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ANTIFUNGAL ACTIVITY OF BIOSYNTHESIZED SILVER NANOPARTICLES AGAINST THREE MAIZE LEAF PHYTOPATHOGENS

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Abstract

Bamboo leaf extract was applied to biosynthesize silver nanoparticles in condition of silver nitrate ($AgNO_3$). Several parameters like extract volume, concentration of $AgNO_3$, and potential of hydrogen (pH) were carried out to confirm the optimized synthesis system. Synthesized silver nanoparticles were characterized through UV-Vis spectrophotometer, transmission electron microscope (TEM), scanning electron microscope (SEM), and energy dispersive X-ray detector (EDX). In addition, inhibition effect of silver nanoparticles towards three maize leaf phytopathogens of *Bipolaris maydis*, *Exserohilum turcicum*, and *Curvularia lunata* was determined. The optimized biosynthesis condition included 15 mL extract, 8 mM $AgNO_3$ at pH 7. The obtained nanoparticles with an average size of 13 nm demonstrated prominent antifungal activity against the three phytopathogens, and the highest inhibition rate reached 93.6% at 200 μg/mL of silver nanoparticles. In addition, when the concentration reached 100 μg/mL the conidia germination of these three pathogens was completely inhibited. The results obtained here provide a neotype pathway for comprehensive control phytopathogens, and it is possible to screen one kind of novel bacteriostatic agents.

Key words: Antifungal activity, Silver nanoparticles, Maize leaf pathogens.

Introduction

In ancient time, silver and its components were used to eliminate inflammation, heal wounds, and ease pain. However, the antimicrobial properties of silver has not fully revealed until rapid development of nanotechnology. Unlike traditional materials, materials at nanoscale possess unique specialities such as physical, chemical, and optical owing to their extremely high activity (You *et al*., 2012; Hajipour *et al*., 2017). For the past few years, preparation, characterization, and use of silver nanoparticles become more widespread. On the whole, there existed three primary synthesis approaches. One is physical method (Nadagoud *et al*., 2011; Zhang *et al*., 2016), another is chemical method (Raja *et al*., 2017; Nasretdinova *et al*., 2017), and the last one is biological method (Hebbalalu *et al*., 2013; Borase *et al*., 2014). Several defects have been proved for physical and chemical approaches, including energy dissipation, complicated operation, requirement of poisonous chemical reagents, etc (Huang *et al*., 2013; Singh *et al*., 2016). In view of these disadvantages, biological method mediated by bacteria (Gurunathan *et al*., 2009; Kalishwaralal *et al*., 2010), actinomycetes (Manivasagan *et al*., 2013), fungi (Qian *et al*., 2013; Huang *et al*., 2018), and plant tissues (Khatami & Pourseyedi, 2015; Logarnjan *et al*., 2016; Javad *et al*., 2017) is coincident with green chemistry. Among these synthesis materials, plant tissues are considered as ideal ones on account of less time, better stability, and large-scale preparation.

As is known to us, various plant diseases that affect agricultural production throughout the growth period, leading to serious economic losses. It's the first choice to use chemical pesticides for agricultural producers. Throughout the ages, excessive and irrational use of chemical pesticides for so long caused environmental pollution, pesticide residue, and drug resistance (Huang *et al*., 2017a; Leadbeater, 2015). In consideration of these problems, it's necessary to exploit neotype substances to replace or assist existing pesticides. During the past decades, silver nanoparticles were confirmed as an efficient antimicrobial agent against bacteria (Velmurugan *et al*., 2014; Khatami *et al*., 2016), fungi (Huang *et al*., 2017b; Khatami *et al*., 2017), and viruses (Elechiguerra *et al*., 2005; Lara *et al*., 2010). It's worth mentioning that the antimicrobial activity of silver nanoparticles revealed three characteristics, wide spectrum, high efficiency, and long lasting. In addition, as a non-antibiotic substance, silver nanoparticles could avoid the appearance of drug-resistant pathogens to the utmost extent (Iqbal & Bakht, 2019).

