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Antifungal mechanism of nanosilver biosynthesized with *Trichoderma longibrachiatum* and its potential to control muskmelon *Fusarium* wilt

Xian Liu[1], Tong Li[1], Xiaohui Cui[1], Ran Tao[1] & Zenggui Gao[2]✉

Fusarium oxysporum **(Schl.) f.sp.** ***melonis*****, which causes muskmelon wilt disease, is a destructive filamentous fungal pathogen, attracting more attention to the search for effective fungicides against this pathogen. In particular, Silver nanoparticles (AgNPs) have strong antimicrobial properties and they are not easy to develop drug resistance, which provides new ideas for the prevention and control of muskmelon** ***Fusarium*** **wilt (MFW). This paper studied the effects of AgNPs on the growth and development of muskmelon, the control efficacy on** ***Fusarium*** **wilt of muskmelon and the antifungal mechanism of AgNPs to** ***F. oxysporum*****. The results showed that AgNPs could inhibit the growth of** ***F. oxysporum*** **on the PDA and in the PDB medium at 100–200 mg/L and the low concentration of 25 mg/L AgNPs could promote the seed germination and growth of muskmelon seedlings and reduce the incidence of muskmelon** ***Fusarium*** **wilt. Further studies on the antifungal mechanism showed that AgNPs could impair the development, damage cell structure, and interrupt cellular metabolism pathways of this fungus. TEM observation revealed that AgNPs treatment led to damage to the cell wall and membrane and accumulation of vacuoles and vessels, causing the leakage of intracellular contents. AgNPs treatment significantly hampered the growth of mycelia in the PDB medium, even causing a decrease in biomass. Biochemical properties showed that AgNPs treatment stimulated the generation of reactive oxygen species (ROS) in 6 h, subsequently producing malondialdehyde (MDA) and increasing protective enzyme activity. After 6 h, the protective enzyme activity decreased. These results indicated that AgNPs destroy the cell structure and affect the metabolisms, eventually leading to the death of fungus.**

Keywords AgNPs, *Trichoderma longibrachiatum*, *Fusarium oxysporum*, Antifungal mechanism, Muskmelon seed germination and growth

Recently, with the rapid development of nanotechnology, biosynthesized nanoparticles (NPs) have attracted more and more attention of researchers due to their wide application in many fields, including agricultural pest and disease control and no any physicochemical factors like high energy, temperature, high toxicity and pressure during the biogenic synthesis process[1–3]. NPs were always termed as "magic bullets" to control plant diseases and promote plant growth because of their properties of nano-pesticides, nano-fertilizers and herbicides[4]. In particular, silver nanoparticles (AgNPs) are one of the most extensively used nanomaterials in the world, and approximately 500 tons of AgNPs are produced annually[5–7]. Biosynthesized AgNPs were widely applied in the agricultural, environmental and medical areas, etc.[8] due to their unique physicochemical properties e.g. cost-efficiency, higher stability, large surface/mass ratio, minimum toxicity and high reaction rate[9–11]. More studies have shown that micromolar doses of AgNPs are sufficient to exhibit strong antifungal activity against fungal pathogens, including *Fusarium* spp.[12], *Candida* sp.[13], *Helminthosporium* sp., *Alternaria alternate*, *Phytophthora arenaria* and *Botrytis* sp.[14]. According to available literatures, AgNPs could potentially replace fungicides to control plant diseases and improve the growth of plants[4], which provides a positive theoretical basis for the practical application of AgNPs.

[1]College of Bioscience and Technology, Shenyang Agricultural University, Shenyang 110866, China. [2]College of Plant Protection, Shenyang Agricultural University, Shenyang 110866, China. ✉email: gaozenggui@syau.edu.cn

The exact mechanism by which AgNPs exert antimicrobial activity is still unclear and a debated topic[15]. There are various mechanisms, including adhesion to the surface of cell wall and membrane to break down them, damaging intracellular structures, inactivating the key enzymes, inducing the production of cellular toxicity and oxidative stress, and modulating the signal transduction pathways[16]. In the meantime, the mode of AgNPs against different microbials (such as Gram-positive and Gram-negative bacteria) may be due to differences in silver uptake pathways into the cell. AgNPs from different ways of production have different physicochemical properties and different ways of bacterial toxicity, resulting in changes of mechanical mode of actions[15]. It is noteworthy that AgNPs biosynthesized with different conditions have different physicochemical properties and antifungal activities[12]. However, relevant studies on AgNPs against phytopathogenic fungi in agricultural practice are insufficient, especially since the mechanisms of AgNPs against pathogenic microbes have not yet been fully elucidated.

