INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17–1355/2018/20–5–1225–1229
DOI: 10.17957/IJAB/15.0595
http://www.fspublishers.org

Full Length Article

Synergistic Antifungal Effect of Biosynthesized Silver Nanoparticles Combined with Fungicides

Weidong Huang[†], Changjin Wang[†], HaiMing Duan, YaLing Bi, Degong Wu, JunLi Du and Haibing Yu[*]
College of Agriculture, Anhui Science and Technology University, Fengyang, 233100, China
[*]For correspondence: haibing50721@163.com
[†]These authors contributed equally

Abstract

Silver nanoparticles (AgNPs) were synthesized by ginkgo fruit extract. UV-vis, TEM, SEM, EDX and AFM were applied for their characterization. The inhibition rate of AgNPs against *Bipolaria maydis* reached 78.7% at the concentration of 200 μg/mL. Prominent synergistic antifungal effect was found when AgNPs were combined with selected fungicides. The toxicity ratio determined by Horsfall's method reached 1.33, 1.34, and 1.23 when the proportion of AgNPs and tebuconazole, propineb, fludioxonil was 1:1, 9:1, and 7:3, respectively. Such compounds not only explore a novel approach to control phytopathogens but also provide possibility to avoid development of drug resistance.

Keywords: Synergistic antifungal effect; Silver nanoparticles; Fungicide; *Bipolaria maydis*

Introduction

In recent years, nanomaterials or nanoparticles have come into more and more people's sight. The size of nanoparticles is in the range of 1 to 100 nm at least in one dimension (Shetty *et al*., 2014). Nano-sized materials have unique properties that differ from bulk counterparts, such as small-sized effect, surface effect, quantum size effect, macroscopic quantum tunneling effect, and so on (Osuwa and Anusionwu, 2011). As an important member of metal nanoparticles, AgNPs emerged potential applications in the fields of heavy metal ion detection (Wang and Chen, 2009; Kirubaharan *et al*., 2012), chemical substances determination (Ping *et al*., 2012; Han *et al*., 2014), surface enhanced Raman scattering (Gil and Lucassen, 2010; Garg and Dhara, 2013), fluorescent signal enhancement (Fu *et al*., 2007, 2008), inhibition of microorganisms (Elechiguerra *et al*., 2005; Morrones *et al*., 2005; Lu *et al*., 2008; Kasprowicz *et al*., 2010; Sotiriou and Pratsinis, 2010; Lamsal *et al*., 2011).

Among these synthesis methods, biological approach using living organism like microorganisms and plants are considered the most popular. There were many research articles focused on biosynthesis of AgNPs, such as *Bacillus licheniformis* (Kalimuthu *et al*., 2008), *Septoria apii* and *Trichoderma koningii* (Huang *et al*., 2013), *Phoenix dactylifera* (Khatami and Pourseyedi, 2015), *Hibiscus rosa-sinensis* (Nayak *et al*., 2015), *Osmanthus fragrans* (Alani *et al*., 2012), *Caulerpa racemosa* (Kathiraven *et al*., 2015), orange peel (Castro *et al*., 2015), etc. Besides, several review articles have summarized green synthesis of AgNPs based on such materials (Borase *et al*., 2014; Asha *et al*., 2016; Mohammadlou *et al*., 2016).

It's well known that phytopathogens that cause plant diseases do severe damage to agroforestry. Under traditional concept, chemical pesticides were used as the first ever unique choice. However, with the growing environmental and drug resistance problems, novel eco-friendly fungistats need to be developed urgently. The rapid development of nanotechnology provides the possibility to resolve such challenge. At nanoscale, antimicrobial activity of silver dramatically enhanced, and it was proved more broad-spectrum for AgNPs including bacteria (Lok *et al*., 2007; Kumari *et al*., 2015), fungi (Khatami *et al*., 2016; Huang *et al*., 2017a), virus (Elechiguerra *et al*., 2005; Lara *et al*., 2010) and pathogenic cell (Mohammad *et al*., 2017) etc.

In this study, ginkgo fruit extract was used to synthesize AgNPs, and TEM, SEM, EDX, and AFM were applied to characterize the synthesized AgNPs in order to provide more information for their application. In addition, antifungal activity of AgNPs alone and synergistic effect of AgNPs and three efficient fungicides (tebuconazole, propineb, fludioxonil) were determined through mycelium growth rate method.

