

## EFFECT OF NANO-ZnO ON ACTIVITY OF WHEAT FUSARIUM HEAD BLIGHT PATHOGEN

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### Abstract

The effects of nano-ZnO on the inhibition efficiency of the activity of wheat Fusarium head blight pathogen, wheat seed germination, and seedling growth were investigated, and the feasibility of using it as a novel seed treatment agent was determined. Results showed that (1) the survival rate of pathogenic conidia was significantly reduced when the nano-ZnO concentration was 50 mg L<sup>-1</sup>. The survival rate was dropped to the lowest when the nano-ZnO concentration was 100 mg L<sup>-1</sup>, and the growth of the mycelium of Fusarium head blight pathogen was significantly inhibited when 100 mg L<sup>-1</sup> was applied. (2) Nano-ZnO treatment increased germination rate, seedling length, root length, and dry matter weight to different degrees. When treated with nano-ZnO at a concentration of 50 mg L<sup>-1</sup>, nano-ZnO significantly improved infested wheat seedlings, while the superoxide dismutase (SOD) and catalase (CAT) *in vivo* activities and malondialdehyde (MDA) content were decreased. (3) Nano-ZnO had no adverse effects on the seed germination and seedling growth of healthy wheat. It also increased SOD and CAT activities in early seedlings and decreased MDA content. Therefore, nano-ZnO not only inhibits pathogen activity and increases seed germination rate but also has a certain role in promoting the growth of healthy seeds. These results show an important application prospect for nano-ZnO that can be applied to crop seed treatment to prevent seed diseases.

**Key words:** Nano-ZnO; Fusarium head blight pathogen; Wheat; Germination rate; Biomass; Antioxidant enzyme.

### Introduction

Fusarium head blight is a worldwide disease mainly caused by fungal pathogen *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schweinitz) Petch] and occurs in mostly warm and humid areas (Goswami & Kistler, 2010). The disease caused by Fusarium head blight is extremely harmful because it affects wheat yield. In the popular year, it can cause 5% to 10% yield loss. Pandemic year can lead to partial crop failure (Cheng *et al.*, 2012). Meanwhile, Fusarium head blight can produce a variety of pathogenic toxins, such as deoxynivalenol (Zhang *et al.*, 2012), that are extremely harmful to humans and animals and seriously affect the quality and commercial value of wheat (Chehri *et al.*, 2011). With regard to the prevention and control of wheat Fusarium head blight, the main measure used at present is spraying pesticides during the flowering period. However, this method produces drug-resistant strains against Fusarium head blight, thereby causing potential environmental pollution and endangering the health of humans and animals (Young *et al.*, 2015). Seed treatment is a cost-effective method of treating seed sickness. Traditional methods of seed treatment include the use of fungicides, placing seeds in boiling water, and chlorine treatment (Wise *et al.*, 2009). However, some deficiencies are observed in traditional treatment methods, such as the poor germicidal effects of seeds and reduction in seed germination and vigor (Du *et al.*, 2005). Therefore, finding an economical and efficient green seed processing method becomes highly important.

Metal oxide nanoparticles are an important class of nanomaterials. Because of their high specific surface area

and unique physical and chemical properties (Aziz *et al.*, 2017), they have gradually become a hot topic with a broad range of applications in energy conversion and storage (Liu *et al.*, 2017; Pan *et al.*, 2016), optoelectronics (Wang & Song, 2006), and biological field (Yadavalli & Shukla, 2017; Golbamaki *et al.*, 2015; Gao *et al.*, 2017). However, studies have found that metal oxide nanomaterials would have either positive or negative effects on organisms (Yang *et al.*, 2006). Among these nanoscale metal oxides, particulate nano-ZnO has found wide applications (Abdolmaleki *et al.*, 2012). Nano-ZnO has the advantages of broad-spectrum antibacterial, high efficiency, low toxicity, and low probability to cause bacterial resistance. The antibacterial effect of nano-ZnO is mainly affected by bacterium type, morphology, concentration, and external environmental conditions (Kuang *et al.*, 2015). At present, the following three processes are the main aspects of nano-ZnO antibacterial mechanism (Mirhosseini & Firouzabad, 2013):

- (1) Release of free Zn<sup>2+</sup>.
- (2) Interaction between nanoparticles and microbial cells.
- (3) Generation of reactive oxygen species (ROS).