Fusarium oxysporum is a major causal agent of *Fusarium* vascular wilt, which leads to great economic losses on muskmelon, tomato, strawberry, and other economically important crops[17]. Among these *Fusarium* sp. *F. oxysporum* f. sp. *melonis* (FOM) causes the *Fusarium* wilt of muskmelon (*Cucumis melo*), a common and serious disease globally. Due to the lack of muskmelon cultivars with high resistance to *F. oxysporum*, chemical fungicides and grafting methods are still the most effective way to manage FOM[18,19]. However, with the frequent and long-term use of fungicides, especially the extensive application in the greenhouse, fungicide-resistant *F. oxysporum* populations in the field are predominant and the soil environment is seriously damaged, which resulting in reduction of control efficacy[20]. The grafting method is both time and labor-intensive, and sometimes the grafts are incompatible[18]. To circumvent this predicament, new effective antifungal agents should be explored and developed. Many literatures have shown that various nanoparticles, including AgNPs, Chitosan NPs, ZnO-NPs, and green or engineered silver nanoparticles, can exhibit their antifungal effects against *F. oxysporum*[21–24]. So, nanomaterials from nanotechnology can be an alternative of fungicides to improve management of soil-borne phytopathogens. Furthermore, it is necessary to evaluate the potential risks of using nanoparticles to the crops for plant disease control.

Here, we biosynthesized AgNPs with *T. longibrachiatum* and investigated the antifungal activity and toxicity mechanisms of AgNPs against FOM pathogen *F. oxysporum*. The main aim is to assess the effect of biosynthesized AgNPs on *F. oxysporum* and its control efficacy on the muskmelon *Fusarium* wilt (MFW), as well as possible antifungal mechanisms of AgNPs by TEM observation of cell structure, leakage of intracellular contents, changes of malondialdehyde (MDA) and activities of protective enzymes. This study will advance the understanding of nanomaterial applications for plant disease control.

Materials and methods

Microorganism and reagents

Trichoderma longibrachiatum was originally isolated from the roots of oak trees and spores have been maintained at 4 °C on silica gel pellets at the culture collection of Sericultural lab, Shenyang Agricultural University, China. Then, it has been re-cultured before use in silver nanoparticles biosynthesis[25].

Fusarium oxysporum was originally isolated from the muskmelon root with the typical symptoms of muskmelon *Fusarium* wilt disease and was identified with *F. oxysporum* specific primers FOF1 and FOR1, then kept at 4 °C in Institute of Plant immunity, Shenyang Agricultural University, China[26].

Muskmelon (*Cucumis melo* L.) seeds (Jingtiang No.1) was purchased from Yue Nong seedling Co., LTD (Shenyang, China).

$AgNO_3$, trichloroacetic acid, thiobarbituric acid brilliant blue G and anthrone (AR Grade) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). A test kit of superoxide dismutase (SOD) was purchased from Shanghai Beyotime Biotechnology Co., LTD (Shanghai, China). Test kids of catalase (CAT) and peroxidase (POD) were purchased from Nanjing Jiancheng Technology Co., LTD (Nanjing, China).

AgNPs biosynthesis

Biosynthesis of AgNPs was performed as described by Cui et al.[12]. The cell-free supernatant of *T. longibrachiatum* was prepared by culturing fungus in potato dextrose broth (PDB) contained (g/L): 200.0, fresh potato cubes, and 20.0, dextrose, at 28 °C and 100 rpm for 72 h, then filtering with Whatman filter paper No.1. Cell-free supernatants (100 mL) were mixed with silver nitrate solution (Final concentration: 2.0 mmol/L) and incubated at 55 °C for 24 h in the light condition. The resultant AgNPs reaction solutions were tested by UV–visible spectrometry in the wavelength range of 300–800 nm. AgNPs in the reaction solution was collected by centrifugation at 10,000 g for 30 min, washed twice with sterile distilled water and with 75% ethanol once stored at − 20 °C. The characterization of biosynthesized AgNPs had been described at paper by Cui et al.[12].

Antifungal activity of biosynthesized AgNPs to *F. oxysporum* and its control for muskmelon wilt

The antifungal activity of synthesized nanoparticles was assayed by controlling the mycelial growth of *F. oxysporum* on the potato dextrose agar (PDA) and in the potato dextrose broth (PDB) respectively. The mycelium disc (d = 1 cm) from the fringe of 4-day-grown fungal colony was transferred to PDA medium containing AgNPs (200 mg/L), in triplicate. Controls were prepared using PDA only. Then the plate was incubated for 4 days at 25 °C and the diameter of colonies were measured to calculate the inhibition ratio of AgNPs to *F. oxysporum*.

One mL spore suspensions of *F. oxysporum* ($> 10^6$) were placed in each of 9 culturing flasks containing PDB medium (150 mL) and cultured by rotary shaker at 100 rpm for 24 h. AgNPs was added into 3 culturing flasks (final concentration: 100 mg/L) and then continued to shake for another 24 h, and the mycelium was filtered by

sterile Whatman filter paper No. 1 and weighed. There are two control group, one is the mycelium fresh weight of 24 h, and the other is 48 h'.

