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Effect of Nano-zinc Oxide on Rapeseed Growth

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Abstract: Landscape plants are an important part of landscape architecture. Landscape plants can not only be used for plant landscaping, but also beautify the environment, improve the environment and regulate the climate, and play an important role in the stability of the ecosystem and the improvement of human living environment. The extensive application of nano-materials in industry, agriculture and other fields has led to a large number of residues in the soil and water environment, affecting the growth and development of garden plants. It has been proved that high concentration of nanomaterials have certain toxic effects on plants, but the research is not sufficient and needs further research. In this study, Rapeseed, an oil crop with ornamental and economic value, was taken as the research object, and nano-zinc oxide was used as the experimental material to explore its mechanism. The main results are as follows: The germination of Rapeseed seeds was studied by using the filter paper culture dish germination method. It was found that the germination rate of Rapeseed seeds under the treatment of nano-zinc oxide was not significantly different from the control. Compared with CK, under 400 mg Ł-1 nano-zinc oxide and the same concentration of zinc oxide, the biomass of Rapeseed significantly decreased, and the photosynthesis of Rapeseed absorbed light energy through chlorophyll, while under 400 mg Ł-1 nano-zinc oxide stress, the chlorophyll content of Rapeseed decreased, and the photosynthetic capacity decreased. Compared with CK, 400 mg Ł-1 nano-zinc oxide and 400 mg ·L-1 zinc oxide significantly increased the hydrogen peroxide content by 73.16% and 67.07%, respectively. Compared with 400 mg Ł-1 nano-zinc oxide, 400 mg Ł-1 zinc oxide significantly decreased by 3.52%; Compared with CK, each treatment significantly increased the content of superoxide anion, and compared with 400 mg Ł-1 nano-zinc oxide, 400 mg Ł-1 zinc oxide significantly decreased 4.81%.

1. Introduction

Nano zinc oxide, as a nano metal oxide with unique properties, is widely used in industry, agriculture and daily life. With the extensive application of nanomaterials, more and more nanomaterials are discharged into the environment. These nanomaterials are easily absorbed and utilized by plants and transmitted through the biological chain. The effect of nano-metal oxides on plants is related to the particle size, concentration and the properties of nano-particles.

Nanoparticles are smaller in size and easier to be absorbed and utilized by plants. According to the literature, nano-metal oxides have certain effects on plants and have duality. Susmita Bandyopadhyay et al. showed that two concentrations of nano-zinc oxide, 500 mg kg^{-1} and 750

mg ŀkg^{-1}, would reduce the growth and dry weight of alfalfa, while 500 mg ŀkg^{-1} zinc oxide would promote the growth of alfalfa [1]. Low concentrations of nano-metal oxides promote plant seed germination and plant growth [2-4], while high concentrations inhibit plant growth [5]. High concentration of nano-zinc oxide will inhibit the growth of cherry radish [6], Chinese cabbage seeds [6], mung bean sprouts [7], asparagus mosaic [8] and corn [9]. Nano-zinc oxide inhibits the elongation of soybean roots and buds, reduces root surface area and root volume, and has a stronger inhibitory effect at high concentrations [10]. When the concentration of nano-zinc oxide is 20 mg Ł$^{-1}$, it can better promote the growth, yield and zinc content of mung bean seeds, but when the concentration is more than 20 mg Ł$^{-1}$, it has no obvious promoting effect [11]. As the concentration of nano-zinc oxide solution increases, it will cause toxicity to rice, and the inhibition rate will also increase. It will reduce the content of soluble sugar in rice, increase the content of soluble protein, and reduce the content of malondialdehyde in rice, which will cause a certain degree of toxicity to plants [12].

2. Materials and methods

2.1 Test materials

'ZS 11' Rapeseed was selected as the test material, and nano-zinc oxide was purchased from Beijing Deke Island Gold Co., Ltd., with a particle size of 30 nm.

