Hindawi
Journal of Nanomaterials
Volume 2020, Article ID 9535432, 7 pages
https://doi.org/10.1155/2020/9535432

Research Article

Synergistic Antifungal Activity of Green Synthesized Silver Nanoparticles and Epoxiconazole against *Setosphaeria turcica*

Weidong Huang, Minhui Yan, Haiming Duan, Yaling Bi, Xinxin Cheng, and Haibing Yu

College of Agriculture, Anhui Science and Technology University, Fengyang 233100, China

Correspondence should be addressed to Haibing Yu; haibing50721@163.com

Received 21 November 2019; Accepted 31 December 2019; Published 12 February 2020

Academic Editor: Thathan Premkumar

Copyright © 2020 Weidong Huang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

It is urgent to develop highly efficient and eco-friendly antimicrobial agents for integrated control of phytopathogens. Silver nanoparticles (AgNPs) were synthesized by *Ligustrum lucidum* leaf extract. UV-vis spectrum showed that there was a strong absorbance at 438 nm. Transmission electron microscopy (TEM) images displayed that synthesized nanoparticles were near spherical with an average size of 13 nm. The antimicrobial effect of AgNPs was evaluated through methods of paper disk diffusion, colony growth, conidia germination, and in vitro inoculation. The 50% inhibition concentration (IC_{50}) of AgNPs against *Setosphaeria turcica* was 170.20 μg/mL calculated by SPSS 13.0. In addition, it displayed a significant synergistic antifungal effect when AgNPs were combined with epoxiconazole at the ratios of 8 : 2 and 9 : 1. The results of this study provide a novel fungistat not only for comprehensive control of plant fungi but also for reducing chemical pesticides use and avoiding drug-resistant phytopathogen generation.

1. Introduction

Silver was known as one kind of efficient disinfectors in the early 19th century. From then on, it was widely applied in the fields of bacteria inhibition, wound dressing, mildew preservation, etc. [1, 2]. However, the disadvantage of silver-like inactivation caused by complexation or precipitation limited its application to some extent [3]. Nanomaterials that are defined in the range of 1-100 nm have been proved to resolve such problem. At nanoscale, particles exhibited unique physical, optical, chemical, and other properties that differ from their bulk counterparts [4, 5].

In the past few years, silver nanoparticles (AgNPs) were verified to inhibit hundreds of pathogens including bacteria, fungi, and virus [6, 7]. In view of the prominent antimicrobial effect, more and more researchers turned their eyes to the synthesis and application of AgNPs. On the whole, physical, chemical, and biological methods could be used to synthesize AgNPs; among these ones, biological approach based on living organisms are proved to be the most popular. It is reported that lots of microorganisms and plant tissues were used to biosynthesize AgNPs [8–13].

Pathogen management is never ceased no matter what occurred in agriculture or medicine, and chemical drugs are always the first choice. However, environmental pollution, drug residue, and drug resistance become more and more prevalent due to long-term, plentiful, and unreasonable use of chemical reagents. Nowadays, it is a great challenge and people are devoting themselves to explore novel approaches to control these diseases in a more scientific way. It seems to provide the possibility to relieve such questions with the development of nanotechnology [14, 15]. It is reported that AgNPs exhibited prominent antimicrobial activity against drug-resistant pathogens [16, 17]. There are two main strategies to limit the use of chemicals: one is using a certain concentration of AgNPs alone to replace chemical use, with lots of articles reported their significant antimicrobial effect against various pathogens [18–21], and the other is conjugating AgNPs and chemicals to assist antibiotics like amoxicillin [22], kanamycin [23], ampicillin [24], and amphotericin [25]. In spite of the significant antimicrobial effect of AgNPs proved by lots of researchers, the mechanism of the toxicity for AgNPs has not been settled until now. Previous reports related to it include membrane damage [26, 27], generation