Materials and Methods

Ginkgo fruits were gathered in Fengyang city, Anhui Province, China. Silver nitrate ($AgNO_3$) was purchased from Sinopharm Chemical Reagent Co., Ltd (China). Tebuconazole, propineb, and fludioxonil were purchased from Shandong Weifang Rainbow Chemical CO., Ltd. The

To cite this paper: Huang, W., C. Wang, H. Duan, Y. Bi, D. Wu, J. Du and H. YU, 201x. Synergistic antifungal effect of biosynthesized silver nanoparticles combined with fungicides. *Int. J. Agric. Biol*., 20: 1225–1229

fungus named *B. maydis* was conserved in the Plant Protection laboratory of Anhui Science and Technology University.

Preparation of Fruit Extract

The extract of Ginkgo fruit was prepared as follows: fruits were denucleated and washed thoroughly in sterile water, then dried on a clean bench. Putting 10 g of fruits into 100 ml of deionized water, then heated at 95°C for 30 min. During the heating process, it's necessary to stir it from time to time. After that, the extract was filtered through filter paper for two times and preserved for further use.

Synthesis of AgNPs

Green synthesis of AgNPs was achieved through adding 10 mL filtrate to 90 mL deioniszed water, followed by reaction with 1 mM $AgNO_3$ at 80°C for 15 min. During this heating process, the solution color changed (Scheme 1).

Characterization of AgNPs

In order to measure optical properties of green synthesized AgNPs, UV-Vis spectroscopy (TU-1950) was applied at the range of 350 nm to 600 nm. The characteristics of AgNPs like morphology, size, and dispersion were measured through TEM, SEM, AFM, etc. TEM (JEM-2100F) analysis of the particles were sonicated for 10 min at first, and then dripped on carbon-coated copper grid to dry completely. For SEM (S-4800) analysis, such synthesized AgNPs were deposited on a sample plate, followed by coating with platinum. AFM (BioScope) was applied to detect AgNPs dried on a mica plate. Furthermore, EDX was used to measure the purity of AgNPs.

Inhibition Rate of AgNPs

Centrifuges and Oven-dried AgNPs were dissolved in sterile water as a stock solution (10 mg/mL). A volume of 5 mL of diluted stock solution was added to 45 mL of PDA medium at an approximate temperature of 55-60°C, and final concentrations of AgNPs of 12.5, 25, 50, 100 and 200 μg/mL were obtained by dilution with sterile water. The control set contained 5 mL of sterile water without silver nanoparticles. A fungus block (φ = 5 mm) was inoculated in the center of each Petri dish with different concentration of AgNPs, followed by incubation at 28°C for 3–5 d. Each control and experimental treatment was performed in three replicates.

Synergetic Antifungal Effect of AgNPs and Fungicides

The 50% effective concentration (EC_{50}) of AgNPs and three fungicides was measured through mycelium growth rate method, respectively. Based on that, various compound proportion was settled as 0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 and 10:0. All compounds were prepared fresh and mixed thoroughly for 5 min. The synergetic effect assessment (toxicity ratio) of AgNPs and fungicides was determined by the follow equations (Horsfall, 1945):

(1) Actual inhibition rate = [(Diameter of control colony-Diameter of treatment colony)/(Diameter of control colony-Diameter of fungus block)]*100%;

(2) Theoretical inhibition rate = (Actual inhibition rate of A at medium concentration* percentage of A +Actual inhibition rate of B at medium concentration* percentage of B)*100%;

(3) Toxicity ratio = Actual inhibition rate/ Theoretical inhibition rate.

The combination effect shows synergistic when toxicity ratio was greater than 1; it shows antagonistic when toxicity ratio was less than 1; it shows additive when toxicity ratio was almost equal 1.