2.2 Test design

Seven treatments of CK, 30 nm nanometer zinc oxide (25 mg Ł$^{-1}$), 30 nm nanometer zinc oxide (400 mg Ł$^{-1}$), zinc oxide (25 mg Ł$^{-1}$), zinc oxide (400 mg Ł$^{-1}$), zinc sulfate (0.8 mg Ł$^{-1}$) and zinc sulfate (3.9 mg Ł$^{-1}$) were set in the test.

Ultrasonic dispersion of nano-zinc oxide for 15 min (KQ2200B, 100 W, 40 kHz, Kunshan Ultrasonic Instrument Co., Ltd.), prepare suspension containing nano-zinc oxide (25, 400 mg Ł$^{-1}$) for standby, and prepare zinc oxide solution (25, 400 mg Ł$^{-1}$) and zinc sulfate solution (0.8, 3.9 mg Ł$^{-1}$) at the same time.

2.3 Measurement items and methods

2.3.1 Seed germination

The seeds were sterilized in 4% sodium hypochlorite solution for 10 min and washed with distilled water for 4 times. Select 30 seeds of the same size and put them in a culture dish. The culture dish is placed in a light cycle of 14 hours, a temperature of 25/20 ℃, a relative humidity of 65%, and a light intensity of 340 μ The best growth effect can be obtained in the incubator with mol s^{-1} m^{-2}. Rapeseed germinated 1 day after sowing, and the number of germinated seeds was recorded 7 days after sowing.

Germination rate=number of germinated seeds/number of tested seeds * 100%

Germination potential=number of germinated seeds in 3 days/number of tested seeds * 100%

2.3.2 Biomass measurement

After 15 days of treatment, take the fresh Rapeseed seedlings in each treatment, set up three repetitions for each treatment, cut the upper part and the underground part, wash the impurities attached to the surface of the plant with distilled water, dry the water on the surface with filter paper, and weigh their fresh weight respectively; After drying the cleaned sample, put it into the oven for sterilization at 105 ℃, and bake it to constant weight at 65 ℃, and weigh its dry weight.

2.3.3 Determination of chlorophyll content

Weigh 0.1 g of fresh Rapeseed leaves and put them into a centrifuge tube, add 10 ml of 95% ethanol solution, cover them and put them in a dark place, extract them at room temperature for 24 hours, and take the supernatant for chlorophyll content determination at 665 and 649 nm wavelength [13]. The calculation formula is:

Ca=13.95A665-6.88A649

Cb=24.96A649-7.32A665

CTotal=Ca+Cb

2.3.4 Determination of physiological indicators

Take fresh Rapeseed plants, wash them with distilled water for three times, remove any external pollutants, weigh 0.5 g of roots in a mortar, add precooled quartz sand and phosphate buffer solution to grind them into pulp on an ice bag, transfer them to a 10 mL centrifuge tube, centrifuge, obtain the supernatant, and determine the physical indicators.

The content of hydrogen peroxide (H_2O_2) was determined by sulfuric acid phthalein precipitation method [14];

The superoxide anion (O^{2-}) was determined by Li method [15].

2.4 Data analysis

The test data were plotted using Origin 2021, and the single factor analysis of variance and multiple comparison tests were performed using SPSS 20.0 statistical software package. The probability of $P \leq 0.05$ is statistically significant.

3. Results and analysis

3.1 Effect of different treatments on Rapeseed seed germination

Table 1: Effect of different treatments on Rapeseed germination

Treatments(mg·L^{-1})	Germination rate	Germination potential	Germination index
CK	1.00 ± 0.00 a	0.99 ± 0.01 a	74.76 ± 0.66 a
25 NPs	1.00 ± 0.00 a	0.97 ± 0.02 a	71.80 ± 0.62 ab
400 NPs	0.99 ± 0.01 a	0.95 ± 0.02 a	73.25 ± 1.56 ab
25 ZnO	0.99 ± 0.01 a	0.95 ± 0.02 a	73.26 ± 1.17 ab
400 ZnO	0.99 ± 0.01 a	0.98 ± 0.02 a	73.90 ± 2.06 a
0.8 $ZnSO_4$	0.98 ± 0.01 a	0.93 ± 0.04 a	72.96 ± 2.32 ab
3.9 $ZnSO_4$	0.97 ± 0.02 a	0.96 ± 0.03 a	68.40 ± 1.03 b