Nano-ZnO can affect plant growth and development. In particular, Raliya and Tarafdar found that the appropriate concentration of nano-ZnO treatment guar bean is able to increase plant biomass, leaf area, chlorophyll and protein content, and the acid and alkaline phosphatase activities (Raliya & Tarafdar, 2013). Previous research has disclosed that nano-ZnO can increase the antioxidant enzyme activity in rice (Anita *et al.*, 2018). But some studies have shown that when the

concentration of nano-materials exceeds 200 mg L<sup>-1</sup>, the growth and development of plants will be inhibited (Di *et al.*, 2014). However, only a few studies have been conducted on the effects of nano-ZnO on pathogenic microorganism activity carried by plant seeds and by the influence of plant seedling stage. Moreover, no clear conclusion has been made about the biological effects of nanomaterials. This study examines the effects of nano-ZnO on the germination and early seedlings of *Fusarium head blight* and wheat seeds to provide references for its improved application in the agricultural field.

## Materials and Methods

Nano-ZnO was purchased from Tangshan Caofeidian Taihong Shengda New Material Co., Ltd. with purity of  $\geq 95\%$ , particle size of  $20 \pm 5$  nm, and specific surface area of  $50 \pm 10$  m<sup>2</sup> g<sup>-1</sup>. A total of 1000 mg L<sup>-1</sup> of nano-ZnO suspension with good dispersion was prepared and measured for dispersion by using UV-vis spectrophotometry (light source  $\lambda = 810$  nm) (Zhang *et al.*, 2008). *F. graminearium* strain PH-1 (NRRL 31084) was purchased from Zhengzhou Jingsiwei Chemical Co., Ltd. Wheat seed variety Jimai 22 purchased from Shandong Academy of Agricultural Sciences.

**Antifungal activity of nano-ZnO:** Growth rate method (Jin *et al.*, 2015) was used for the detection of the antifungal activity of nano-ZnO. The specific operations are as follows: dilution of nano-ZnO concentrations to 50, 100, 150, and 200 mg L<sup>-1</sup> with potato dextrose agar (PDA); incubation of 5 mm diameter cake for 5 days, which was then gently placed on a medium plate (with the mycelial side down), containing different concentrations of nano-ZnO as described above; A PDA plate without nano-ZnO was used as the blank control and its culture in a 28 °C incubator. Each treatment was repeated three times. On the 3rd and 5th day of culture, the diameter of the pathogen was measured by the cross method, and the growth inhibition rate was calculated using the following equation:

$$\text{Inhibition rate (\%)} = [(D_1 - D_0) - (D_2 - D_0)] / (D_1 - D_0) \times 100\%$$

where  $D_0$  is the diameter of initial mycelia cake,  $D_1$  is the expansion diameter of mycelia in the blank control, and  $D_2$  is the expansion diameter of mycelia in the treatment.

**Inhibition of nano-ZnO on conidia of *F. graminearium*:** Conidia suspensions of *F. graminearium* strain PH-1 (NRRL 31084) were cultured and collected for cryogenic preservation. The suspension concentration was adjusted to 10<sup>6</sup> mL<sup>-1</sup>. The spore suspension was mixed with different concentrations of nano-ZnO solution at a volume ratio of 1:99. The nano-ZnO concentrations were 50, 100, 150, and 200 mg L<sup>-1</sup>, and the spore suspension concentration was 10<sup>4</sup> mL<sup>-1</sup>. The suspension with no nano-ZnO as a control was incubated at a constant temperature of 28°C. After exposing in the box for 2 h, the mixture was diluted to a suitable multiple, and 100  $\mu$ L

of each mixture was transferred in a PDA fixation medium. They were placed in a constant temperature incubator at 28°C for 3 days. The number of colonies was counted to calculate the growth inhibition rate by using the following equation:

$$\text{Inhibition rate (\%)} = [(N_0 - N_1) / N_0] \times 100\%$$

where  $N_0$  is the number of germination in control group, and  $N_1$  is the number of germination in treatment group.