Results

Biosynthesis of AgNPs

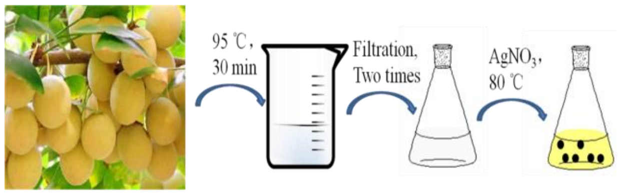
As shown in Fig. 1, the color of diluted filtrate changed from white to yellow after heating at 80°C for 15 min under the condition of 1 mM $AgNO_3$, indicating formation of AgNPs, while the color remained unchanged for filtrate without $AgNO_3$ (Fig. 1a). UV-Vis spectroscopy indicates that maximum absorbance is at 426 nm (Fig. 1b), corresponding to the surface plasmon resonance of AgNPs (Lok *et al*., 2007). However, there were no characteristic absorption peak of silver on account of $AgNO_3$ (green line) or filtrate (red line) alone.

Characterization of AgNPs

TEM analysis: As shown in Fig. 2, characterizations of morphology, dispersion, size and size distribution of AgNPs emerged. Easy to see such synthesized AgNPs were spherical or near spherical with favorable dispersion (Fig. 2a, b and c). In order to determine particle size and size distribution, 200 typical AgNPs were selected randomly from several TEM micrographs. The particle size was in the range of 8~24 nm with average size of about 14 nm. 6.5% of particles distributed between 5~10 nm, 60% of particles distributed between 10~15 nm, while 32%fell between 15~20 nm, and 1.5% between 20~25 nm (Fig. 2d).

SEM and EDX Analysis: When illuminated by SEM, particles appeared on the substrate, which represent AgNPs (Fig. 3a). Peaks at about 3 keV indicate the existence of elemental silver, while other peaks may be attributed to the elements of ginkgo fruit extract and reagents (Fig. 3b). The results showed synthesized AgNPs was pure, and could be used for further experiments.

AFM Analysis: The specified morphological features of the green synthesized nanoparticles were investigated by AFM. All of the particles on the mica were



Scheme 1: Schematic diagram of AgNPs synthesis

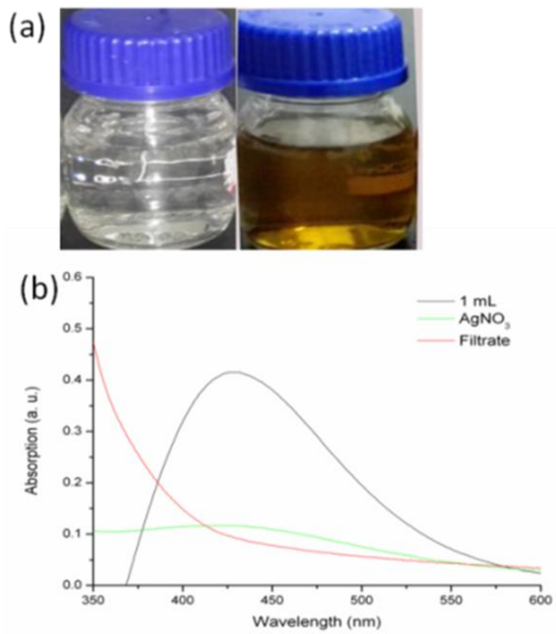


Fig. 1: Green synthesis of AgNPs, based on 1 mL ginkgo fruit extract: (a), color change of diluted filtrate before and after adding 1 mM $AgNO_3$ at 80°C; (b), UV-Vis spectrum of AgNPs

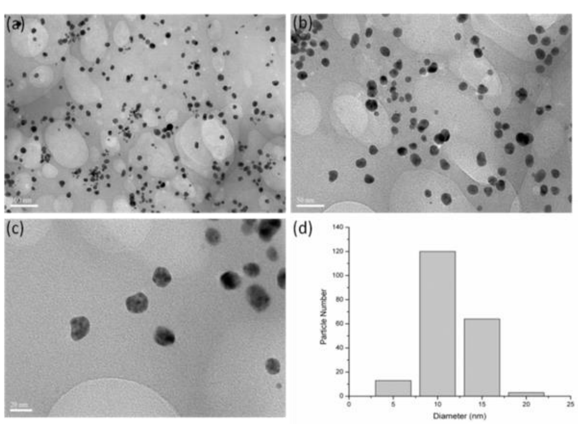


Fig. 2: TEM micrographsat different magnifications (a-c) and size distribution (d) of AgNPs

monodispersed (Fig. 4a), the surface morphology of the sample can be better visualized by their respective 3Dtopographic view (Fig. 4b).