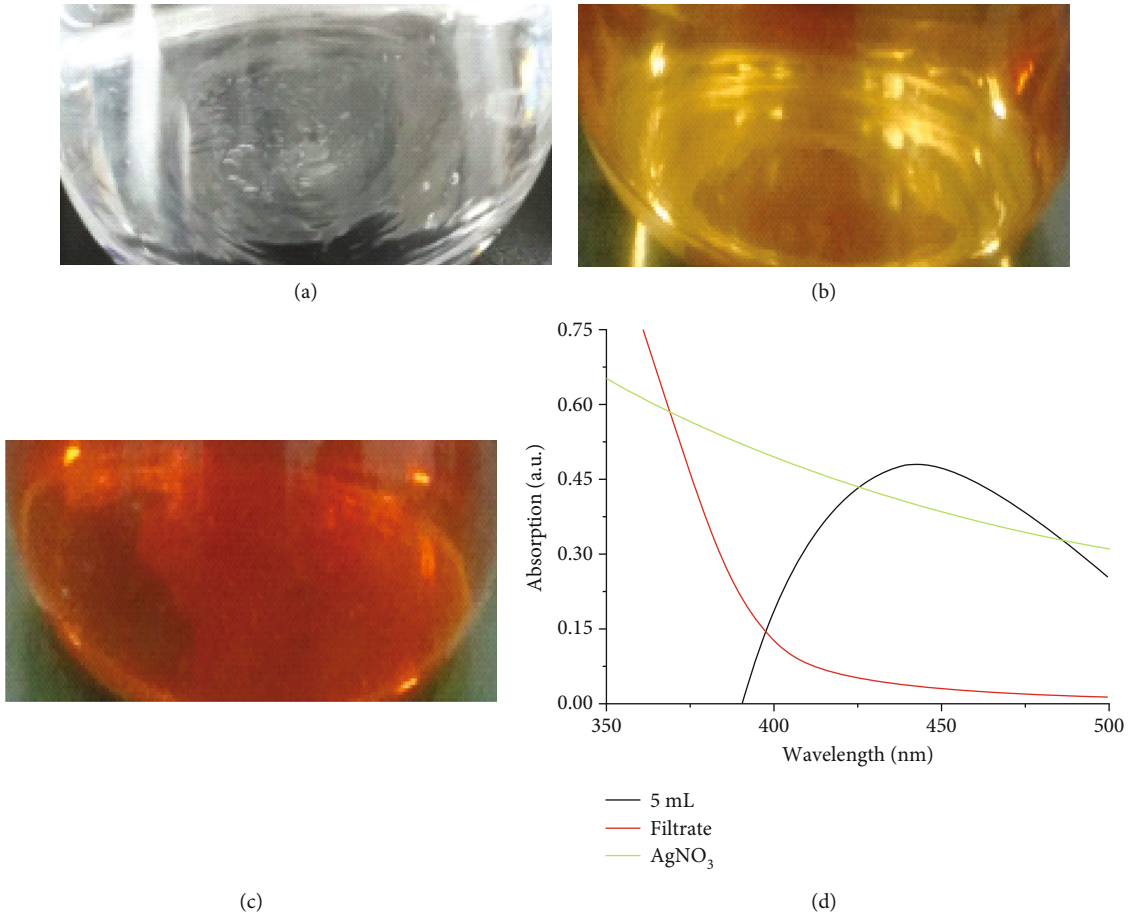


Figure 1: Biosynthesis of AgNPs based on 5 mL of *Ligustrum lucidum* leaf extract. (a) $AgNO_3$ solution, (b) leaf filtrate, (c) leaf extract with 1 mM $AgNO_3$, and (d) corresponding UV-vis absorption spectra.

of reactive oxygen species (ROS) [28, 29], and dissolution of Ag ions (Ag^+) [30, 31]. In recent years, there is a tendency to accept the viewpoint that degree of dissolution and delivery of Ag^+ made a great contribution to the antimicrobial activity of AgNPs [32].

Although there were lots of articles about the combination of AgNPs and chemicals, focusing on bacteriostatic agents, fungistats were rarely reported [33, 34]. In this study, the antifungal activity of AgNPs biosynthesized by *Ligustrum lucidum* leaf extract against *Setosphaeria turcica* (*S. turcica*) was evaluated alone, and the synergistic inhibition effect was also measured at various conjugation ratios of AgNPs and epoxiconazole. These results would provide a reference for the integrative control of phytopathogens, and it also paves the way to explore nanopesticides to assist or replace chemical pesticides.

2. Materials and Methods

2.1. Material and Reagent. *Ligustrum lucidum* leaves were gathered in the campus of Anhui Science and Technology University, China. Silver nitrate ($AgNO_3$) was purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Epoxiconazole was purchased from Jiangsu Dongbao Agrochemical Co. Ltd. The single spore isolate named S. turcica was preserved in the Plant Protection Laboratory of Anhui Science and Technology University.

2.2. Biosynthesis and Characterization of AgNPs. 10 g of thoroughly washed dry *Ligustrum lucidum* leaves was taken into 100 mL deionized water, followed by heating at 95°C for 30 min. Then the solution was filtrated by a Whatman No. 1 paper and a Millipore filter ($\varphi = 0.22\ \mu$m). AgNPs were biosynthesized by mixing 20 mL filtrate and 1 mM $AgNO_3$, and the total volume was 100 mL. It was heated at 80°C until the solution colour changed. UV-vis spectroscopy (TU-1950, PERSEE, China) and transmission electron microscopy (TEM, JEM-2100F, JEOL, Japan) were applied to characterize synthesized nanoparticles.

2.3. Antifungal Activity of AgNPs. Antifungal activity of AgNPs against *S. turcica* was conducted through paper disk diffusion, colony growth, conidia germination, and in vitro inoculation.