## Effect of nano-ZnO on seed germination and seedling growth of wheat infected with *F. graminearium*:

The preparation of contaminated wheat seeds adopted the method as previously reported (Young *et al.*, 2015) and with a slight modification. The wheat seeds with complete shape and same size were selected, washed with ultrapure water, and air-dried. Clean wheat seeds were soaked with spore suspension. The ratio of the volume of the solution (mL) to the seed mass (g) was 10:3. These seeds were placed in a constant temperature incubator at 28°C for 12 h to allow the affection of bacteria. After 12 h, the bacteria solution was discarded, and the seeds were air-dried. After the surface moisture of seeds was dried, seeds infected with *F. graminearium* were soaked in spore suspension. The ratio of the volume of the solution (mL) to the seed mass (g) was 10:3. The nano-ZnO solution concentrations were 12.5, 25, 50, 100, and 200 mg L<sup>-1</sup>, and ultrapure water was used as the blank control, which was incubated in a constant temperature incubator at 28°C for 12 h. Then, the seeds were air-dried. After the seed surface moisture was air-dried, the treated seeds were sown in a petri dish containing 2 layers of 15 cm filter paper. A total of 100 seeds were sown per dish, and the treatment was repeated thrice. The culture dish was placed in a constant temperature incubator with temperature of 20°C, illumination intensity of 6400 lx, humidity of 50%, and daily light and dark time of 12 h. Water was added once a day. On the 7th day, the germination rate was measured, and the root length, seedling length, dry weight above the ground, and dry weight under the ground were measured. On the 10th day, the SOD activity, CAT activity, and MDA content in wheat leaves were measured.

## Effect of nano-ZnO on normal wheat seeds and seedlings:

The wheat seeds with complete shape and same size were selected, washed with ultrapure water, and air-dried. After the surface moisture of seeds was dried, wheat seeds were soaked with spore suspension. The ratio of the volume of the solution (mL) to the seed mass (g) was 3:1. The nano-ZnO solution concentrations were 12.5, 25, 50, 100, and 200 mg L<sup>-1</sup>, and the ultrapure water was used as the blank control. The treatment groups and the control group were placed in a constant temperature incubator at 28°C for 12 h, and then air-dried. After the seed surface moisture was air-dried, the seeds were sown in a petri dish containing 2 layers of filter paper (15 cm in diameter). A total of 100 seeds were sown per dish, and the treatment was repeated thrice. The culture conditions and test indicators are the same as that mentioned in Section 2.2.3.

### Statistical analysis

Statistical analysis was performed using SPSS software (version 22.0, IBM Corp., Armonk, NY, USA). Significance of difference was analysed using one-way analysis of variation (ANOVA), followed by Duncan's test with a level of significance of  $p = 0.05$ .

### Results and Discussion

**Antifungal activity of nano-ZnO:** The pathogens were grown on different nano-ZnO concentrations on the PDA plates for 3 days. When using the concentration of 50 mg L<sup>-1</sup>, the inhibition rate of nano-ZnO on mycelial growth was 7.69% (Fig. 1, Table 1). However, compared with the control group (0 mg L<sup>-1</sup> nano-ZnO), no significant difference was observed ( $P > 0.05$ ). When the concentration was 100 mg L<sup>-1</sup>, the inhibition rate of nano-ZnO to mycelial growth reached 21.20%, and the growth diameter of mycelium was significantly lower than that of the control group ( $p < 0.05$ ). When the concentration continued to increase, the inhibition rate of nano-ZnO on mycelial growth of the pathogenic fungus was further increased. This result was significantly different from result in the control group ( $p < 0.05$ ), thereby indicating that the growth of pathogenic mycelium was effectively inhibited. However, the inhibition effects of 100, 150, and 200 mg L<sup>-1</sup> concentrations showed no significant