The full muskmelon seeds are washed with distilled water and immersed in a 10% sodium hypochlorite solution for 10 min to kill pathogens on the seeds surface, then rinsed three times with sterile water. The disinfected seeds were wrapped in gauze soaked with sterile water for germination at 28℃. After the cotyledon had expanded and the young roots had emerged, the seedlings were transferred to tube boxes for water culture in a light incubator at 28 °C. Muskmelon seedlings (7-day old) with minor injury roots were immersed in *F. oxysporum* spore suspension (about 1×10^6 cfu/mL) for 12 h, then transferred to tube boxes for continuing water culture in a light incubator for 14 d. The culturing water is changed every two days. There are three treatments, one is water culture of muskmelon seedlings without *Fusarium* inoculation, and the other two are water culture after *Fusarium* inoculation, in which AgNPs is added to one of the aqueous solutions (25 mg/L). There are 15 muskmelon seedlings in each treatment in triplicate. The disease plants were investigated 2 weeks later and disease incidence (numbers of disease plants / numbers of all treated plants) was calculated.

Effect of AgNPs on the development and growth of muskmelon

After seeds were disinfected with the method used in 2.3, they were soaked in sterile water, and nanoparticle suspensions for about 12 h after being rinsed three times with sterile water. One piece of filter paper was put into each 15 mm Petri dish, and 5 mL of sterile water or 25 mg/L AgNPs was added. Seeds were then transferred onto the filter paper with 20 seeds per dish, in triplicate. Petri dishes were covered, and placed in a light incubator at 26 °C and 4,000 lx with a 16-h light/8-h dark cycle. At 3 and 7 days, seed germination potential and rate are calculated respectively. After 7 days, the germination experiments were stopped and length of seedling stem and root and the number of fibrous roots was measured[27].

The antifungal mechanism of AgNPs

Transmission electron microscopy (TEM) assays

To observe the effect of AgNPs on the fungal morphology of *F. oxysporum* hyphae, mycelia cultured in PDB medium for 2 days were treated with AgNPs with 100 mg/mL for 24 h. Then, the mycelia were collected and fixed with 2.5% (v/v) glutaraldehyde solution, stained with 1% (w/v) osmium tetroxide and dehydrated with a series of ethanol solutions (30–100%) as described by Shi et al.[28]. The morphology of mycelia was captured with TEM (HT7700, HITACHI, Japan).

Measurement of MDA, soluble protein and carbohydrates content

To measure the effect of AgNPs on the physiological and biochemical properties of *F. oxysporum* hyphae, mycelia cultured with PDB medium for 48 h were filtered with sterile Whatman filter paper No. and rinsed with sterile water three times, then blotted with sterile filter paper. 5 g mycelia were immersed into 20 mL sterile water and 100 mg/L AgNPs solution respectively for a certain period time (0, 6, 12 and 24 h), then mycelia and culture liquid were collected by filtering with sterile filter paper respectively, in triplicate.

MDA content was measured using a 2-thiobarbituric acid reaction method. 0.5 g of fresh mycelium was homogenized in 5 mL of 5% (W/V) trichloroacetic acid and the homogenate was centrifuged at 10,000 g for 15 min at room temperature. The supernatant was mixed with an equal volume of 2-thiobarbituric acid (0.67% in 20% [w/v] trichloroacetic acid) and the mixture was boiled for 30 min at 100 °C, followed by centrifugation for 10 min at 7500 g to clarify the solution.

The absorbance of the supernatant was measured at 532 nm and corrected for non-specific turbidity by subtracting the A600. MDA contents were calculated using the equation $\text{MDA content}(\text{nmol/g}) = 6.452 \times (\text{A532} - \text{A600}) \times \frac{V1 \times V}{V2 \times W}$ ($V1$: the reaction volume; $V2$: the supernatant volume in the reaction system; V: the total supernatant volume; W: the weight of fresh mycelium)[29].

The contents of soluble proteins, soluble sugars were determined spectrophotometrically (UV2000, UNICO, Shanghai, China) as previously described by Bradford[30] and Yin et al.[31] respectively. Briefly, 1.0 g (fresh weight, FW) of the samples were ground and combined with 50 mmol/L of phosphoric acid buffer. After centrifugation at 12,000 g for 30 min at 4 °C, the supernatant (1 mL) was mixed with 4 mL 0.01% (w/v) Coomassie Brilliant Blue G-250 to determine the soluble protein content by measuring the absorbance at 595 nm. The samples (1.0 g FW) were ground and combined with 15 mL of distilled water and boiled for 20 min. After cooling and filtering, the supernatant (2 mL) was mixed with 0.5 mL 2% (w/v) anthrone and 5 mL sulfuric acid (1.84 g/cm^3) to determine the soluble sugar content by measuring the absorbance at 620 nm.

Determination of enzyme activity

For protective enzyme activity assays, mycelia samples (1 g) were homogenized in liquid nitrogen before the addition of 5 ml of pre-cooled phosphate buffer (50 mmol/L, pH 7.0). After centrifugation at 10,000 g (15 min, 4 °C), the supernatants are taken as crude enzyme extracts. The activity superoxide dismutase (SOD) was measured by the nitroblue tetrazolium reduction method, peroxidase (POD) activity was measured by the guaiacol method, while catalase (CAT) activities were measured by ultraviolet colorimetry[32].