In this research, the Leaf extract of *bamboo* was used to biosynthesize silver nanoparticles, and the optimized biosynthetic process was ascertained by regulating extract volume, concentration of $AgNO_3$, and solution pH. UV-Vis spectrophotometer, TEM, SEM, and EDX were conducted to characterize synthetic silver nanoparticles to reveal their particular information. In addition, the antifungal effect of silver nanoparticles against three phytopathogens occurred on maize leaf was measured by agar well diffusion, inhibition rate, and conidia germination.

Materials and Methods

Microorganism strains: The three microorganism strains used here were *Bipolaris maydis*, *Exserohilum turcicum*, and *Curvularia lunata* separated from pathogenic *Zea mays* leaves in Fengyang, Anhui province, China. The monospore strains were conserved in the Environmental and Ecological laboratory.

Production of plant leaf filtrate: *Bamboo* leaves were fully flushed using sterile water and dried in aseptic condition, followed by cutting into small fragments. About 10 g of leaf was appended to a blue cap bottle containing 100 mL of deionized water, boiling at 100°C for 20 min. Then, the extract was filtered through Whatman No. 1 filter paper and conserved at 4°C.

Optimized biosynthesis of silver nanoparticles: Volume ratio (1: 9) of filtrate and deionized water was mixed to synthesize silver nanoparticles. The mixture was then reacted with $AgNO_3$ at 60°C. Solution color change indicated generating of silver nanoparticles. The optimization process was conducted, it included varied volumes of leaf extract from 5 to 50 mL, concentrations of $AgNO_3$ between 1 to 8 mM, and pH value from 3 to 11. UV-Vis absorbance spectra were recorded to verify silver nanoparticles gained by such conditions.

Characterization of silver nanoparticles: After incubation for 10 min, 3 mL of cool solution was measured through UV-Vis spectrometer (TU-1950) at the range of 350 nm to 600 nm, respectively. For TEM (JEM-2100F) analysis, 20 µL of colloid was dripped on copper substrate, and dried completely. About 30 µL of colloid was added and scanned after coating with platinum for SEM (S-4800). EDX was applied to analyze the components of synthesized silver colloid.

Antifungal activity of silver nanoparticles

Agar well diffusion assay: About 150 µL of conidia suspension at the density of 10^6/mL was smeared evenly on PDA (Potato Dextrose Agar) mediums. Three wells were made with sterile borer into inoculated agar mediums. About 30 µL of different treatments of silver nanoparticles (100 and 200 µg/mL) and *bamboo* filtrate were appended into the agar wells. The mediums were thermostatic incubated 3 d at 28°C after setting aside for 5 min. Each treatment was conducted three replicates.

Colony growth inhibition: Synthetic silver nanoparticles were centrifuged at 14000 r/min for 30 min, and Oven-dried at 80°C. A stock solution of 10 mg/mL was made by dissolving obtained powder in sterile water. A volume ratio (1: 9) of diluted stock solution and PDA medium was mixed uniformly at about 55°C, and the final concentrations of silver nanoparticles were settled between 12.5 and 200 µg/mL multiplied by two times. The solution added equal volume of sterile water with no silver nanoparticles as control. In the middle of each medium containing varied concentrations of silver nanoparticles, one fungus block with diameter of 8 mm was inoculated, and then incubated 5 d at 28°C. Three replicates were conducted for each.

Conidia germination effect: The density of conidia was regulated to 10^6/mL by a hemocytometer. Then, conidia suspensions containing varied concentrations of silver nanoparticles (12.5-200 µg/mL) and sterile water were transfered into sterile centrifugal tubes at 1:9 (v/v), respectively. Finally, the mixed culture was incubated 1-2 d at 25°C. Conidia germination of each treatment was recorded by a microscope (100 X).

Results

Green synthesis of silver nanoparticles: Fig. 1 showed the color of plant extract turned from yellow green into light brown in condition of 1 mM $AgNO_3$, reflecting production of silver nanoparticles. UV-Vis spectra revealed that there was a strong resonance peak at 462 nm (Fig. 1b), and synthesis of silver nanoparticles was further confirmed (Khatami *et al*., 2018). It is worth mention that synthetic solution emerged different color and corresponding absorption peak change according to various synthesized materials and parameters.