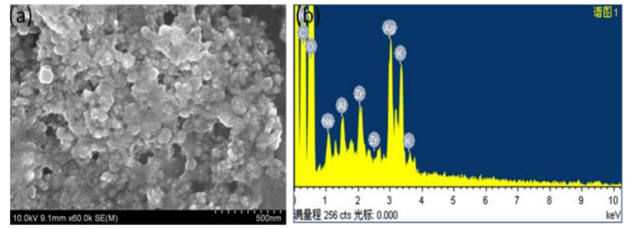


Fig. 3: SEM micrograph (a) and EDX spectroscopy (b) of AgNPs

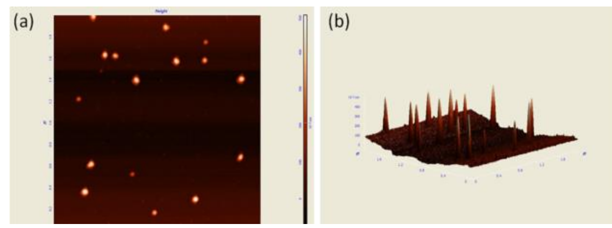


Fig. 4: AFM images of AgNPs synthesized by ginkgo fruit extract: (a), morphological feature of the nanoparticles; (b), 3Dtopographic view of the nanoparticles

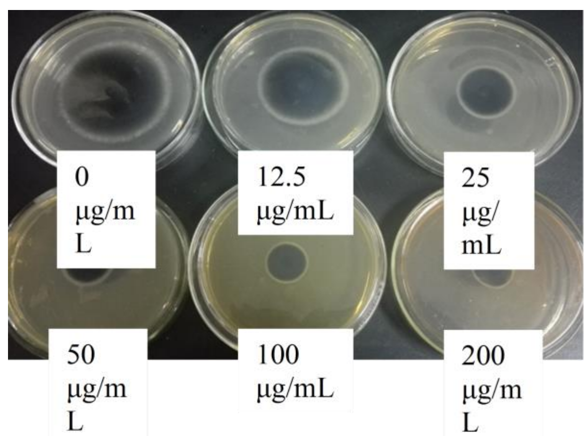


Fig. 5: Inhibition of the colony growth of *B. maydis* by AgNPs with various concentrations

Inhibition Rate of AgNPs

As shown in Fig. 5, AgNPs exhibited a prominent inhibition effect on colony growth. The diameter of *C. lunata* without AgNPs measured by cross method was 6.37 cm, and it decreased gradually with the rise in the concentration of AgNPs. The diameter of the AgNPs reached its minimum value (1.75 cm) at 200 μg/mL. The data from three replicates of the treatments with different concentrations were averaged and used to calculate by SPSS 13.0 the 50% effective concentration (EC_{50}), which was 31.73 μg/mL. The inhibition rate caused by the varied AgNPs concentrations (12.5–200 μg/mL) in the control and experimental treatments of *B. maydis* was in the range 27.6–78.7%.

Synergistic Antifungal Activity of AgNPs and Fungicides

As shown in Table 1, 2 and 3, there exhibited obvious synergistic antifungal activity between AgNPs and three fungicides against *B. maydis*. The combination of AgNPs and tebuconazole showed universal synergistic antifungal effect at various proportions, and the maximum reached 1.33 when the two components were equal. The compounds of AgNPs and propineb presented synergistic antifungal effect at the volume ratio of 8:2 and 9:1, and the maximum reached 1.33 at 9:1, the rest ones were additive or antagonistic. The compounds of AgNPs and fludioxonil showed synergistic antifungal effect at the volume ratio of 7:3, 8:2 and 9:1, and the maximum reached 1.23 at 7:3, the rest ones were additive or antagonistic.

Discussion

In recent years, biosynthesis of AgNPs attracts more and more researchers' attention in view of their unique properties. Among these materials, plant tissues proved to be optimal. As a famous medicinal material, Ginkgo leaf extract was used to prepare AgNPs in previous studies (Song and Kim, 2009; Ren *et al*., 2016; Huang *et al*., 2017b). To our knowledge, it's the first time to synthesize AgNPs using ginkgo fruit extract.