Statistical analyses

Statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS) for Windows, Version 22.0 and Graphpad Prism 6.0 (GraphPad, San Diego, USA). All the experimental data were presented as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was conducted to compare the differences of the means. Dunnett's test was performed for multiple comparisons and Student's *t*-test for each

dataset compared with the control data. Statistical significance for all tests was considered at a probability level of 0.05 ($p < 0.05$) or 0.01($p < 0.01$).

Results

The inhibitory effect of AgNPs on the *F. oxysporum* and incidence of *Fusarium* wilt of muskmelon

Fungal pathogen, *F. oxysporum*, was used to identify the antifungal activity of AgNPs biosynthesized with *T. longibrachiatum*. The mycelial growth of *F. oxysporum* on the PDA plates or in liquid PDB amended with AgNPs (200 mg/L and 100 mg/L respectively) were evaluated to analyze the effect of AgNPs on *F. oxysporum*. After 7 days of incubation on the plates, the mycelial growth was inhibited by AgNPs with a diameter of 3.4 ± 0.4 cm, which displayed stronger antifungal activity than that of CK (7.8 ± 0.2 cm) (Fig. 1a, b). In the liquid culture, AgNPs was added after incubation for 24 h to assess the inhibitory/damaging effect on the mycelium. Continuously shaking culture at 24 h, the fresh weight of mycelia treated with AgNPs was significantly lower than that of CK and slightly lower than that at 24 h (Fig. 1c). Quantitative data confirmed that AgNPs had an antifungal activity to *F. oxysporum*.

To determine whether AgNPs affect the virulence of *F. oxysporum*, AgNPs was supplemented when the spores was inoculated on the slight-damaged root of muskmelon (cultivar No.1 Jingtian). The *F. oxysporum* strain without AgNPs served as a control (CK-F). The assessment of disease symptoms at 2 weeks post-inoculation showed that there were fewer roots inoculated with AgNPs showing the typical *Fusarium* symptom (necrosis to water-soaked or dry lesions in brown color) and fewer withered leaves compared with CK-F. The incidence of muskmelon seedlings treated with AgNPs was significantly lower than that of CK-F (Fig. 1d, e).

The effect of AgNPs on the growth and development of muskmelon

Effects of nanoparticles at 25 mg/L on seed germination and stem/root growth of seedlings are shown in Table 1. Seed germinations were affected by the nanoparticles. Germination rate and germination potential both increased by about 10%. The effect of nanoparticle suspensions at 25 mg/L on root and stem growth were significant ($p < 0.05$) compared with the CK. The root and stem length of muskmelon treated with AgNPs reached to 9.13 ± 0.87 cm and 1.79 ± 0.12, longer than these of CK (5.53 ± 0.66 cm and 1.35 ± 0.22 cm) respectively. At the same time, AgNPs promoted muskmelon root to grow more lateral roots, which were 2 times more than the CK. The biomass of muskmelon seedlings was not affected by the nanoparticles. Although the fresh weight of seedlings with AgNPs was higher than that of CK, the difference showed no significant ($p < 0.05$).

The mechanism of AgNPs against *F. oxysporum*

To further understand how AgNPs interact with fungal cells, the ultrastructural structures of *F. oxysporum* mycelium were determined using TEM analysis. Compared with the cell wall and internal structure of cell treated without AgNPs (Fig. 2a, b), the craters could be seen on the cell walls and cell walls became thinner when AgNPs were present, which implied that AgNPs treatment could lead to defects in the cell wall of *F. oxysporum*. The structural damages of *F. oxysporum* cells treated with AgNPs were clearly visible, including the accumulation of vacuoles or vesicles in the cytoplasm (Fig. 2c, d). In conclusion, TEM observations revealed that both the surface and intracellular organelles of fungal cells were disrupted by AgNPs, resulting in AgNPs being toxic to *F. oxysporum*.

The changes in protein and carbohydrate contents in mycelia and culture medium can also be used to further elucidate the damage of fungal cell walls and membranes by AgNPs. The craters in the cell wall and cell membrane of mycelium can make the proteins and carbohydrates leak into the medium, resulting in the imbalance of nutrients in the cell and cause the cell death. After the mycelium was treated with AgNPs for 12 h, the contents of proteins and carbohydrates in the cell decreased, while these in the culture medium increased. The contents of proteins and carbohydrates in culture medium were higher than these in the cell at the AgNPs-treated mycelium, while the results were reversed with the controls (Fig. 3a, b).

Malondialdehyde (MDA) is one of the commonly used biomarkers of lipid peroxidation of cell membrane, which can indicate levels of the damage of cell membrane. The MDA content in the mycelium treated with AgNPs was significantly higher than that without AgNPs treatments (Fig. 3c), which showed that AgNPs could induce more reactive oxygen species (ROS) to oxidize the lipid which increased the membrane permeability. As a reaction to this condition, antioxidative enzymes (or protective enzymes) such as catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) could be produced to remove these excess ROS. Under water stress (CK), the activities of protective enzymes in mycelium increased, however the activities of enzymes increased firstly, then decreased with AgNPs treatment (Fig. 4). The results showed that treatment of *Fusarium* mycelium with silver nanoparticles for a short time (less than 6 h) can improve the activity of protective enzymes, but with the extension of treatment time, the activity of protective enzymes decreases, indicating that treatment of mycelium for a long time can reduce the activity of protective enzymes, thereby affecting the removal of reactive oxygen species and causing the death of mycelium cells.