Optimized green synthesis of silver nanoparticles

Influence of varied volumes of leaf extract: The solution color altered from light brown to deep brown as the extract volume increased from 5 mL to 50 mL (Fig. 2a). From 5 mL to 50 mL, maximum absorption increased, and it reached the maximum at 50 mL (Fig. 2b). As a result, 50 mL is optimum volume of *bamboo* leaf extract for this optimization procedure. On the whole, the optimal extract volume varied according to different plant species and tissues.

Influence of different concentrations of $AgNO_3$: The solution color blackened with increasing concentration of $AgNO_3$ from 1 mM to 8 mM (Fig. 3 a). The corresponding absorbance peak increased gradually, and it reached the maximum when the concentration was 8 mM (Fig. 3b). As a result, 8 mM is optimum concentration of $AgNO_3$ for this optimization procedure. Concentration of $AgNO_3$ is an important parameter that affects formation of silver nanoparticles, optimal concentration could not only synthesize more nanoparticles but maintain it in favorable dispersibility.

Influence of different pH values: It's found that the pH value influenced production of silver nanoparticles. Solution with different pH present various color. For this experiment, solution presented pale brown when its pH was 3 and 5, while it darkened as pH increased to 7 or higher (Fig. 4a). Corresponding spectra exhibited that the maximum absorbance was increased gradually from 3 to 11, while the spectra of solution 9 and 11 were notoscale (beyond 9.99) and was prone to aggregation. As a result, the optimized solution pH was 7.

Characterization of silver nanoparticles

TEM detection: As shown in Fig. 5, the synthetic silver nanoparticles were near spherical (Fig. 5a). In order to definite more details of particles, 200 particles were selected and measured randomly. The result showed that the particles were in the range of 3 to 34 nm, and the average diameter was around 13 nm (Fig. 5b).

SEM and EDX analysis: As shown in Fig. 6a, plenty of particles were visible on the substrate. At the location of about 3 Kev, there existed several peaks of silver. Element Pt should be the gold plating before scanning, the other peaks might be owing to Cu grid and components of *bamboo* leaf extract.

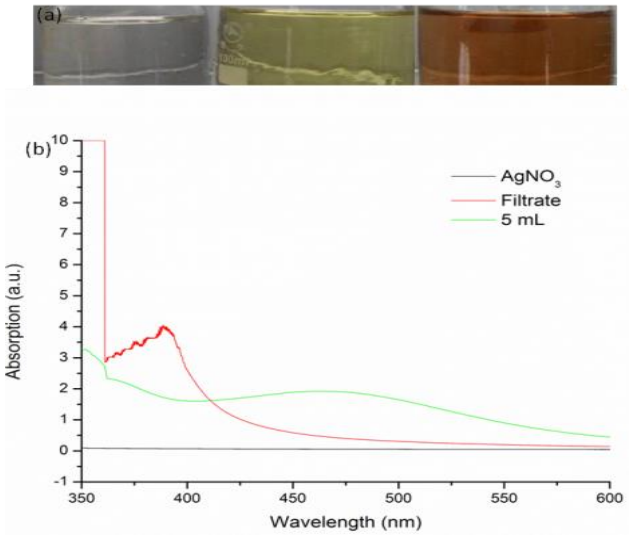

Fig. 1. Biosynthesis of Silver nanoparticles. (a), Color change of diluted extract with and without 1 mM $AgNO_3$; (b), Relevant full-wave absorption spectra.

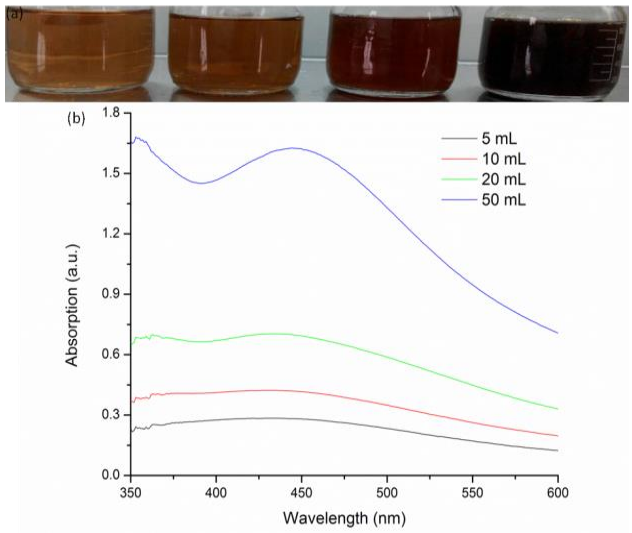

Fig. 2. Influence of different volumes of *bamboo* leaf extract on silver nanoparticles formation. (a), Color of silver nanoparticles on account of 5 to 50 mL of leaf extract. (b), Relevant full-wave absorption spectra.