Note: Different lowercase letters in the same column indicate significant differences at 0.05 level

The study found that from Table 1, it can be seen from the germination situation that the germination rate is between 97% and 99%, and the germination potential is between 93% and 97%. There is no significant difference between each treatment. Compared with CK, under 400 mg·L^{-1} nano-zinc oxide, zinc oxide and zinc sulfate treatment, the germination rate and germination potential of Rapeseed decreased slightly, but did not reach a significant level. Compared with CK, the germination index of nano-zinc oxide, zinc oxide and 0.8 mg·L^{-1} zinc sulfate treatment was different, but the difference was not significant. 3.9 mg·L^{-1} zinc sulfate treatment significantly reduced the germination index of Rapeseed. The germination results showed that each treatment had little effect on the germination rate and bud potential of Rapeseed, which may be due to the

selective absorption of substances by the seed coat of Rapeseed, and thus had no significant effect on the germination of Rapeseed seeds [16-17]. This is similar to the study of Yu Jingbo and others [12].

3.2 Effect of different treatments on Rapeseed biomass

Figure 1 shows the comparison of dry and fresh biomass of underground and aboveground Rapeseed under different treatments. The results showed that compared with CK, 25 mg Ł$^{-1}$ nano-zinc oxide, 400 mg Ł$^{-1}$ nano-zinc oxide, 25 mg Ł$^{-1}$ zinc oxide, 400 mg Ł$^{-1}$ zinc oxide and 3.9 mg Ł$^{-1}$ zinc sulfate had significant differences in underground fresh weight, 3.9 mg Ł$^{-1}$ zinc sulfate significantly increased by 33.41%, and other treatments decreased by 21.48%, 61.76%, 41.87%, 35.27% and 3.38% respectively. Compared with 25 mg Ł$^{-1}$ nano-zinc oxide, 400 mg Ł$^{-1}$nano-zinc oxide reached a significant level, and the fresh weight of Rapeseed roots decreased by 51.31%. At the concentration of 25 mg Ł$^{-1}$, the difference between nano-zinc oxide treatment and zinc oxide treatment is significant, and the zinc oxide treatment is significantly reduced by 25.97% compared with nano-zinc oxide treatment. At the concentration of 400 mg Ł$^{-1}$, the difference between nano-zinc oxide treatment and zinc oxide treatment was significant, and the zinc oxide treatment increased by 69.30% compared with nano-zinc oxide treatment. For underground dry weight, compared with CK, except 0.8 mg Ł$^{-1}$ zinc sulfate treatment, all treatments significantly reduced the underground dry weight of Rapeseed by 33.35%, 69.93%, 56.42%, 56.84% and 33.82%, respectively. Compared with 25 mg Ł$^{-1}$ nano-zinc oxide, 400 mg Ł$^{-1}$ nano-zinc oxide significantly reduced the root dry weight of Rapeseed by 54.89%. At the concentration of 25 mg Ł$^{-1}$, the difference between nano-zinc oxide and zinc oxide is significant. Compared with nano-zinc oxide treatment, zinc oxide treatment significantly reduces 34.62%. At the concentration of 400 mg Ł$^{-1}$, compared with nano-zinc oxide treatment, the dry weight of Rapeseed root under zinc oxide treatment significantly increased 43.53%.