difference ( $p > 0.05$ ). The results indicate that the pathogens were grown on PDA plates containing different nano-ZnO concentrations for 5 days. When the nano-ZnO concentration was 50 mg L<sup>-1</sup>, no significant difference was observed from the control group. When the concentration was greater than or equal to 100 mg L<sup>-1</sup>, the growth diameter of the pathogenic mycelia containing nano-ZnO plates was significantly lower than that of the control group, and the difference between the three treatment groups also reached a significant difference ( $p < 0.05$ ). These results show that a certain concentration of nano-ZnO can effectively inhibit the growth of *Fusarium head blight* pathogen and can effectively continue to play this kind of role (Fig. 1, Table 1).

**Inhibition of nano-ZnO on conidia of *F. graminearum*:** Different concentrations of nano-ZnO treatment can significantly reduce the survival rate of pathogenic conidia ( $p < 0.05$ ) and decrease with the increase in nano-ZnO concentration of pathogenic conidia survival (Fig. 2). After the concentration exceeding 100 mg L<sup>-1</sup>, nano-ZnO tended to flatten the survival inhibition rate of conidia, which result may be the concentration of nanoparticles adsorbed by cell walls per unit area of the spores reached saturation (Durán *et al.*, 2015). If the concentration was continued to increase, then its effective concentration should have been constant. Thus, its performance tends to be flattened.

**Table 1. Inhibition rates on the mycelium of *F. graminearum* on the 3rd and 5th day from different nano-zno concentrations (Mean  $\pm$  SD).**

Nano-ZnO treatment concentrations (mg L <sup>-1</sup> )	Diameter of mycelium (cm)		Inhibition rate (%)	
	Incubation time of 3 d	Incubation time of 5 d	Incubation time of 3 d	Incubation time of 5 d
0	3.88 $\pm$ 0.09a	6.42 $\pm$ 0.66a	—	—
50	3.62 $\pm$ 0.16a	5.93 $\pm$ 0.08a	7.69	8.31
100	3.16 $\pm$ 0.26b	5.07 $\pm$ 0.47b	21.20	22.82
150	2.69 $\pm$ 0.13bc	4.17 $\pm$ 0.21c	35.11	38.03
200	2.30 $\pm$ 0.13c	3.45 $\pm$ 0.23d	46.75	50.14

Note: Different lowercase letters within column indicate significant difference at the 0.05 level

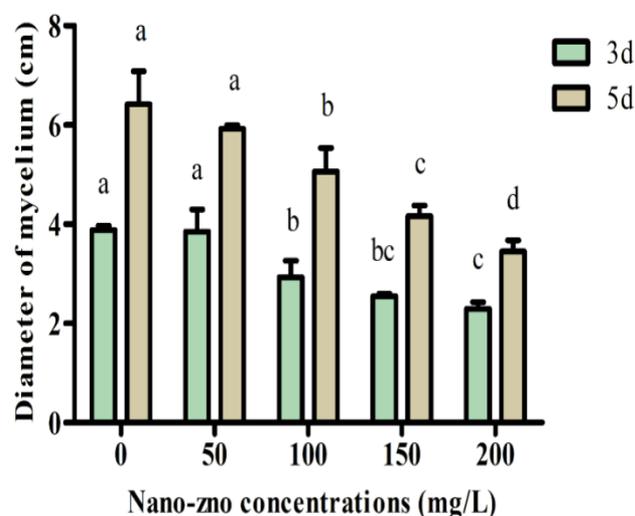


Fig. 1. Diameter of mycelium under different nano-zno concentrations on the 3rd and 5th day.