Discussion

Various types of nanoparticles, such as AgNPs, AuNPs, CuNPs, MgONPs, and TiO_2-NPs with their antifungal, antibacterial or antiviral properties have been applied to resistance against plant pathogens[33,34]. Nanoparticle technology is a promising alternative to pesticides in the control of plant diseases, which is sustainable in agriculture and food science[35]. Biogenic NPs have favorable biodegradability, biocompatibility, non-allergenicity, and antimicrobial activity, with low toxicity to animals and humans compared with chemical-synthesized NPs[36]. Trichogenic Nanoparticles showed the broad-spectrum and higher antifungal activity compared with chemically

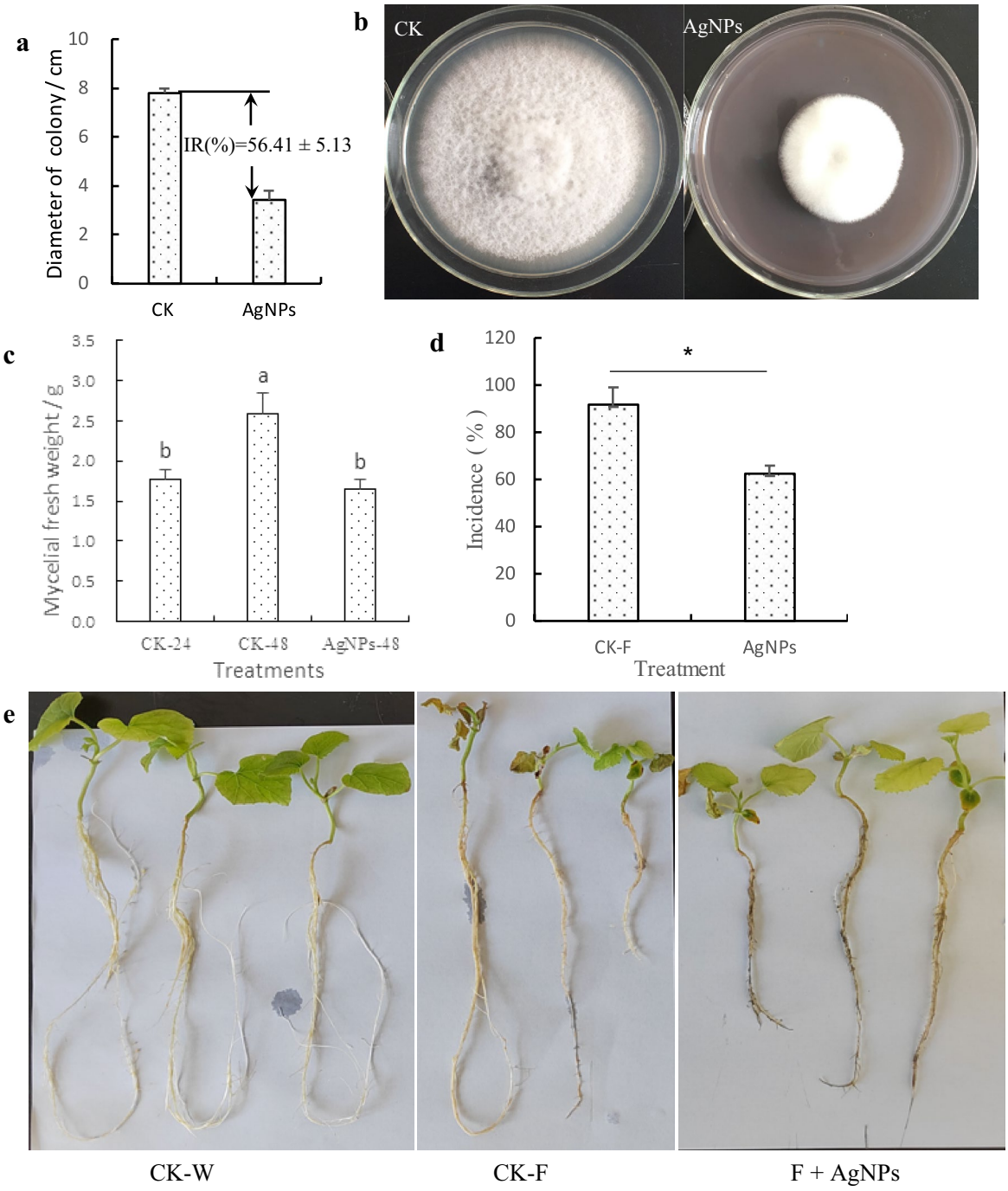

Fig. 1. Inhibitory effect of AgNPs against *F. oxysporum* (**a** and **b** on PDA medium, **c** in PDB medium) and control of AgNPs on the muskmelon *Fusarium* wilt (**d**, **e**). CK-W represents muskmelon seedling without inoculation with *F. oxysporum* and culturing in culture water without AgNPs. CK-F represents muskmelon seedling inoculated with *F. oxysporum* and culturing in culture water without AgNPs. F + AgNPs represents muskmelon seedling inoculated with *F. oxysporum* and culturing in AgNPs solution (25 mg/L). Different letter(s) in the same column differ significantly according to Duncan's test ($p < 0.05$) in **c**. Statistically significant differences are shown by $^{*}p < 0.05$ in (**d**).

