌پی‌ام به‌صورت درون شیشه‌ای بررسی شد. سپس تأثیر غلظت ۶ پی‌پی‌ام نانو ذرات نقره روی *Macrophomina phaseolina* در گلخانه بررسی شد. برای آزمایش‌های درون شیشه‌ای جدایه‌های قارچ روی محیط عصاره سیب‌زمینی- دکستروز آگار حاوی نانو ذرات نقره، کشت شدند. رشد پرگنه‌ها ۱، ۲، ۳، ۵ و ۱۰ روز بعد از کشت ثبت و میزان مهار رشد محاسبه شد. حساس‌ترین قارچ به نانو ذرات نقره *Pythium aphanidermatum* بود به‌گونه‌ای که رشد در همه غلظت‌ها بعد از ۱۰ روز کاملاً مهار شده بود. دومین قارچی که بالاترین حساسیت را داشت، *Sclerotinia sclerotiorum* بود به‌طوری‌که فقط در ۶ پی‌پی‌ام رشد کرد و سومین درجه حساسیت مربوط به *M. phaseolina* بود که در تمام غلظت‌ها بعد از سه روز رشد متوقف شد. در آزمایش‌های گلخانه‌ای، پنج تیمار شامل کنترل منفی (بدون بیمارگر و نانوسیلور)، کنترل مثبت (بدون نانوسیلور همراه با بیمارگر)، ۶ پی‌پی‌ام نانوسیلور بدون بیمارگر، ۶ پی‌پی‌ام نانوسیلور همراه بیمارگر، کربوکسین-تیرام (۰/۱۵ درصد) همراه بیمارگر بود. وزن خشک و تر ریشه و اندام‌های هوایی اندازه‌گیری شد. براین اساس تیمارهای دارای نانوسیلور و قارچ‌کش بالاترین رشد را نسبت به کنترل مثبت داشتند. کنترل شیمیایی بالاترین میزان رشد را نشان داد. میزان رشد در تیمار نانوسیلور همراه بیمارگر مشابه کنترل منفی بود. براین اساس پیشنهاد می‌شود که از نانوسیلور به‌عنوان جانشینی ایمن‌تر برای قارچ‌کش‌های شیمیایی برای کنترل *M. phaseolina* استفاده شود.

واژگان کلیدی: *Rhizoctonia solani*، *Sclerotinia sclerotiorum*، *Pythium aphanidermatum*