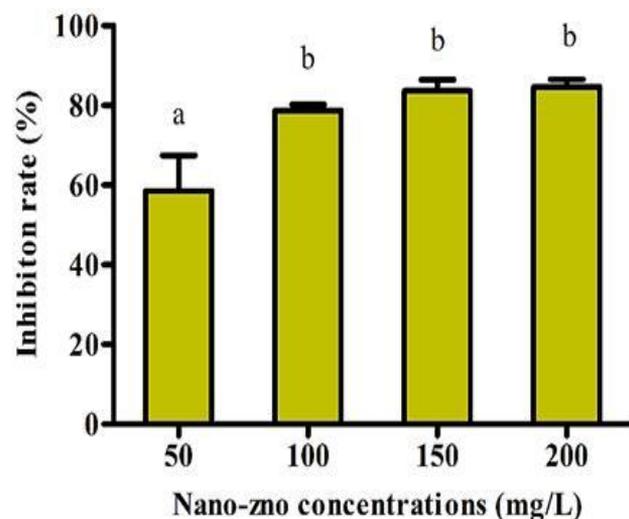


Fig. 2. Inhibition rates of different nano-zno concentrations on pathogenic conidia.

**Effect of nano-ZnO on seed germination and seedling of wheat infected with *F. graminearum*:** The results shown in Table 2 indicate that compared with the control group, the treatment of the infested seeds with nano-ZnO solution can increase the germination rate to various degrees. Moreover, the germination rate was the highest when the concentration was 100 mg L<sup>-1</sup>. Nano-ZnO significantly promoted seedling growth at a concentration of 50 mg L<sup>-1</sup> ( $p < 0.05$ ). Compared with the control group, when the concentration of nano-ZnO at 50 and 200 mg L<sup>-1</sup>, the root growth of infested wheat was significantly promoted ( $p < 0.05$ ). The treatment of seeds with nano-ZnO can increase the dry biomass weight of the infested wheat above-ground and underground, and its maximum biomass can be reached at a specific nano-ZnO concentration. When the nano-ZnO treatment concentration was 50 mg L<sup>-1</sup>, the dry above-ground biomass reached a maximum, and the dry underground biomass reached its maximum at the concentration of 25 mg L<sup>-1</sup>. The dry underground biomass value will decrease when the nano-ZnO concentration is higher than 25 mg L<sup>-1</sup>. Therefore, suitable concentration of nano-ZnO solution to treat the infested wheat seeds can effectively alleviate the damages, such as reduced germination rate and reduced dry matter accumulation, caused by pathogenic toxins to wheat seeds and seedlings.

As shown in Table 3, except for concentrations of 50 and 200 mg L<sup>-1</sup>, the MDA content treated with other nano-ZnO concentrations was significantly lower than that of the control group ( $p < 0.05$ ). This result indicates that the treatment of infested seeds with low nano-ZnO concentration can reduce the MDA content in wheat seedlings. Table 3, shows that compared with the control group, the effect of different nano-ZnO solution

concentrations on the SOD activity of the seedlings in the treated nano-ZnO solution was different. In addition, the concentration of 50 mg L<sup>-1</sup> significantly increased the SOD activity ( $p < 0.05$ ). The concentration of 200 mg L<sup>-1</sup> showed no significant difference from the control group, and the remaining concentrations significantly reduced SOD activity. Except for concentrations of 12.5 mg L<sup>-1</sup>, The CAT activity was significantly increased when the nano-ZnO concentrations were 25, 50 100 and 200 mg L<sup>-1</sup> ( $p < 0.05$ ).

**Effect of nano-ZnO on the seed and seedling of wheat without *F. graminearum*:** The treatment of healthy seeds with different nano-ZnO solution concentrations showed no significant difference in terms of germination rate, seedling length, root length, and dry weight compared with the control group (Fig. 3). The results showed that nano-ZnO treatment had no adverse effect on the germination rate, seedling morphology, and biomass of healthy wheat seeds. These results were the same as the findings of previous research (Young *et al.*, 2015).