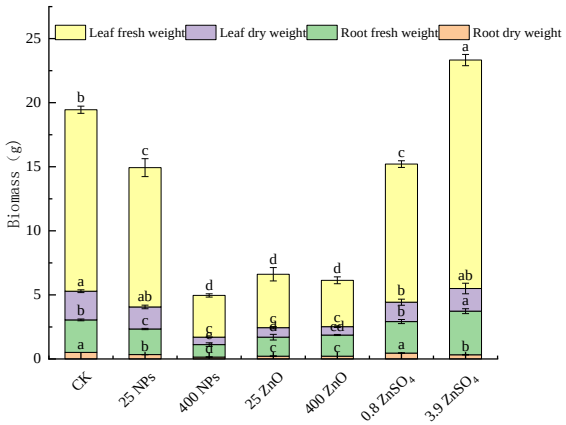

Figure 1: Effects of different treatments on Rapeseed biomass

As for the fresh weight of shoot, compared with CK, except 3.9 mg Ł$^{-1}$ zinc sulfate treatment, the fresh weight of shoot of Rapeseed was significantly reduced by other treatments, 3.9 mg Ł$^{-1}$ zinc sulfate significantly increased by 25.92%, and other treatments decreased by 23.22%, 77%, 70.54%, 70.54% and 23.84% respectively. Compared with 25 mg Ł$^{-1}$ nano-zinc oxide, 400 mg Ł$^{-1}$ nano-zinc oxide significantly decreased by 70.05%, and 25 mg Ł$^{-1}$ nano-zinc oxide significantly decreased by 61.62%. For shoot dry weight, compared with CK, except 25 mg Ł$^{-1}$ nano-zinc oxide and 3.9 mg Ł$^{-1}$ zinc sulfate, other treatments all reduced shoot dry weight of Rapeseed, and the difference was significant, reducing 74.23%, 66.98%, 70.98% and 32.67% respectively. Compared

with 25 mg Ł$^{-1}$ nano-zinc oxide, the dry weight of Rapeseed leaves under 400 mg Ł$^{-1}$ nano-zinc oxide treatment significantly decreased by 66.49%, and the dry weight of Rapeseed leaves under 25 mg Ł$^{-1}$ zinc oxide treatment significantly decreased by 57.06%. This may be due to the smaller particle size of nano-zinc oxide, which is easier to enter the body of Rapeseed and has a stronger toxic effect on Rapeseed.

3.2.1 Effect of nano-zinc oxide on chlorophyll content of Rapeseed

It can be seen from Table 2 that compared with CK, 400 mg Ł$^{-1}$ nano-zinc oxide and 400 mg Ł$^{-1}$ zinc oxide significantly reduced the content of chlorophyll a, which were 50.97% and 18.17% respectively. Compared with 25 mg Ł$^{-1}$ nano-zinc oxide, 400 mg Ł$^{-1}$ nano-zinc oxide significantly decreased by 53.25%, and compared with 25 mg Ł$^{-1}$ zinc oxide, 400 mg Ł$^{-1}$ zinc oxide significantly decreased by 19.47%. At the concentration of 400 mg Ł$^{-1}$, compared with nano-zinc oxide, the content of chlorophyll a in zinc oxide treatment was significantly increased by 66.92%. In terms of chlorophyll b content, compared with CK, except for 25 mg Ł$^{-1}$ nano-zinc oxide treatment, other treatments reached significant levels, with 25 mg Ł$^{-1}$ zinc oxide, 0.8 mg Ł$^{-1}$ zinc sulfate and 3.9 mg Ł$^{-1}$ zinc sulfate significantly increased by 15.79%, 30.01% and 55.56%. Compared with 25 mg Ł$^{-1}$ nano-zinc oxide, 400 mg Ł$^{-1}$ nano-zinc oxide significantly decreased by 50.73%, and compared with 25 mg Ł$^{-1}$ zinc oxide, 400 mg Ł$^{-1}$ zinc oxide significantly decreased by 34.26%. At the concentration of 400 mg Ł$^{-1}$, compared with nano-zinc oxide, zinc oxide significantly increased by 36.99%. For the total amount of chlorophyll, compared with CK, 25 mg Ł$^{-1}$ nano-zinc oxide, 25 mg Ł$^{-1}$ zinc oxide, 0.8 mg Ł$^{-1}$ zinc sulfate and 3.9 mg Ł$^{-1}$ zinc sulfate were significantly increased by 9.23%, 9.43%, 28.29 and 51.09%, respectively. Compared with 25 mg Ł$^{-1}$ nano-zinc oxide, 400 mg Ł$^{-1}$ nano-zinc oxide significantly decreased 51.82%, and compared with 25 mg Ł$^{-1}$ zinc oxide, 400 mg Ł$^{-1}$ zinc oxide significantly decreased 28.09%. At the concentration of 400 mg Ł$^{-1}$, compared with nano-zinc oxide, zinc oxide treatment significantly increased 49.51%. This shows that zinc sulfate can promote the increase of chlorophyll content within a certain concentration range, and the higher the concentration, the more significant the effect.