2.3.1. Paper Disk Diffusion Assay. The diameter of the inhibition zone created on an agar plate reflected the antimicrobial effect of AgNPs. About 100 μL conidial suspension (10^6/mL) was uniformly spread on potato dextrose agar (PDA) plates. Then, 10 μL of varied concentrations (12.5, 25, 50, 100, and

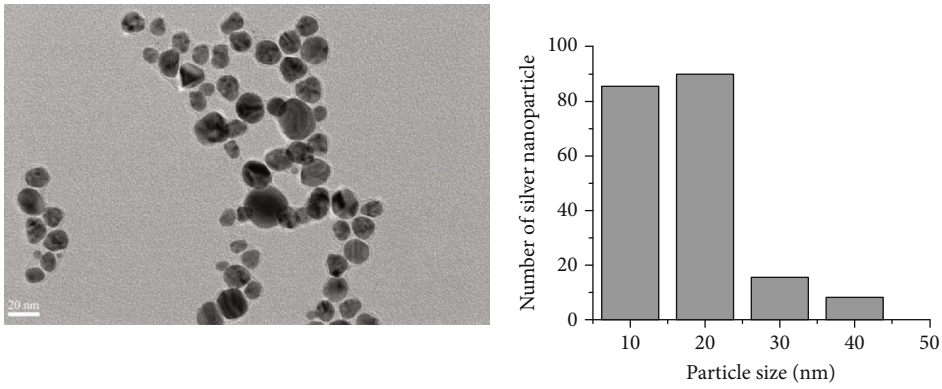


Figure 2: TEM image (a) and size distribution (b) of AgNPs.

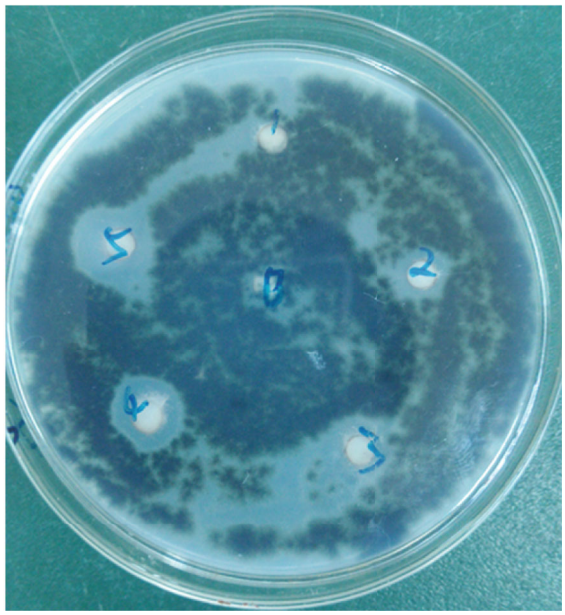


Figure 3: Inhibition zone created by AgNPs against *S. turcica*. The label of 0 represents sterile water, and 2-5 correspond to different concentrations (12.5, 25, 50, 100, and 200 μg/mL) of AgNPs.

200 μg/mL) of AgNPs was added onto the sterile filter paper disks (φ = 5 mm) that are fixed on plates, taking an equal volume of sterile as the control. The plates were incubated at 28°C for 2-3 days after standing for 5 min.

2.3.2. Colony Growth Assay. AgNP powders obtained by centrifugation and oven drying were dissolved in sterile water, and the concentration was set as 10 mg/mL. Then 5 mL of diluted AgNPs was added to 45 mL of PDA medium at about 55°C, making the final concentration of AgNPs as 12.5, 25, 50, 100, and 200 μg/mL separately. The control contained 5 mL of sterile water without AgNPs. A fungus block (φ = 6 mm) was inoculated in the center of each PDA plates, followed by incubating at 28°C for 5-7 d.

2.3.3. Conidia Germination. The density of *S. turcica* conidia was adjusted to 10^6/mL using a counting chamber. AgNPs and conidial suspension were mixed at a volume ratio of 1 : 9, and the final concentration of AgNPs was 12.5, 25, 50, 100, and 200 μg/mL, respectively. Conidial suspension without AgNPs was set as the control. Finally, incubation was at 25°C for 12 h.

2.3.4. In Vitro Inoculation. Maize leaves (Zhengdan 958, ZD958) were rinsed thoroughly by sterile water; surface sterilization was conducted by immersing in 75% ethyl alcohol (v/v) and 1% sodium hypochlorite (v/v) for 30 s and 1 min. After that, leaves were immersed in sterile water for 2 min to remove solvent residue. Finally, 15 μL of AgNPs and conidial suspension mixture made as 2.3.3 was dripped on air-dried leaves placed on solidified PDA plates; an equal volume of conidia suspension without AgNPs was added as the control. Treated leaves along with control were incubated at 28°C for 3-5 d.