Except for the nano-ZnO concentration of 100 and 200 mg L<sup>-1</sup>, the MDA content of wheat was significantly lower than that of the control (Table 4). Other concentrations were not significantly different from the controls. All the treatment with nano-ZnO showed significantly difference ( $p < 0.05$ ) compare with control, and except for the concentration of 100 mg L<sup>-1</sup>, treatment of other concentrations significantly reduced the SOD activity. The effect of nano-ZnO on the CAT activity in seedlings exhibited significantly increased compared with that of the control group and showed a regular increase and then decrease, and the CAT enzyme activity of wheat seedlings was the highest at the concentration of 50 mg L<sup>-1</sup> (Table 4).

**Table 2. Effects of different nano-zno concentrations on the germination and growth of seeds infected with the *F. graminearum* (Mean  $\pm$  SD).**

Nano-ZnO concentrations (mg L <sup>-1</sup> )	Germination rate (%)	Seedling length (cm)	Root length (cm)	Dry aboveground biomass (mg)	Dry underground biomass (mg)
0	85.67 $\pm$ 1.67c	11.13 $\pm$ 0.51b	8.81 $\pm$ 1.73c	9.27 $\pm$ 0.23c	7.63 $\pm$ 0.22b
12.5	94.67 $\pm$ 0.58a	11.78 $\pm$ 0.70ab	11.01 $\pm$ 1.26abc	10.34 $\pm$ 0.58b	8.23 $\pm$ 0.06b
25	92.00 $\pm$ 3.00ab	11.82 $\pm$ 0.47ab	10.17 $\pm$ 1.47abc	10.42 $\pm$ 1.30ab	9.41 $\pm$ 0.29a
50	90.00 $\pm$ 173b	12.08 $\pm$ 0.68a	9.33 $\pm$ 0.84ab	11.39 $\pm$ 0.36a	9.01 $\pm$ 0.01a
100	96.00 $\pm$ 1.00a	11.83 $\pm$ 0.56ab	10.74 $\pm$ 1.67abc	10.64 $\pm$ 1.69ab	7.97 $\pm$ 0.90b
200	92.33 $\pm$ 3.79ab	11.91 $\pm$ 0.82ab	11.67 $\pm$ 1.67a	10.88 $\pm$ 1.28ab	8.07 $\pm$ 0.25b

Note: Different lowercase letters within column indicate significant difference at the 0.05 level

**Table 3. MDA, SOD, and CAT activities of the infested wheat seedlings with different concentrations of nano-zno.**

Nano-ZnO concentrations (mg L <sup>-1</sup> )	SOD activity (U/ mgprot )	CAT activity (U/ mgprot )	MDA content (nmol/ mgprot )
0	214.19 $\pm$ 0.63b	9.07 $\pm$ 1.61c	4.62 $\pm$ 0.07b
12.5	187.40 $\pm$ 2.72c	7.59 $\pm$ 0.27d	3.39 $\pm$ 0.21c
25	181.72 $\pm$ 4.73c	11.35 $\pm$ 0.11b	3.49 $\pm$ 0.06c
50	264.98 $\pm$ 4.87a	14.65 $\pm$ 0.39a	5.52 $\pm$ 0.4a
100	181.10 $\pm$ 2.32c	10.94 $\pm$ 0.36b	3.67 $\pm$ 0.06c
200	214.16 $\pm$ 3.65b	11.02 $\pm$ 1.00b	4.47 $\pm$ 0.30b

**Table 4. MDA, SOD, and CAT activities of uninfected wheat seedlings with different nano-zno concentration.**

Nano-ZnO concentrations (mg L <sup>-1</sup> )	SOD activity (U/ mgprot)	CAT activity (U/ mgprot)	MDA content (nmol/ mgprot)
0	188.17 ± 2.96 <sup>b</sup>	4.48 ± 0.73 <sup>d</sup>	3.95 ± 0.22 <sup>ab</sup>
12.5	166.63 ± 1.76 <sup>e</sup>	7.07 ± 1.22 <sup>c</sup>	2.95 ± 0.11 <sup>d</sup>
25	181.00 ± 1.89 <sup>cd</sup>	8.72 ± 1.48 <sup>b</sup>	3.29 ± 0.20 <sup>cd</sup>
50	178.97 ± 1.37 <sup>d</sup>	10.66 ± 0.42 <sup>a</sup>	3.32 ± 0.17 <sup>cd</sup>
100	197.73 ± 0.77 <sup>a</sup>	9.94 ± 0.17 <sup>ab</sup>	4.22 ± 0.22 <sup>a</sup>
200	183.05 ± 0.54 <sup>c</sup>	9.11 ± 0.05 <sup>ab</sup>	3.56 ± 0.55 <sup>bc</sup>