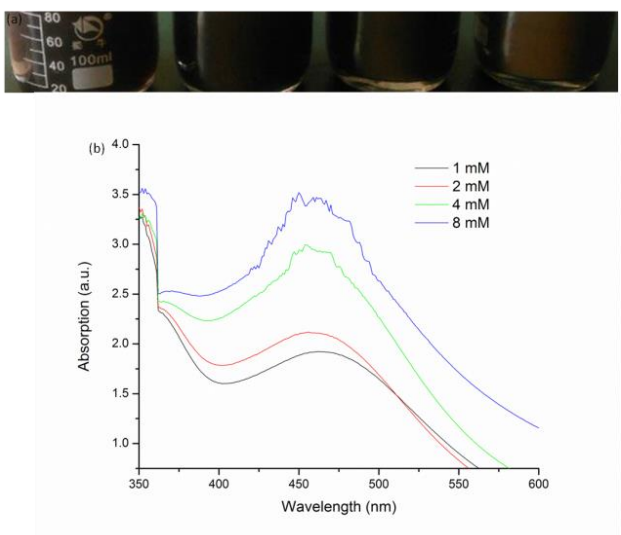

Fig. 3. Influence of different concentrations of $AgNO_3$ on Silver nanoparticles production. (a), Color of silver nanoparticles with 1 to 8 mM $AgNO_3$. (b), Relevant full-wave absorption spectra.

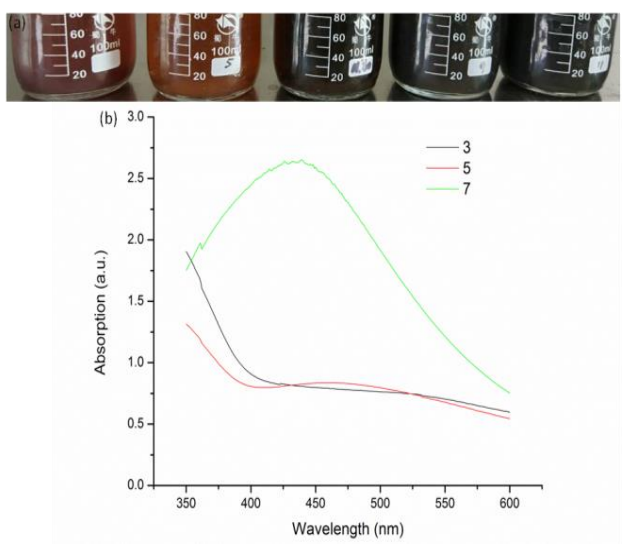

Fig. 4. Influence of varied pH values on Silver nanoparticles production. (a), Color of silver nanoparticles with pH of 3 to 11. (b), Relevant full-wave absorption spectra.