Table 2: Effect of ZnO NPs on chlorophyll content of Rapeseed

Treatments(mg Ł$^{-1}$)	Chl a(mg/g)	Chl b(mg/g)	Chl(a+b)(mg/g)	Chla/b
CK	6.569 ±1.039 c	8.058 ±0.856 d	14.627 ±0.192 d	0.816 ±0.021 a
25 NPs	6.888 ±0.138 c	9.089 ±0.781 cd	15.977 ±0.709 c	0.758 ±0.008 a
400 NPs	3.221 ±0.631 f	4.478 ±1.186 f	7.698 ±0.556 f	0.721 ±0.032 a
25 ZnO	6.676 ±1.113 c	9.331 ±3.375 c	16.006 ±2.276 c	0.718 ±0.037 a
400 ZnO	5.376 ±1.740 d	6.134 ±6.368 e	11.510 ±4.639 e	0.904 ±0.136 a
0.8 $ZnSO_4$	8.289 ±1.090 b	10.477 ±1.500 b	18.766 ±0.751 b	0.791 ±0.021 a
3.9 $ZnSO_4$	9.564 ±2.350 a	12.536 ±0.984 a	22.100 ±1.392 a	0.763 ±0.024 a

3.2.2 Effect of different treatments on active oxygen system of Rapeseed

Hydrogen peroxide in plants has stable properties, high transmembrane permeability and rapid diffusion. When plants are under stress, hydrogen peroxide can produce defense response to stress and regulate plant growth and development [18]. It can be seen from Figure 2 A that, compared with CK, all treatments except 25 mg Ł$^{-1}$ zinc oxide reached significant levels, with 25 mg Ł$^{-1}$ nano-zinc oxide, 400 mg Ł$^{-1}$ nano-zinc oxide, 400 mg Ł$^{-1}$ zinc oxide and 3.9 mg Ł$^{-1}$ zinc sulfate increased by 2.92%, 73.16%, 67.07% and 30.02% respectively. 0.8 mg Ł$^{-1}$ zinc sulfate decreased by 1.37%. Compared with 25 mg Ł$^{-1}$ nano-zinc oxide, 400 mg Ł$^{-1}$ nano-zinc oxide significantly increased by 68.25%. Compared with 25 mg Ł$^{-1}$ zinc oxide, 400 mg Ł$^{-1}$ zinc oxide significantly increased by

68.51%. At the concentration of 25 mg Ł$^{-1}$, compared with 25 mg Ł$^{-1}$ nano-zinc oxide, 25 mg Ł$^{-1}$ zinc oxide significantly decreased by 3.67%. At the concentration of 400 mg Ł$^{-1}$, compared with 400 mg Ł$^{-1}$ nano-zinc oxide, 400 mg Ł$^{-1}$ zinc oxide significantly decreased by 3.52%.

Superoxide anion is one of the active oxygen species in plants. Under normal conditions, the content of superoxide anion in plants is kept in balance to ensure the normal physiological state of plants [19]. When plants are poisoned, the content of superoxide anion in plants increases rapidly to cope with the toxicity of stress on plants. It can be seen from Figure 2 B that compared with CK, the differences of each treatment are significant, with the increase of 9.18%, 96.79%, 3.64%, 87.32%, 3.79% and 5.69% respectively for 25 mg Ł$^{-1}$ nano-zinc oxide, 400 mg Ł$^{-1}$ nano-zinc oxide, 25 mg Ł$^{-1}$ zinc oxide, 400 mg Ł$^{-1}$ zinc oxide, 0.8 mg Ł$^{-1}$ zinc sulfate and 3.9 mg Ł$^{-1}$ zinc sulfate. Compared with 25 mg Ł$^{-1}$ nano-zinc oxide, 400 mg Ł$^{-1}$ nano-zinc oxide significantly increased by 80.24%. Compared with 25 mg Ł$^{-1}$ zinc oxide, 400 mg Ł$^{-1}$ zinc oxide significantly increased by 80.73%. At the concentration of 25 mg Ł$^{-1}$, compared with 25 mg Ł$^{-1}$ nano-zinc oxide, 25 mg Ł$^{-1}$ zinc oxide significantly decreased by 5.07%. At the concentration of 400 mg Ł$^{-1}$, compared with 400 mg Ł$^{-1}$ nano-zinc oxide, 400 mg Ł$^{-1}$ zinc oxide significantly decreased by 4.81%. This indicates that 400 mg Ł$^{-1}$ nano-zinc oxide and zinc oxide are more toxic than other treatments.