Treatments	Germination rate/%	Germination potential/%	Root length/cm	Stem length/cm	Number of lateral roots	Fresh weight/mg
CK	86.67 ± 5.77[a]	73.33 ± 11.55[a]	5.53 ± 0.66[b]	1.35 ± 0.22[b]	5.07 ± 1.33[b]	98.0 ± 10.99[a]
AgNPs	96.67 ± 8 2.88[a]	83.33 ± 2.89[a]	9.13 ± 0.87[a]	1.79 ± 0.12[a]	10.83 ± 1.78[a]	112.70 ± 5.83[a]

Table 1. Effect of AgNPs on the seed germination and stem/root growth of seedlings. Different letter (a and b) in the same column differ significantly according to Duncan's test ($p < 0.05$).

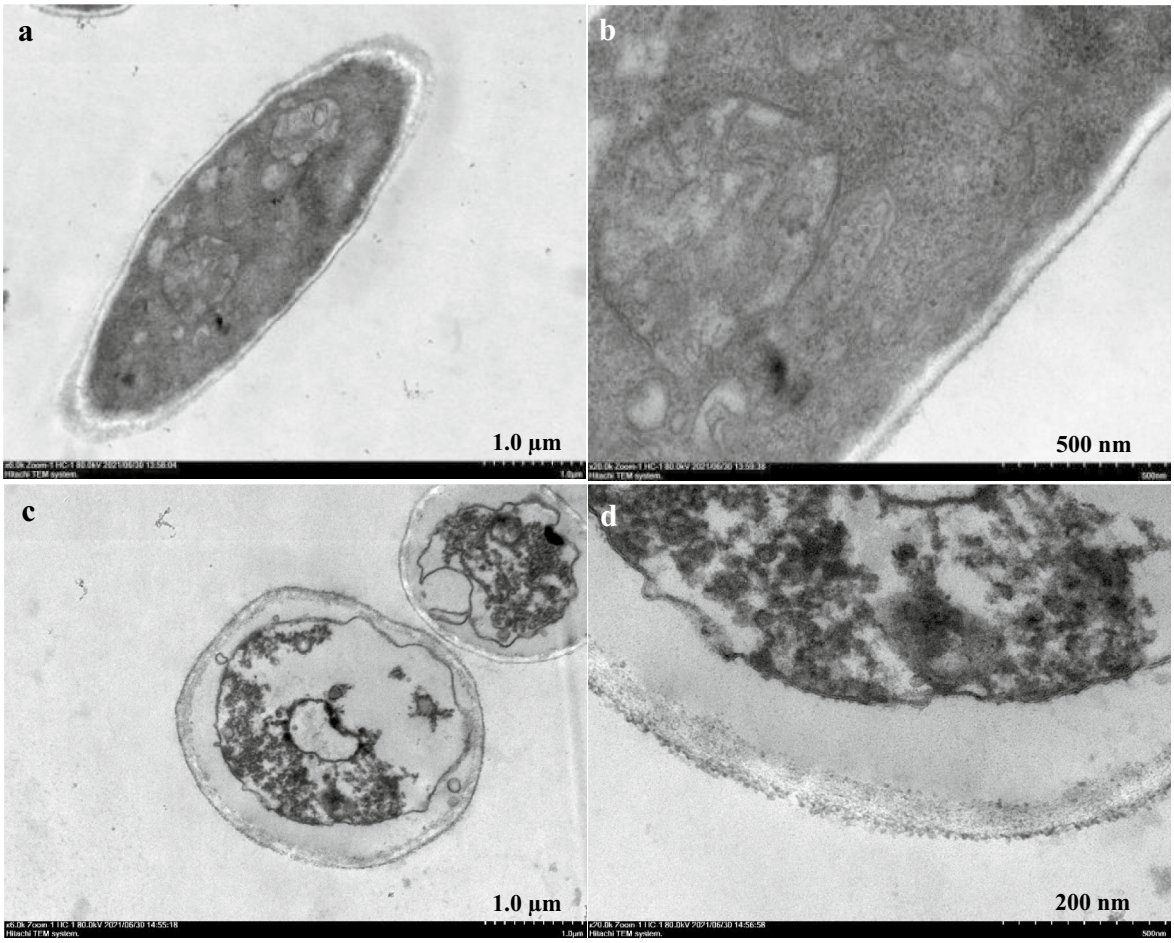

Fig. 2. AgNPs treatment disturbs fungal morphology of *F. oxysporum*. Mycelia treated without AgNPs (**a**, **b**) and with AgNPs (**c**, **d**) were imaged by transmission electronic microscopy (TEM).