2.3.5. Synergistic Inhibition Effect of AgNPs and Epoxiconazole. The 50% inhibition concentration (IC_{50}) of AgNPs and epoxiconazole was measured through a mycelium growth rate method, respectively. On account of that, a different mixed proportion was set 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 mixtures were prepared fresh and blended thoroughly for 5 min. The synergistic activity assessment (toxicity ratio) of AgNPs and epoxiconazole was determined by the following equations [34]:

(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 activity shows synergistic when the toxicity0020ratio was greater than 1; it shows antagonistic when

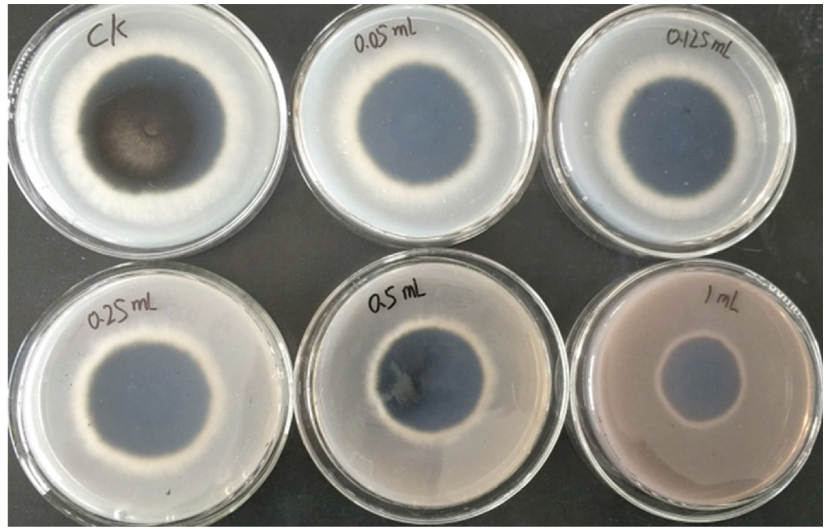


Figure 4: Colony growth inhibition of *S. turcica* in the presence of AgNPs.

the toxicity ratio was less than 1; it shows additive when the toxicity ratio was almost equal to 1.

3. Results and Discussion

3.1. Biosynthesis of AgNPs. $AgNO_3$ (Ag^+) could be reduced into elemental silver (Ag^0) under the action of reductase, and the solution changed from pale yellow to reddish brown in the presence of filtrate and $AgNO_3$ (Figure 1(c)); the obvious colour change indicated the formation of AgNPs [35], while the solution of $AgNO_3$ (Figure 1(a)) or *Ligustrum lucidum* leaf filtrate (Figure 1(b)) alone maintained their primary colour. The UV-vis spectrum showed a strong absorption peak at 438 nm, corresponding to the surface plasmon resonance of AgNPs [36]. However, there was no obvious characteristic absorbance peak of AgNPs for $AgNO_3$ (green line) or leaf filtrate (red line) alone. It is proved that the two portions were both needed for the biosynthesis of AgNPs, and the biological method also avoided the disadvantages of chemical and physical approaches such as the usage of additional chemical reagents, high energy consumption, and high cost [37].

3.2. TEM Analysis. Figure 2 shows the morphology, dispersity, and size distribution of synthesized AgNPs. Most of the nanoparticles were spherical or near spherical, and several ones were triangular, polygonal, or irregular shaped; the particles dispersed well on the substrate (Figure 2(a)). The size of AgNPs measured by ImageJ was in the range of 4-42 nm, and the average size was about 13 nm (Figure 2(b)). It is reported that there were several parameters that affected the shape, dispersity, and size of nanoparticles such as biological species, materials proportion, solution pH, reaction time, and so on [38, 39].

3.3. Paper Disk Diffusion. There exhibited positive correlation between the diameter of inhibition zone and antimicrobial activity. Figure 3 shows that the obvious inhibition zone appeared in the presence of AgNPs and it enlarged along with increasing concentration of AgNPs. The diameter was 7, 9, 12, 14, and 17 mm when the concentration of AgNPs was 12.5, 25, 50, 100, and 200 μg/mL, respectively. However, there was no inhibition zone created for sterile water. It is reported that the agar well diffusion method was also used to measure the diameter of inhibition zone besides paper disk diffusion approach and the diameter tended to be larger compared with this method owing to more volume capacity [40].

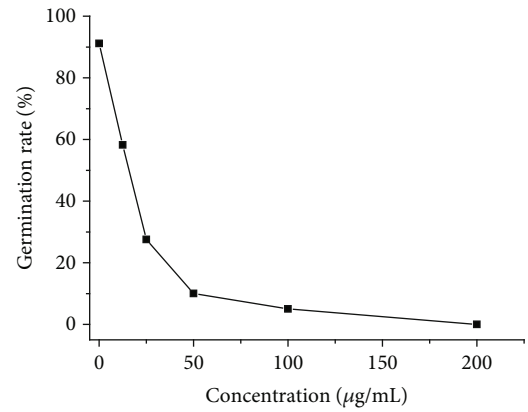


Figure 5: Effect of AgNPs on conidia germination of *S. turcica*.