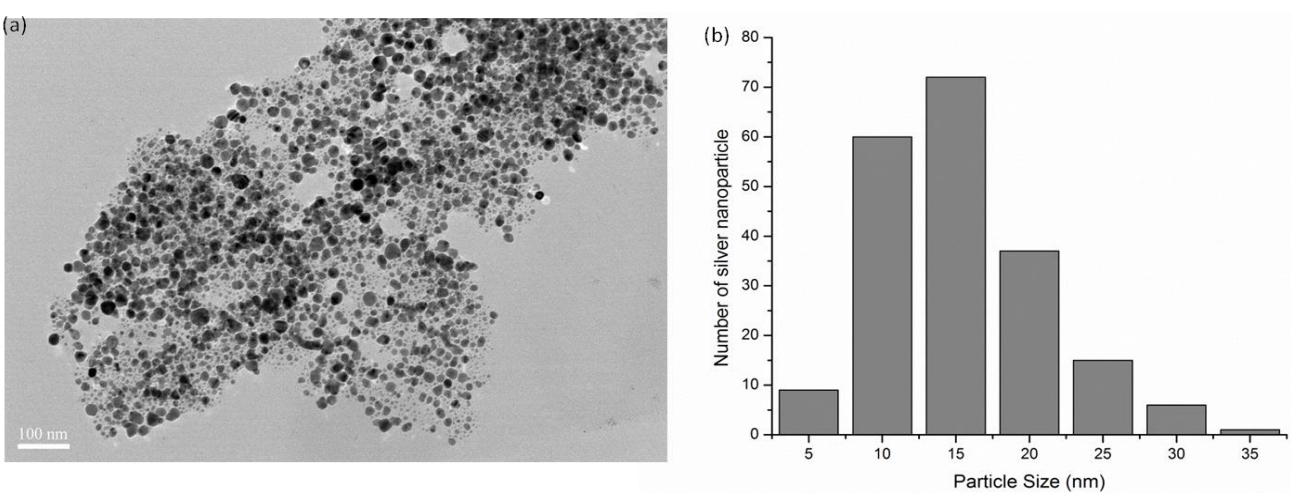

Fig. 5. TEM image (a) and size distribution (b) of silver nanoparticles.

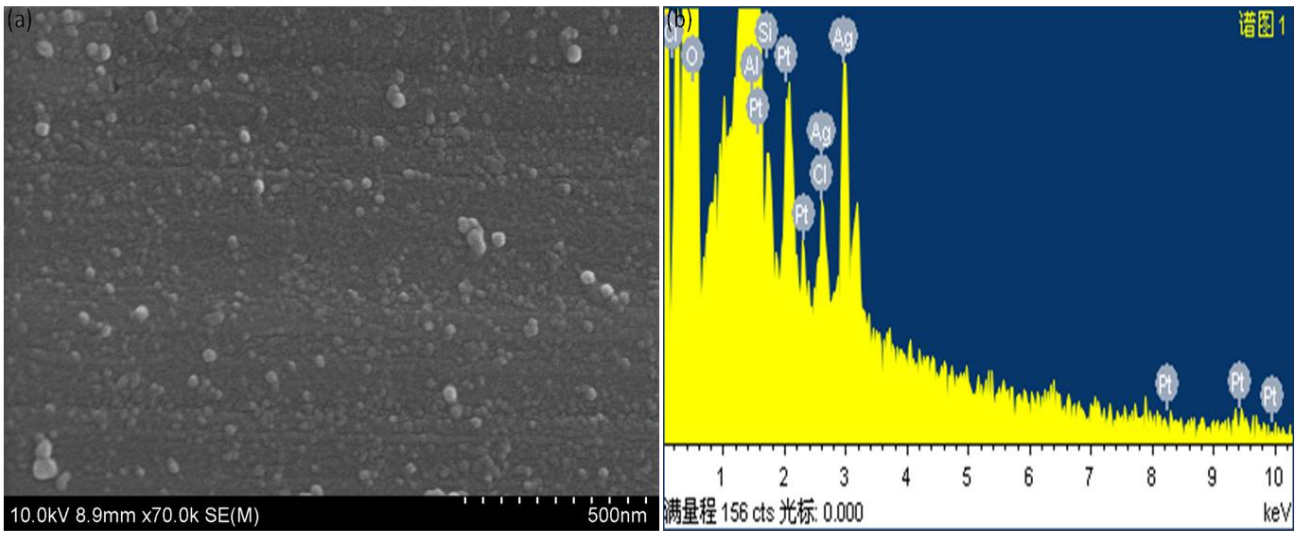

Fig. 6. SEM image (a) and EDX spectrum (b) of silver nanoparticles.