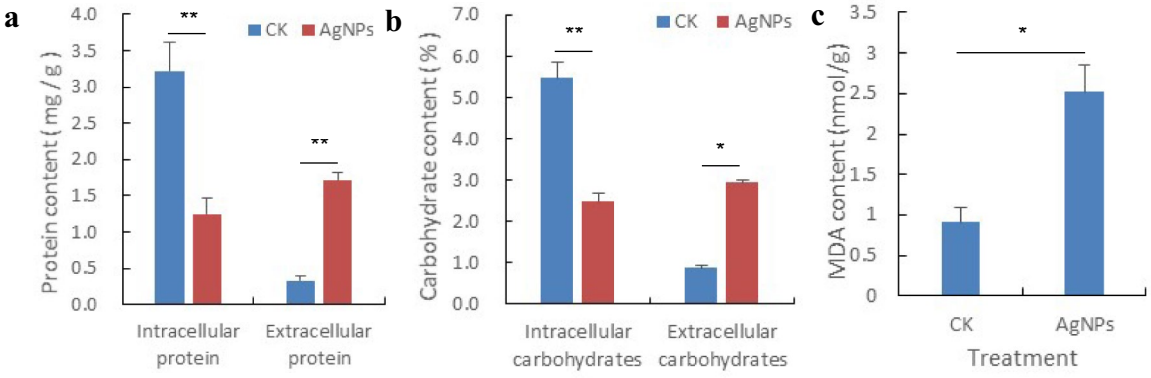

Fig. 3. Effect of AgNPs on soluble proteins content (**a**), soluble sugars content (**b**) and MDA content (**c**). Data are presented as mean ± standard error of the mean (SEM) of 3 individuals. Statistically significant differences are shown by $*p < 0.05$ and $**p < 0.01$ from two-way ANOVA.

synthesized NPs of similar shape and size. Biomolecules from *Trichoderma* sp. act as capping agents, which prevent the NPs from aggregating and releasing harmful substances and increase the NPs properties toward plant pathogens to stabilize the NPs, make them less toxic and present greater biological activity[37]. In these NPs, Silver nanoparticles (AgNPs) are one of the most widely studied and used metal nanoparticles because of their unique physical and chemical properties, including small particle size, large surface area, various shapes and porosity[7]. Earlier studies have found that AgNPs could kill many kinds of harmful microbes including bacteria, fungi, viruses etc. and had a short time in killing pathogens compared with other forms of silver[38]. Wen et al.[39]

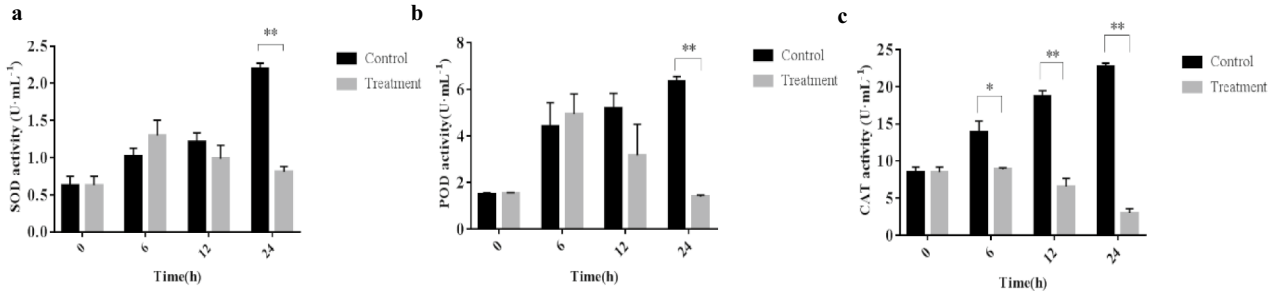

Fig. 4. Effect of AgNPs on the activity of protective enzymes (**a**) SOD; (**b**) POD; (**c**) CAT. Data are presented as mean ± standard error of the mean (SEM) of 3 individuals. Statistically significant differences are shown by $^{*}p < 0.05$ and $^{**}p < 0.01$ from two-way ANOVA.