3.4. Colony Growth Inhibition. As shown in Figure 4, the colony of *S. turcica* was significantly inhibited by AgNPs. The diameter of control measured by the cross crossing method was 7.2 cm, and it gradually decreased along with increasing concentration of AgNPs. The diameter descended to its minimum value (3.7 cm) at 200 μg/mL. The IC_{50} of AgNPs calculated by SPSS 13.0 was 170.2 μg/mL, and the 95% confidence interval was between 98.82 and 590.29 μg/mL. The inhibition rate caused by various AgNPs concentrations (12.5-200 μg/mL) of *S. turcica* was in the range of 2.31%-52.22%. The inhibition effect is never the same; it may be influenced by several parameters including AgNP species, AgNP shape, AgNP size, AgNP concentration, and pathogen species [19, 38].

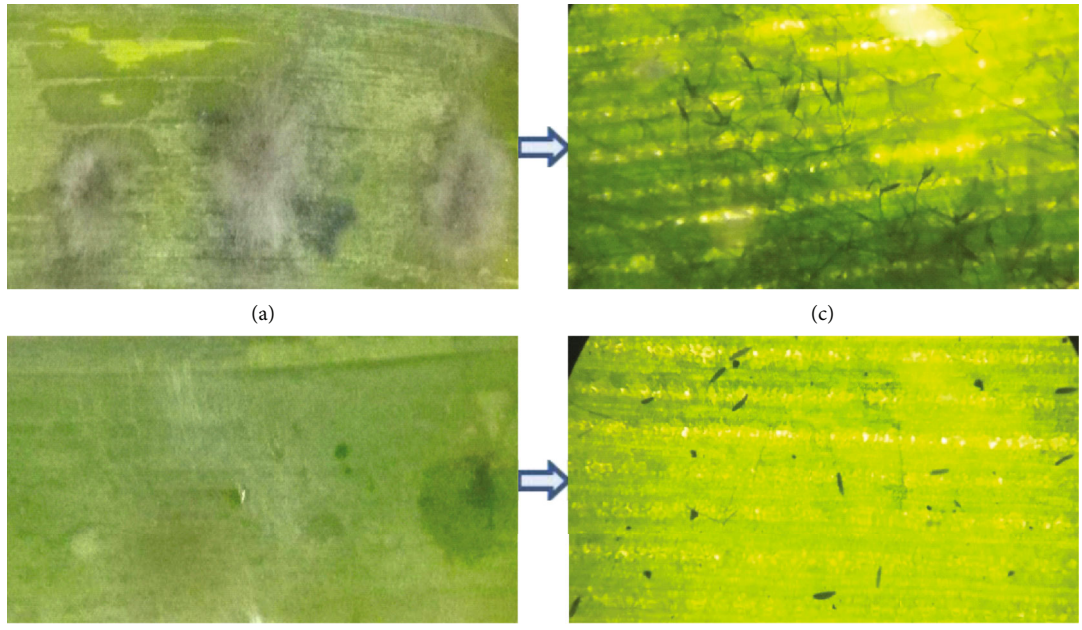


Figure 6: In vitro inoculation assay of AgNPs against *S. turcica*. (a) Maize leaf inoculated with conidia suspension. (b) Maize leaf inoculated with conidia suspension and AgNPs. (c) and (d) represent microscopic images of the local area of (a) and (b).

3.5. Conidia Germination Inhibition. Quantities of germinated conidia are vital for fungi to infect plant tissue successfully, so it is the key to inhibit germination of conidia to alleviate or avoid appearance of plant diseases. Figure 5 shows that AgNPs could prominently inhibited germination of conidia of *S. turcica*. Under the proper temperature and humidity, the germination rate of conidia suspension without AgNPs was 91.17%, while it decreased sharply in the presence of varied concentrations of AgNPs, and the conidia were completely inhibited at 200 μg/mL.

3.6. In Vitro Inoculation Assay. In vitro inoculation was also conducted to evaluate the antifungal activity of AgNPs, and obvious lesion appeared for the maize leaf that inoculated with conidia suspension alone (Figure 6(a)). However, the leaf inoculated with the compound of conidia suspension and AgNPs (100 μg/mL) looked to be healthy as normal leaf (Figure 6(b)). In addition, the corresponding microscopic images showed germinated conidia interweaved throughout the whole leaf tissues (Figure 6(c)), while germination of the conidia treated with AgNPs was absolutely inhibited (Figure 6(d)). In view of the external observation and microexamination, conidia of S. turcica were limited to germinate and spread in maize leaf by AgNPs. The similar result was reported before that AgNPs limiting the conidia of Bipolaris sorokiniana germinate and infect wheat leaf [41].