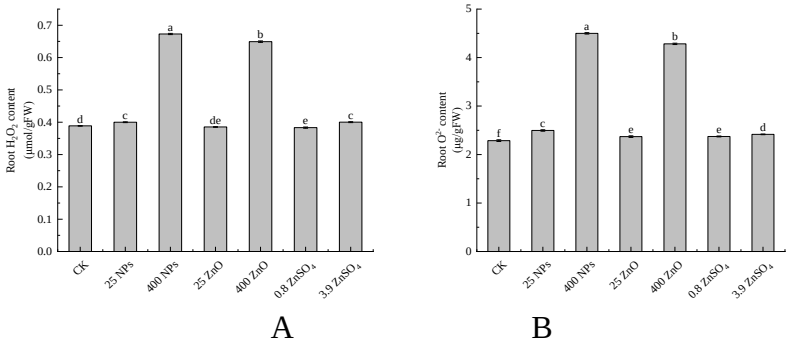

Figure 2: Effect of different treatments on ROS of Rapeseed root

3.3 Discussion and conclusion

The study found that nano-zinc oxide had no significant effect on the germination rate and germination potential of Rapeseed seeds. Compared with CK, nano-zinc oxide, zinc oxide and 0.8 mg Ł$^{-1}$ zinc sulfate decreased the germination index of Rapeseed seeds, but the difference was not significant. 3.9 mg Ł$^{-1}$ zinc sulfate significantly decreased the germination index of Rapeseed seeds. Compared with CK, except 3.9 mg Ł$^{-1}$ zinc sulfate treatment, all treatments reduced the fresh weight of Rapeseed underground and aboveground. For the fresh weight of Rapeseed underground, 0.8 mg Ł$^{-1}$ zinc sulfate had no significant difference, while other treatments had significant differences. For the underground part, each treatment has significant differences. For the dry weight of Rapeseed, all treatments reduced the dry weight of Rapeseed underground. Except 0.8 mg Ł$^{-1}$ zinc sulfate, all treatments had significant differences. For the dry weight of Rapeseed aboveground, all treatments had significant differences.

It was found that 25 mg Ł$^{-1}$ nano-zinc oxide promoted the chlorophyll synthesis of Rapeseed, 400 mg Ł$^{-1}$ nano-zinc oxide inhibited the chlorophyll synthesis of Rapeseed, and 400 mg Ł$^{-1}$ nano-zinc oxide had a stronger inhibitory effect than 400 mg Ł$^{-1}$ zinc oxide. Zinc sulfate promoted chlorophyll synthesis, of which 3.9 mg Ł$^{-1}$ zinc sulfate had the most obvious effect.

Through the determination of the content of hydrogen peroxide and superoxide anion in Rapeseed plants, this study found that when Rapeseed was affected by 400 mg Ł$^{-1}$ nano-zinc oxide and 400 mg Ł$^{-1}$ zinc oxide, the content of hydrogen peroxide and superoxide anion in Rapeseed

increased significantly, which showed that when Rapeseed was under stress, the content of hydrogen peroxide and superoxide anion in Rapeseed was increased to resist the toxicity and keep the physiological state of Rapeseed as balanced as possible, This is consistent with Cui Jian's research [20].

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