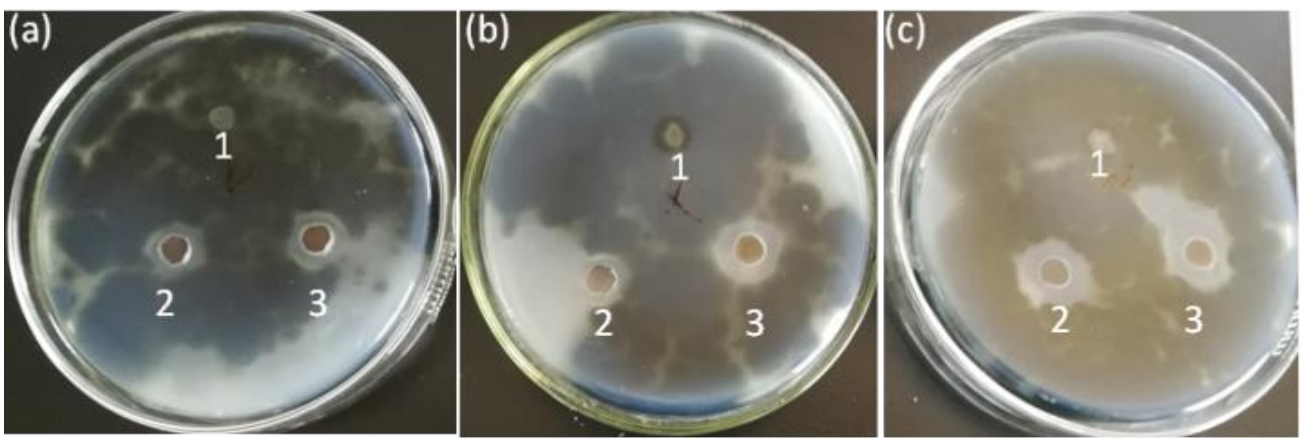

Fig. 7. Inhibition zones caused by silver nanoparticles against *E. turcicum* (a), *B. maydis* (b), and *C. lunata* (c). In each plate, the labels of 1, 2, and 3 correspond to leaf extract, silver nanoparticles of 100 and 200 µg/mL.

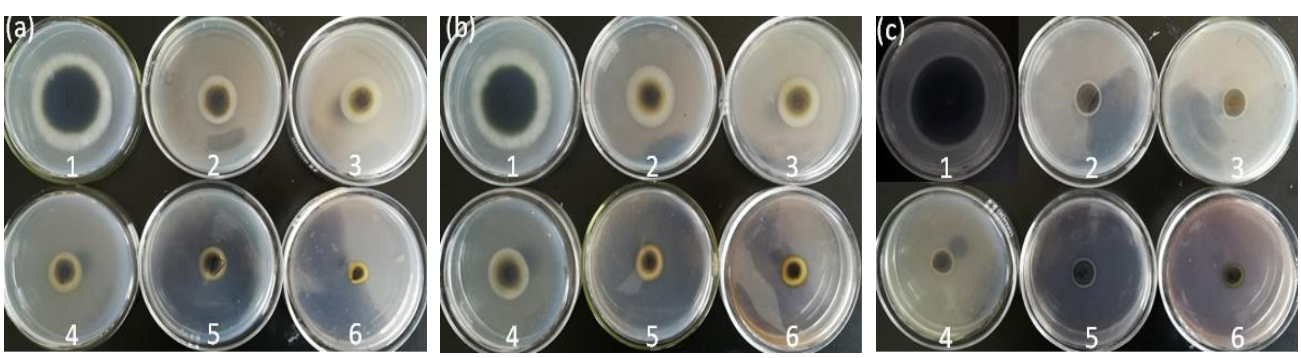

Fig. 8. Colony growth inhibition of silver nanoparticles against *E. turcicum* (a), *B. maydis* (b), and *C. lunata* (c). In each plate, the labels of 1, 2, 3, 4, 5, and 6 correspond to the concentration of silver nanoparticles was 0, 12.5, 25, 50, 100, and 200 µg/mL, seperately.

Antifungal activity of silver nanoparticles against three phytopathogens

Agar well diffusion study: Fig. 7 indicated great antifungal effect of silver nanoparticles against three phytopathogens through agar well diffusion assay. In each plate, apparent inhibition zones emerged corresponding to varied concentrations of nanoparticles. The inhibition zone of 100 µg/mL silver nanoparticles against *E. turcicum, B. maydis, and C. lunata* was 11, 12, and 14 mm, respectively. It was 12, 13, and 16 mm when it was 200 µg/mL. Nevertheless, it appeared no inhibition zone when the treatment was leaf extract. The result showed that *C. lunata* was more sensitive to synthetic silver nanoparticles among the three tested maize leaf pathogens.