Drug resistance by pathogenic bacteria and fungi has been continuously increasing, so it is necessary to develop new antimicrobial agents. Researches in recent years proved that AgNPs was a promising candidate, and the enhanced antibacterial activity of AgNPs and antibiotics was verified by Li *et al.* (2005) and Dar *et al.* (2013), while synergistic antifungal effect of AgNPs and chemical substances was rarely reported (Monteiro *et al*., 2013).

Conclusion

There appeared synergistic antifungal activity of AgNPs and three different fungicides, and the optimal proportion of each varied with fungicides category. These results would not only provide a new way for pathogens inhibition but also reduce drug resistance to the utmost extent.

Acknowledgements

This work was supported by the Key Discipline of Plant Protection in University of Science and Technology of Anhui (AKZDXK2015C04), Natural Science Fund of Education Department of Anhui Province (KJ2017A510), Talent Introduction Project in Anhui Science and Technology University (NXYJ201602), Outstanding Talent Cultivation Project in Colleges and Universities of Anhui Province (gxbjZD23), Prize in National Crop Variety Regional Experiment Stations Project of Fengyang, and Award Program of Base Construction in Anhui Province (1701r07010008).

Table 1: Toxicity ratio between AgNPs and tebuconazole against *B. maydis*

Volume ratio	Actual inhibition rate (%)	Theoretical inhibition rate (%)	Toxicity ratio
0:10	52.41	52.41	1.00
1:9	63.35	52.00	1.22
2:8	60.05	55.39	1.08
3:7	63.50	55.23	1.15
4:6	63.03	55.07	1.14
5:5	73.08	54.91	1.33
6:4	70.88	54.74	1.29
7:3	67.59	54.58	1.24
8:2	69.78	54.42	1.28
9:1	64.76	54.26	1.19
10:0	48.28	48.28	1.00

Table 2: Toxicity ratio between AgNPs and propineb against *B. maydis*

Volume ratio	Actual inhibition rate (%)	Theoretical inhibition rate (%)	Toxicity ratio
0:10	57.16	57.16	1.00
1:9	45.92	56.17	0.82
2:8	53.48	55.18	0.97
3:7	17.56	54.19	0.32
4:6	20.25	53.20	0.38
5:5	44.03	52.22	0.84
6:4	54.57	51.23	1.07
7:3	51.59	50.24	1.03
8:2	59.70	49.25	1.21
9:1	64.56	48.26	1.34
10:0	47.27	47.27	1.00

Table 3: Toxicity ratio between AgNPs and fludioxonil against *B. maydis*

Volume ratio	Actual inhibition rate (%)	Theoretical inhibition rate (%)	Toxicity ratio
0:10	46.73	46.73	1.00
1:9	43.49	47.27	0.92
2:8	35.39	47.81	0.74
3:7	32.41	48.35	0.67
4:6	49.70	48.89	1.02
5:5	38.90	49.43	0.79
6:4	48.62	49.97	0.97
7:3	62.13	50.51	1.23
8:2	61.32	51.05	1.20
9:1	59.43	51.59	1.15
10:0	52.13	52.13	1.00

References

Alani, F., M. Moo-Young and W. Anderson, 2012. Biosynthesis of silver nanoparticles by a new strain of *Streptomyces* sp. compared with *Aspergillus fumigatus*. *World J. Microbiol. Biotechnol.*, 28: 1081–1086

Asha, A., T. Sivaranjani, P. Thirunavukkarasu and S. Asha, 2016. Green Synthesis of Silver Nanoparticle from Different Plants-A Review. *Int. J. Pure Appl. Biosci.*, 4: 118–124

Borase, H.P., B.K. Salunke, R.B. Salunkhe, C.D. Patil, J.E. Hallsworth, B.S. Kim and S.V. Patil, 2014. Plant Extract: A Promising Biomatrix for Ecofriendly,Controlled Synthesis of Silver Nanoparticles. *Appl. Biochem. Biotechnol.*, 173: 1–29

Castro, L., M.L. Blazquez, F. Gonzalez, J.A. Munoz and A. Ballester, 2015. Biosynthesis of silver and platinum nanoparticles using orange peel extract: characterisation and applications. *IET Nanobiotechnol*., 9: 252–258