3.7. Synergistic Inhibition Effect Assay. Recently, more and more researchers pay their attention to the compound of two or more different antimicrobial substances in consideration of environment pollution and drug resistance. Table 1 shows that biosynthesized AgNPs and epoxiconazole could be combined amicably, and there exhibited no obvious antagonistic effect among these mixed proportions. The prominent synergistic antifungal effect occurred at 8 : 2 and 9 : 1 for AgNPs and epoxiconazole, and the inhibition toxicity ratio reached 1.22 and 1.24, respectively. The antimicrobial activity showed general synergistic for conjugation of AgNPs and antibacterial agents reported previously [23, 24]. However, it diversified when AgNPs combined with fungistats, additive and antagonistic effects were also found when AgNPs were mixed with propineb and fludioxonil against *Bipolaris maydis* [34].

Table 1: Toxicity ratio between AgNPs and epoxiconazole against *S. turcica*.

Volume ratio	Actual inhibition rate (%)	Theoretical inhibition rate (%)	Toxicity ratio
0 : 10	49.12	49.12	1.00
1 : 9	52.63	49.47	1.06
2 : 8	45.61	49.83	0.92
3 : 7	50.88	50.17	1.01
4 : 6	54.39	50.52	1.08
5 : 5	52.63	50.88	1.03
6 : 4	56.14	51.23	1.10
7 : 3	59.65	51.58	1.16
8 : 2	63.16	51.92	1.22
9 : 1	64.91	52.28	1.24
10 : 0	52.63	52.63	1.00

4. Conclusions

S. turcica is an important phytopathogen that caused severe foliar disease on maize around the world. It is a great challenge to control that disease in an ecofriendly way. In this

study, AgNPs biosynthesized by *Ligustrum lucidum* leaf extract exhibited prominent antifungal activity against *S. turcica*. In addition, the synergistic antifungal effect was also confirmed when AgNPs and epoxiconazole conjugated at proper proportions. These results not only provide a new approach for integrative control of plant pathogens but also reduce or avoid drug resistance to the utmost extent. The modification of AgNPs such as surface charge, acid-base property, and aggregation behavior would be carried out to evaluate their influence on the antimicrobial activity of AgNPs in the near future.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

MY and DM carried out the experiments and measurements and drafted the manuscript. BL and CX participated in the discussion. WH and HY contributed to the design of the experiment and analysis of the results in this paper. All authors read and approved the final manuscript.

Acknowledgments

This work was supported by the Natural Science Fund of the Education Department of Anhui Province (KJ2019ZD57, KJ2019A0814, and KJ2018A0543), Talent Introduction Project in Anhui University of Science and Technology (NXYJ201602), and Innovation Project of University Students (201910879021).

References

[1] A. Oloffs, C. Grosse-Siestrup, S. Bisson, M. Rinck, R. Rudolph, and U. Gross, "Biocompatibility of silver-coated polyurethane catheters and silvercoated Dacron® material," *Biomaterials*, vol. 15, no. 10, pp. 753–758, 1994.

[2] P. Mohanpuria, N. K. Rana, and S. K. Yadav, "Biosynthesis of nanoparticles: technological concepts and future applications," *Journal of Nano Research*, vol. 10, no. 3, pp. 507–517, 2008.

[3] B. S. Atiyeh, M. Costagliola, S. N. Hayek, and S. A. Dibo, "Effect of silver on burn wound infection control and healing: review of the literature," *Burns*, vol. 33, no. 2, pp. 139–148, 2007.

[4] J. C. Osuwa and P. C. Anusionwu, "Some advances and prospects in nanotechnology: a review," *Asian Journal of Information technology*, vol. 10, no. 2, pp. 96–100, 2011.

[5] M. Bakshi, H. B. Singh, and P. C. Abhilash, "The unseen impact of nanoparticles: more or less?," *Current Science*, vol. 106, no. 3, pp. 350–352, 2014.

[6] K. Chaloupka, Y. Malam, and A. M. Seifalian, "Nanosilver as a new generation of nanoproduct in biomedical applications," *Trends in Biotechnology*, vol. 28, no. 11, pp. 580–588, 2010.

[7] W. Huang, Y. Bao, H. Duan, Y. Bi, and H. Yu, "Antifungal effect of green synthesised silver nanoparticles against *Setosphaeria turcica*," *IET Nanobiotechnology*, vol. 11, no. 7, pp. 803–808, 2017.