Note: Different lowercase letters within column indicate significant difference at the 0.05 level

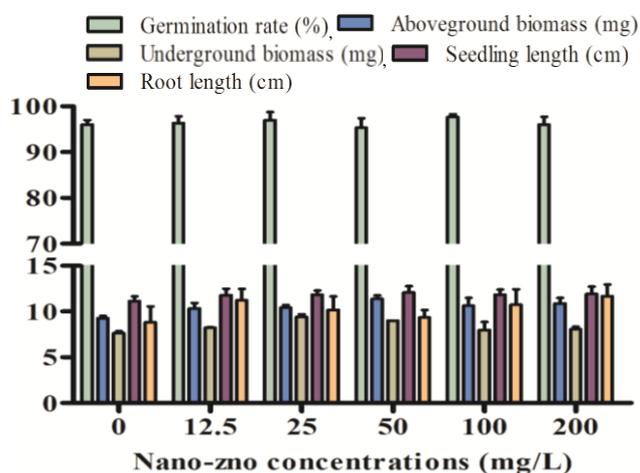


Fig. 3. Effects of different nano-ZnO concentrations on the seed germination and growth without the *F. graminearum* (Mean ± SD).

## Discussion

The experimental data show that the suitable nano-ZnO concentration had certain ability to prevent and control Fusarium head blight. If the concentration is too low, the antibacterial effect cannot be achieved. Meanwhile, if the concentration is too high, the antibacterial performance will not show significant change or will decrease. This phenomenon can be explained by one of the viewpoints of the nano-ZnO antibacterial mechanism, that is, nano-ZnO adsorbs on the cell membrane and attacks cells, inducing the cells to produce excessive ROS and indirectly leading to cell death (Sawai & Yoshikawa, 2004). When the nano-ZnO concentration is too low, only a scarce amount of nanoparticles were adsorbed to attack the cell membrane, and the amount of reactive oxygen generated is not enough to cause cell apoptosis. Meanwhile, when the nanoparticles adsorbed on the membrane per unit area reach saturation, the antibacterial effect is the strongest. The continuous rising of ion concentration will cause the system ionic strength to become too high, leading to particle precipitation. Thus, the antibacterial effect will decrease instead when the nanoparticles concentration exceeds its saturation number (Umadevi *et al.*, 2011). Therefore, attention shall be paid to the appropriate application of nano-ZnO concentration in actual production.

In addition, the results of this study show that nano-ZnO can alleviate the damage of *F. graminearum* to wheat seeds and seedlings to a certain extent. Nano-ZnO has no adverse effect on the germination and seedling growth of healthy seeds and can increase the antioxidant enzyme activity and reduce the MDA content. But when