Dar, M.A., A. Ingle and M. Rai, 2013. Enhanced antimicrobial activity of silver nanoparticles synthesized by *Cryphonectria* sp. evaluated singly and in combination with antibiotics. *Nanomedicine*, 9: 105–110

Elechiguerra, J.L., J.L. Burt, J.R. Morones, A. Camacho-Bragado, X. Gao, H.H. Lara and M.J. Yacaman, 2005. Interaction of silver nanoparticles with HIV-1. *J. Nanobiotechnol.*, 3: 1–6

Fu, Y., J. Zhang and J.R. Lakowicz, 2008. Reduced blinking and long-lasting fluorescence of single fluorophores coupling to silver nanoparticles. *Langmuir*, 24: 3429–3433

Fu, Y., J. Zhang and J.R. Lakowicz, 2007. Plasmonic enhancement of single-molecule fluorescencenear a silver nanoparticle. *J. fluoresc*., 17: 811–816

Garg, P. and S. Dhara, 2013. Single molecule detection using SERS study in PVP functionalized Agnanoparticles. *AIP Conf. Proc*., 1512: 206–207

Gil, R. and G.W. Lucassen, 2010. SERRS-based detection of dye-labeled DNA usingpositively-charged Ag nanoparticles. *Anal. Meth*., 2: 445–447

Han, C., K. Xu, Q. Liu, X. Liu and J. Li, 2014. Colorimetric sensing of cysteine using label-free silver nanoparticles. *Sensor Actuat. B-Chem.*, 202: 574–582

Horsfall, J.G., 1945. *Fungicides and their action*. Chronica Botanica Co., Waltham, Mass

Huang, W.D., J.J. Yan, Y. Wang, C.L. Hou, T.C. Dai and Z.M. Wang, 2013. Biosynthesis of Silver Nanoparticles by *Septoria apii* and *Trichoderma koningii*. *Chin. J. Chem*., 31: 529–533

Huang, W.D., Y. Bao, H.M. Duan, Y.L. Bi and H.B. Yu, 2017a. Antifungal effect of green synthesized silver nanoparticles against *Setosphaeria turcica*. *IET Nanobiotechnol*., 11: 803–808

Huang, W.D., X.Y. Chen, H.M. Duan, Y.L. Bi and H.B. Yu, 2017b. Optimized Biosynthesis and Antifungal Activity of *Osmanthus fragrans* Leaf Extract-mediated Silver Nanoparticles. *Int. J. Agric. Biol.*, 19: 668–672

Kalimuthu, K., R.S. Babu, D. Venkataraman, M. Bilal and S. Gurunathan, 2008. Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. *Colloids Surf. B: Biointerf.*, 65: 150–153

Kasprowicz, M.J., M. Koziol and A. Gorczyca, 2010. The effect of silver nanoparticles onphytopathogenic spores of *Fusarium culmorum. Can. J. microbiol*., 56: 247–253

Kathiraven, T., A. Sundaramanickam, N. Shanmugam and T. Balasubramanian, 2015. Green synthesis of silver nanoparticles using marine algae *Caulerpa racemosa* and their antibacterial activity against some human pathogens. *Appl. Nanosci.*, 5: 499–504

Khatami, M. and S. Pourseyedi, 2015. *Phoenix dactylifera* (date palm) pit aqueous extract mediated novel route for synthesis high stable silver nanoparticles with high antifungal and antibacterial activity. *IET Nanobiotechnol*., 9: 184–190

Khatami, M., R. Mehnipor, M.H.S. Poor and G.S. Jouzani, 2016. Facile Biosynthesis of Silver Nanoparticles Using *Descurainia sophia* and Evaluation of Their Antibacterial and Antifungal Properties. *J. Clust. Sci.*, 27: 1601–1612

Kirubaharan, C.J., D. Kalpana, Y.S. Lee, A.R. Kim, D.J. Yoo, K.S. Nahm and G.G. Kumar, 2012. Biomediated Silver Nanoparticles for the Highly Selective Copper (II) Ion Sensor Applications. *Ind. Eng. Chem. Res.*, 51: 7441–7446

Kumari, A., A. Guliani, R. Singla, R. Yadav and S.K. Yadav, 2015. Silver nanoparticles synthesized using plant extracts show strong antibacterial activity. *IET Nanobiotechnol*., 9: 142–152