[8] N. Pugazhenthiran, S. Anandan, G. Kathiravan, N. K. Udaya Prakash, S. Crawford, and M. Ashokkumar, "Microbial synthesis of silver nanoparticles by *Bacillus* sp," *Journal of Nanoparticle Research*, vol. 11, no. 7, pp. 1811–1815, 2009.

[9] K. Vahabi, G. A. Mansoori, and S. Karimi, "Biosynthesis of Silver Nanoparticles by Fungus Trichoderma Reesei (A Route for Large-Scale Production of AgNPs)," *Insciences Journal*, vol. 1, no. 1, pp. 65–79, 2011.

[10] W. Huang, J. Yan, Y. Wang, C. Hou, T. Dai, and Z. Wang, "Biosynthesis of silver nanoparticles bySeptoria *apiiandTrichoderma koningii*," *Chinese Journal of Chemistry*, vol. 31, no. 4, pp. 529–533, 2013.

[11] M. Khatami and S. Pourseyedi, "*Phoenix dactylifera* (date palm) pit aqueous extract mediated novel route for synthesis high stable silver nanoparticles with high antifungal and antibacterial activity," *IET Nanobiotechnology*, vol. 9, no. 4, pp. 184–190, 2015.

[12] M. Mohammadlou, H. Maghsoudi, and H. Jafarizadeh-Malmiri, "A review on green silver nanoparticles based on plants: synthesis, potential applications and eco-friendly approach," *International Journal of Food Research*, vol. 23, no. 2, pp. 446–463, 2016.

[13] W. Huang, H. Li, H. Duan et al., "Antifungal effect of *Buxus sinica* leaf extract-mediated silver nanoparticles against *Curvularia lunata*," *International Journal of Agriculture and Biology*, vol. 20, pp. 2395–2400, 2018.

[14] A. M. Allahverdiyev, K. V. Kon, E. S. Abamor, M. Bagirova, and M. Rafailovich, "Coping with antibiotic resistance: combining nanoparticles with anti-biotics and other antimicrobial agents," *Expert Review of Anti-Infective Therapy*, vol. 9, no. 11, pp. 1035–1052, 2011.

[15] J. K. Schluesener and H. J. Schluesener, "Nanosilver: application and novel aspects of toxicology," *Archives of Toxicology*, vol. 87, no. 4, pp. 569–576, 2013.

[16] S. Mohanty, S. Mishra, P. Jena, B. Jacob, B. Sarkar, and A. Sonawane, "An investigation on the antibacterial, cytotoxic, and antibiofilm efficacy of starch-stabilized silver nanoparticles," *Nanomedicine-Nanotechnology Biology and Medicine*, vol. 8, no. 6, pp. 916–924, 2012.

[17] M. K. Rai, S. D. Deshmukh, A. P. Ingle, and A. K. Gade, "Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria," *Journal of Applied Microbiology*, vol. 112, no. 5, pp. 841–852, 2012.

[18] M. Khatami, R. Mehnipor, M. H. S. Poor, and G. S. Jouzani, "Facile biosynthesis of silver nanoparticles using Descurainia sophia and evaluation of their antibacterial and antifungal properties," *Journal of Cluster Science*, vol. 27, no. 5, pp. 1601–1612, 2016.

[19] M. S. Nejad, G. H. S. Bonjar, M. Khatami, A. Amini, and S. Aghighi, "In vitro and in vivo antifungal properties of silver nanoparticles against *Rhizoctonia solani*, a common agent of rice sheath blight disease," *IET Nanobiotechnology*, vol. 11, no. 3, pp. 236–240, 2017.

[20] M. Khatami, I. Sharifi, M. A. L. Nobre, N. Zafarnia, and M. R. Aflatoonian, "Wastegrass-mediated green synthesis of silver nanoparticles and evaluation of their anticancer, antifungal and antibacterial activity," *Green Chemistry Letters and Reviews*, vol. 11, no. 2, pp. 125–134, 2018.

[21] S. Gurunathan, "Rapid biological synthesis of silver nanoparticles and their enhanced antibacterial effects against *Escherichia fergusonii* and *Streptococcus mutans*," *Arabian Journal of Chemistry*, vol. 12, no. 2, pp. 168–180, 2019.

[22] P. Li, J. Li, C. Wu, Q. Wu, and J. Li, "Synergistic antibacterial effects of β-lactam antibiotic combined with silver nanoparticles," *Nanotechnology*, vol. 16, no. 9, pp. 1912–1917, 2005.

[23] A. M. Fayaz, K. Balaji, M. Girilal, R. Yadav, P. T. Kalaichelvan, and R. Venketesan, "Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria," *Nanomedicine Nanotechnology Biology and Medicine*, vol. 6, no. 1, pp. 103–109, 2010.

[24] I. S. Hwang, J. H. Hwang, H. Choi, K. J. Kim, and D. G. Lee, "Synergistic effects between silver nanoparticles and antibiotics and the mechanisms involved," *Journal of Medical Microbiology*, vol. 61, Part 12, pp. 1719–1726, 2012.