the concentration of nano-materials is higher than 200 mg L<sup>-1</sup>, it will significantly inhibit the growth and development of plants. Therefore, the concentration of seeds treated with nano-materials should not be higher than 200 mg L<sup>-1</sup> (Di *et al.*, 2014). The results show that the germination rate, seedling length, root length, and dry matter accumulation after treatment with different nano-ZnO solution concentrations tend to follow the decrease-increase-decrease trend. The crest mostly appears in 50–100 mg L<sup>-1</sup>, thereby indicating that nano-ZnO can effectively alleviate seed injury caused by infesting bacteria in this concentration range. At the same time, nano-ZnO has the weakest toxicity to the plants themselves. When the concentration is low, nano-ZnO cannot improve the damage of seeds or plants caused by the bacteria. Moreover, the nanoparticles will stimulate the pathogens, which will be in a more active stress state and increase the damage to the plants. When the concentration is too high, it will lead to precipitation (Umadevi *et al.*, 2011) and may induce excessive free radicals in the plant and cause poisoning to the plant, resulting in the trend decrease-increase-decrease (Lu *et al.*, 2002). From the trend of CAT activity and MDA content in the treated group with both bacteria and normal seedlings, appropriate nano-ZnO concentration can increase CAT activity and reduce MDA content to different degrees after treatment with bacteria or healthy seeds (Abdelaal *et al.*, 2020). This result indicates that under the dual stimulation of nanoparticles and pathogenic bacteria or nanoparticle stimulation alone, the plants respond to the adverse external environment, self-regulate within the plant tolerable range, and activate CAT to scavenge active oxygen and reduce the MDA content (Li *et al.*, 2013). The SOD activity in the two groups showed a decrease-increase-decrease trend. This result may be due to the different effects of the different enzyme types. When faced with external environmental stimuli, the trend of different antioxidant enzymes varies (Liu *et al.*, 2006). SOD is the first line of defense for plants against oxidation. The SOD activity, CAT activity, and MDA content in wheat seedlings were all in a relative optimal state after treatment by using 50 mg L<sup>-1</sup> nano-ZnO with bacteria. The appropriate concentration of nano-ZnO particles in contact with plant cells will stimulate more peroxidation in the plant, resulting in a rise in free radical content within a certain range (Lin & Xing, 2008), which stimulates the plant antioxidant enzymes to increase their activity and reduce MDA content in vivo. If the nano-ZnO concentration is too high, then the induced free radicals are excessive, and the antioxidant system cannot be cleared in time. The free radicals will directly attack proteins, DNA, lipids, and other biological macromolecules, as well as damage

organisms, inhibit plant growth, and even cause plant death (Lu *et al.*, 2002). From the microscopic perspective, this phenomenon preliminarily explains that the suitable nano-ZnO concentration can improve the possible growth of the infested seedlings. Therefore, in the appropriate concentration range, nano-ZnO can inhibit pathogen invasion and protect plant growth. The results of this study indicate that nano-ZnO has a strong antibacterial potential against wheat pathogens and has no adverse effects on the germination and seedling growth of wheat seeds. Thus, nano-ZnO can be used as a new material for seed treatment to prevent or control wheat disease. In addition, nano-ZnO can be used in other crop seeds to prevent or control seed disease. However, nanomaterials can probably threaten human health and have a certain risk (Nel *et al.*, 2006). Therefore, conducting in-depth studies on the migration and accumulation of nanoparticles in plants and their effects on human health in the future is necessary.

### Conclusions

This study reveals that when the concentration of nano-ZnO was 50 mg L<sup>-1</sup>, the survival rate of pathogenic conidia was considerably reduced. The growth of mycelium of gibberellin pathogen was inhibited when 100 mg L<sup>-1</sup> was applied. Varying rates of increase in germination rate, seedling length, root length, and dry matter weight were observed in infested wheat seeds treated with nano-ZnO. When treated with nano-ZnO at a concentration of 50 mg L<sup>-1</sup>, nano-ZnO significantly improved the SOD and CAT activities in infested seedlings and reduced MDA content. Nano-ZnO had no adverse effects on the seed germination and seedling growth of healthy wheat, and increased SOD and CAT activities in early seedlings while decreasing MDA content. Therefore, nano-ZnO not only reduces pathogen activity and increases seed germination rate but also plays a critical role in promoting the growth of healthy seeds. These results show an important application prospect of nano-ZnO as a new seed treatment agent.

### Acknowledgement

Researchers supporting project: the Science and Technology Service Network Initiative of Chinese Academy of Sciences (KFJ-ST-S-QYZD-020), Science and Technology Projects of Anhui Province (KJ2018A0140) and the Program of Research on Coating Material for Improving the Resistance of the Seed.

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(Received for publication 5 March 2019)