Lamsal, K., S.W. Kim, J.H. Jung, Y.S. Kim, K.S. Kim and Y.S. Lee, 2011. Inhibition Effects of Silver Nanoparticles against Powdery Mildews on Cucumber and Pumpkin. *Mycobiology*, 39: 26–32

Lara, H.H., N.V. Ayala-Nunez, L. Ixtepan-Turrent and C. Rodriguez-Padilla, 2010. Mode of antiviral action of silver nanoparticles against HIV-1. *J. Nanobiotechnol*., 8: 1–6

Li, P., J. Li, C.Z. Wu, Q.S. Wu and J. Li, 2005. Synergistic antibacterial effects ofβ-lactam antibiotic combined with silvernanoparticles. *Nanotechnology*, 16: 1912–1917

Lok, C.N., C.M. Ho, R. Chen, Q.Y. He, W.Y. Yu, H. Sun, P.K. Tam, J.F. Chiu and C.M. Che, 2007. Silver nanoparticles: partial oxidation and antibacterial activities. *J. Biol. Inorg. Chem*., 12: 527–534

Lu, L., R.W.Y. Sun, R. Chen, C.K. Hui, C.M. Ho, J.M. Luk, J.K.K. Lau and C.M. Che, 2008. Silver nanoparticles inhibit hepatitis B virus replication. *Antivir. Ther.*, 13: 253–262

Mohammad, H., S. Poor, M. Khatami, H. Azizi and Y. Abazari, 2017. Cytotoxic activity of biosynthesized Ag Nanoparticles by *Plantago major* towards a human breast cancer cell line. *Rend. Lincei*, 28: 693–699

Mohammadlou, M., H. Maghsoudi and H. Jafarizadeh-Malmiri, 2016. A review on green silver nanoparticles based on plants: Synthesis, potentialapplications and eco-friendly approach. *Inter. J. Food Res.*, 23: 446–463

Monteiro, D.R., S. Silva, M. Negri, L.F. Gorup, E.R. de Camargo, R. Oliveira, D.B. Barbosa and M. Henriques, 2013. Antifungal activity of silver nanoparticles in combination withnystatin and chlorhexidine digluconate against *Candida albicans* and *Candida glabrata* biofilms. *Mycoses*, 56: 672–680

Morrones, J.R., J.L. Elechiguerra, A. Camacho, K. Hot, J.B. Kouri, J.T. Ramirez and M.J. Yacaman, 2005. The bactericidal effect of silver nanoparticles, *Nanotechnology*, 16: 2346–2353

Nayak, D., S. Ashe, P.R. Rauta and B. Nayak, 2015. Biosynthesis, characterisation and antimicrobial activity of silver nanoparticles using *Hibiscus rosa-sinensis* petals extracts. *IET Nanobiotechnol*., 9: 288–293

Osuwa, J.C. and P.C. Anusionwu, 2011. Some advances and prospects in nanotechnology: a review. *Asian J. Inform. Technol.*, 10: 96–100

Ping, H., M. Zhang, H. Li, S. Li, Q. Chen, C. Sun and T. Zhang, 2012. Visual detection of melamine in raw milk by label-free silver nanoparticles. *Food Contr.*, 23: 191–197

Ren, Y.Y., H. Yang, T. Wang and C. Wang, 2016. Green synthesis and antimicrobialactivity of monodisperse silver nanoparticles synthesized using *Ginkgo biloba* leaf extract. *Phys. Lett. A*, 380: 3773–3777

Shetty, P., N. Supraja, M. Garud and T.N.V.K.V. Prasad, 2014. Synthesis, characterization and antimicrobial activity of *Alstonia scholaris* bark-extract-mediated silver nanoparticles. *J. Nanostr. Chem*., 4: 161–170

Song, J.Y. and B.S. Kim, 2009. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. *Bioproc. Biosyst. Eng*., 32: 79–84

Sotiriou, A. and S.E. Pratsinis, 2010. Antibacterial Activity of Nanosilver ions and particles. *Environ. Sci. Technol.*, 44: 5649–5654

Wang, G.Q. and L.X. Chen, 2009. Aptameric SERS sensor for Hg^{2+} analysis using silvernanoparticles. *Chin. Chem. Lett*., 20: 1475–1477

(Received 29 November 2017; Accepted 28 December 2017)