found that 2 nm AgNPs could decrease *Ustilaginoidea virens* growth and virulence to inhibit it infection to rice plants. AgNPs mycosynthesized with fresh cultures of *Trichoderma* sp. and *Cephalosporium* sp. showed a higher antifungal activity against *F. oxysporum* (FOC) in vitro at 100 µg/mL and could reduce *Fusarium* wilt incidence of chickpea by 73.33%[40]. Antifungal activity of AgNPs on phytopathogenic fungi was inspected based on colony formation via in vitro Petri dish assay. The extension of colony was inhibited and antifungal efficiency of AgNPs was lowered at 24 h, which suggested inhibiting diseases development was depended on the exposure of AgNPs with spores and germination tube. At the same time, the growth chamber inoculation assays further confirmed that AgNPs significantly reduced these two fungal diseases on *Lolium perenne* when AgNPs was applied at 3 h before spore inoculation, whereas the efficacy significantly diminished with an application at 24 h after inoculation[41,42]. In our study, AgNPs mycosynthesized with *T. longibrachiatum* could inhibit the growth of *F. oxysporum* hyphae on the PDA medium, which closed to the results of the foregoing studies. In the PDB culture, the weight of mycelia slightly decreased after AgNPs addition in medium, which showed that AgNPs could not only suppress the growth of pathogenic fungi, but also destroy the cell wall and membrane, resulting in the leakage of protoplasm, causing the weight of mycelia to decrease. Growth in light incubator inoculation assay further confirmed that AgNPs decreased the incidence of muskmelon seedlings, which indicated AgNPs could be a fungicide to control plant pathogens.

Numerous studies have reported adverse effects of AgNPs[43–45] or beneficial effects on plants, such as promoting germination of seed and plant growth, enhancing the biochemical traits[4,46], increasing the yield of wheat[47]. The progressive and destructive effects of AgNPs on the plants may vary depending on their properties and origins, exposure concentration, and dispersed exposure media, as well as test species[34,45]. Different types of nanofertilizers (including silver, iron, zinc, titanium, carbon nanotubes, molybdenum and silica) have been developed for plant growth regulator and immunity inducer and applied on various crop systems, showing efficient impact on root elongation, shoot elongation, plant biomass, chlorophyll content and seed germination at certain concentrations[33]. Our results showed that AgNPs at 25 mg/L had progressive effect on seed germination, growth of seedlings of muskmelon, which were similar to the results of Matras et al.[47]. The results suggested that AgNPs biosynthesized with *T. longibrachiatum* could improve the growth of muskmelon seedlings, which may enhance the resistance of muskmelon to FOC.

AgNPs show the various modes of action on which they perform their antimicrobial activity including: (i) damaging cell wall and cell membrane, causing leakage of cellular content, (ii) denaturation of protein, causing metabolism dysfunction and organelles destabilization inside the cell, (iii) inducing ROS production to oxidize lipids, proteins and mediate cellular and ROS toxicity[16,48]. In our study, after fungal mycelia were immersed in AgNPs solution for 24 h, TEM observation found fungal cell disintegration, separation of hyphal wall and cell membrane, micropore or fissure formation on cell wall, deformation of some organelles, which were in agreement with these reported by Min et al.[49] and Tomah et al.[50]. The increase of protein and carbohydrate content in the culture medium containing AgNPs also indirectly indicated that silver nanoparticles could damage the cell wall and membrane, resulting in leakage of intracellular substances. Malondialdehyde (MDA) is produced by free-radical-mediated chain of reactions and an important marker of lipid peroxidation to measure level of disruption of the cell membrane[51,52]. MDA content in mycelia treated by AgNPs increased significantly, showing that AgNPs induced more ROS to oxidize the lipid. Response to the more ROS, activities of the protective enzymes (SOD, POD and CAT) changed obviously, showing an inverted U-shaped curve of first rising and then decreasing in 24 h, whereas activities of these enzymes continued increasing in sterile water (CK) in 24 h (Fig. 4). Qian et al.[53] believed that the balance between ROS production and its scavengers, such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) might be upset following exposure to metal compounds, which resulted in change of MDA content in cells because of MDA was an indicative of the degree of balance between ROS and antioxidative substances. Our results showed that CAT was sensitive to AgNPs, consistent with reports by Li et al. about tea plant stressed by cold, salt and drought[54]. This meant that CAT might be one of the key enzymes involved in response to AgNPs.

Conclusions

Biosynthesized AgNPs using *T. longibrachiatum* could be considered as an excellent antifungal agent against fungal pathogens, such as *F. oxysporum* and promote seed germination, plant growth and development at low dose. The present study showed that AgNPs had the ability to inhibit the growth of *F. oxysporum* or eliminate fungus and 25 mg/L AgNPs have the capacity to promote the seed germination, growth of muskmelon seedlings and reduce the incidence of muskmelon *Fusarium* wilt. The antifungal mechanism of AgNPs to *F. oxysporum* should depend on impairing the development, damaging cell structure, and interrupting cellular metabolism pathways. The results will supply useful information for the broad application of silver nanoparticles. However, antifungal mechanism of biosynthesized AgNPs needs to be further studied at the molecular level.

Data availability

The data used to support the findings of this study are included within the article.

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Author contributions

X.L., X.C. and T.L.: Conceptualization; X.L. and X.C.: Methodology; R.T., Z.G. and X.L.: Validation; T.L. and X.C.: Formal Analysis; X.C., X.L. and Z.G.: Investigation; X.C. and T.L.: Data curation; T.L., R.T. and X.L.: Writing—Original Draft Preparation; X.L.: Writing—Review & Editing; T.L. and X.L.: Visualization; X.L. and Z.G.: Supervision; Z.G.: Funding acquisition.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to Z.G.