[25] M. A. Dar, A. Ingle, and M. Rai, "Enhanced antimicrobial activity of silver nanoparticles synthesized by *Cryphonectria* sp. evaluated singly and in combination with antibiotics," *Nanomedicine*, vol. 9, no. 1, pp. 105–110, 2013.

[26] I. Sondi and B. Salopek-Sondi, "Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria," *Journal of Colloid and Interface Science*, vol. 275, no. 1, pp. 177–182, 2004.

[27] J. P. Ruparelia, A. K. Chatterjee, S. P. Duttagupta, and S. Mukherji, "Strain specificity in antimicrobial activity of silver and copper nanoparticles," *Acta Biomaterialia*, vol. 4, no. 3, pp. 707–716, 2008.

[28] E. T. Hwang, J. H. Lee, Y. J. Chae et al., "Analysis of the toxic mode of action of silver nanoparticles using stress-specific bioluminescent bacteria," *Small*, vol. 4, no. 6, pp. 746–750, 2008.

[29] J. S. Kim, E. Kuk, K. N. Yu et al., "Antimicrobial effects of silver nanoparticles," *Nanomedicine: Nanotechnology, Biology and Medicine*, vol. 3, no. 1, pp. 95–101, 2007.

[30] S. Kittler, C. Greulich, J. Diendorf, M. Köller, and M. Epple, "Toxicity of silver nanoparticles increases during storage because of slow dissolution under release of silver ions," *Chemistry of Materials*, vol. 22, no. 16, pp. 4548–4554, 2010.

[31] S. Chernousova and M. Epple, "Silver as antibacterial agent: ion, nanoparticle, and metal," *Angewandte Chemie International Edition*, vol. 52, no. 6, pp. 1636–1653, 2013.

[32] A. Ivask, A. ElBadawy, C. Kaweeteerawat et al., "Toxicity mechanisms in Escherichia coli vary for silver nanoparticles and differ from ionic silver," *ACS Nano*, vol. 8, no. 1, pp. 374–386, 2013.

[33] D. R. Monteiro, S. Silva, M. Negri et al., "Antifungal activity of silver nanoparticles in combination with nystatin and chlorhexidine digluconate against *Candida albicans* and *Candida glabrata* biofilms," *Mycoses*, vol. 56, no. 6, pp. 672–680, 2013.

[34] W. Huang, C. Wang, H. Duan et al., "Synergistic antifungal effect of biosynthesized silver nanoparticles combined with fungicides," *International Journal of Agriculture and Biology*, vol. 20, pp. 1225–1229, 2018.

[35] H. P. Borase, B. K. Salunke, R. B. Salunkhe et al., "Plant extract: a promising biomatrix for ecofriendly, controlled synthesis of silver nanoparticles," *Applied Biochemistry and Biotechnology*, vol. 173, no. 1, pp. 1–29, 2014.

[36] H. Bar, D. K. Bhui, G. P. Sahoo, P. Sarkar, S. Pyne, and A. Misra, "Green synthesis of silver nanoparticles using seed extract of *Jatropha curcas*," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 348, no. 1-3, pp. 212–216, 2009.

[37] S. P. Dubey, M. Lahtinen, and M. Sillanpaa, "Tansy fruit mediated greener synthesis of silver and gold nanoparticles," *Process Biochemistry*, vol. 45, no. 7, pp. 1065–1071, 2010.

[38] V. Sarsar, M. K. Selwal, and K. K. Selwal, "Biogenic synthesis, optimisation and antibacterial efficacy of extracellular silver nanoparticles using novel fungal isolate *Aspergillus fumigatus* MA," *IET Nanobiotechnology*, vol. 10, no. 4, pp. 215–221, 2016.

[39] D. Amerasan, T. Nataraj, K. Murugan et al., "Mycosynthesis of silver nanoparticles using Metarhizium anisopliae against the rural malaria vector Anopheles culicifacies Giles (Diptera: culicidae)," *Journal of Pest Science*, vol. 89, no. 1, pp. 249–256, 2016.

[40] S. O. Hasson, M. J. al-Awady, A. H. al-Hamadani, and I. H. al-Azawi, "Boosting antimicrobial activity of imipenem in combination with silver nanoparticles towards *S. fonticola* and *Pantoea* sp," *Nano Biomedicine and Engineering*, vol. 11, no. 2, pp. 200–214, 2019.

[41] S. Mishra, B. R. Singh, A. Singh, C. Keswani, A. H. Naqvi, and H. B. Singh, "Biofabricated silver nanoparticles act as a strong fungicide against *Bipolaris sorokiniana* causing spot blotch disease in wheat," *PLoS ONE*, vol. 9, no. 5, p. e97881, 2014.