Inhibition rate measurement: Fig. 8 showed excellent antifungal activity of silver nanoparticles against colony growth of three tested phytopathogens. The diameter of colony reduced step by step along with growing concentration of silver nanoparticles (label 1-6), and it achieved a minimum value at 200 µg/mL. The inhibition rate caused by varied concentrations (12.5-200 µg/mL) of silver nanoparticles against *E. turcicum*, *B. maydis*, and *C. lunata* was in the range of 52.17-80.43%, 60.42-83.33%, and 77.08-93.75%. *C. lunata* was further confirmed as the most sensitive pathogen to silver nanoparticles.

Effect on conidia germination: As shown in Fig. 9, silver nanoparticles influenced conidia germination of *E. turcicum*, *B. maydis*, and *C. lunata*. For control, the germination rate was 94.33%, 95.00%, and 90.33%. However, it reduced sharply with increased concentration of silver nanoparticles. To be specific, conidia germination of *C. lunata* was absolutely inhibited at 50 µg/mL, and those of the other two were inhibited totally at 100 µg/mL. Conidia germination is the key process for pathogen invasion, high inhibition rate at low concentration of silver nanoparticles against conidia germination could induce effective pathogen quantity even avoid its infection on plants.

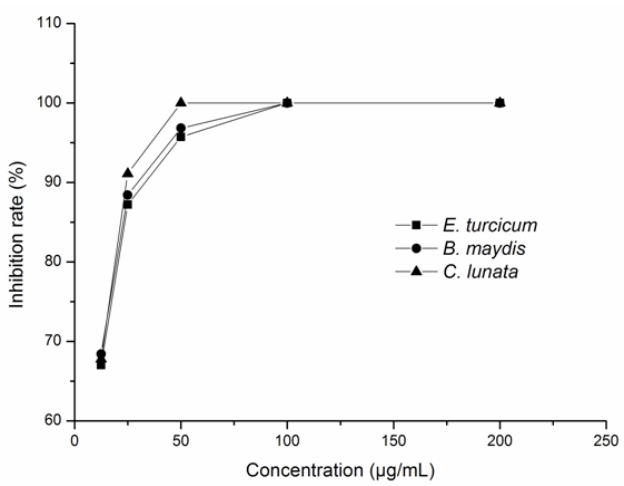

Fig. 9. Effect of silver nanoparticles on spores germination of *E. turcicum*, *B. maydis*, and *C. lunata*.

Discussion

Preparation of nanoparticles based on plant tissues is considered as an eco-friendly synthesis approach, and more and more researchers focus on production, manifestation, and use of nanoparticles. As one kind of important metal nanoparticles, silver nanoparticles could be synthesized by *bamboo* leaf extract in short time, and optimal synthesis condition was ascertained in this experiment. Results of agar well diffusion assay, inhibition rate measurement, and conidia germination inhibition showed that optimized silver nanoparticles exhibited prominent antifungal activity against three tested pyhtopathogens occurred on maize leaf.

Since the past decades, nanomaterials serve an important role in many areas, and that will not be separated from production and our daily life (Hajipour *et al.,* 2017). Among the three main synthesis approaches, biosynthesis method based on plant tissues is proved to be the ideal one owing to its particular advantages. So far as we know, *bamboo* leaf extract was applied to synthesize silver nanoparticles, and the optimized biosynthesis condition was determined for the first time.

Antimicrobial effect of metal nanoparticles especially for silver nanoparticles has been widely described (Khatami *et al*., 2015; Khatami *et al*., 2018). It proved that the antimivrobial effect of the same silver nanoparticles against various pathogens differentiated (Sarsar *et al*., 2016), and silver nanoparticles produced through different approaches, microbes or plant species showed diverse inhibition effect even against the same pathogen (Khatami *et al*., 2015; Khatami *et al*., 2016). During the growth period of maize, there are several phytopathogens caused leaf diseases that do severe loss in yield such as *E. turcicum, B. maydis, and C. lunata.* The inhibition rate of optimized synthetic silver nanoparticles based on bamboo leaf extract against tested three phytopathogens was higher than 80%. The result showed prominent antifungal effect against such three leaf phytopathogens, and it will provide a novel avenue to manage these plant diseases.

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