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Study on the Effect of Sodium Nano Lignin Sulfonate on the Growth of Mung Bean Seedlings under Zinc Stress

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Abstract: Nanotechnology can effectively improve the environmental hazards caused by petrochemical industry, so it is widely used in environmental protection field. Among them, the pollution of heavy metal Zn accounts for a larger proportion and the pollution degree is deeper.The investigation of heavy metal pollution in some bay area water shows that there are other heavy metals besides Zn in the estuary.Mining non-ferrous metal smelting, electroplating wastewater and some chemical enterprises discharged from the wastewater contains a large amount of zinc.With the rapid development of industry, the wastewater discharged into the environment by tannery, electroplating and zinc salt production has produced far-reaching zinc pollution to the environment. Therefore, the treatment of zinc-containing wastewater has received strong attention nowadays.Ligninsulfonate, a by-product of pulp or fiber pulp production using the sulfite method, is directly extracted in its paper mill waste red liquor and is a non-toxic resource with a wide range of sources, low cost and renewable.In this paper, nano-sodium lignosulfonate was prepared by combining low-cost and abundant lignosulfonate to improve its adsorption capacity of zinc ions.The effects of nano-sodium lignosulfonate and non-nano-sodium lignosulfonate on the growth and development of mung bean seedlings after adsorbing zinc ions were studied by measuring the morphology and physiological and biochemical indexes of mung bean seedlings.The research will provide technical support for the treatment of heavy metal pollution in water environment, the agricultural application of zinc pollution treatment solution, and the prevention of secondary pollution after heavy metal treatment in water.The main research works were (1) preparation of nano-sodium lignosulfonate formulations; (2) characterization of morphological structure of nano-sodium lignosulfonate formulations by laser pointer, scanning electron microscope and optical microscope; (3) treatment solution with nano- and non-nano-sodium lignosulfonate formulations adsorbed with Zn2+, treatment of mung bean seeds, and study of its effect on wheat germination and seedling growth under Zn ion stress .The results showed that (1) the prepared nano-adsorbents exhibited homogeneous fine granularity and uniform dispersion with particle size in the range of about 20 nm to 60 nm under room temperature neutral conditions; (2) the adsorption of the nano-formulations was higher than that of the non-nanoproducts under the same conditions. (3) The effect of nano-sodium lignin sulfonate was found to be better than that of non-nanopreparations for the alleviation of Zn stress by seedling trials in wheat, and the best effect was obtained when the addition amount was 30 ml.

1. Literature Review

1.1 The rise and development of nanotechnology

The first scanning tunneling microscope (STM) was developed at a laboratory in Zurich, Switzerland, in the 1980s[1]. Nanotechnology has provided mankind with precise and fine products and also introduced a new way of thinking. By using nanotechnology in agriculture and medicine, it has transformed the face of agriculture and medicine and has had a significant impact on increasing the productivity of society and even fundamentally solving the huge environmental crisis facing humanity. Ultra-fine particles with nanoscale size have various characteristics such as small particle size, large relative surface area and macroscopic quantum tunneling, and thus can exhibit different qualities and functions. Nanotechnology itself has become highly important and valuable for research because of the practical applications and economic value of nanoparticles in many aspects of physical properties [2].

At the same time, a "Nano Engineering and Technology Group" was formed, consisting of leaders from various disciplines. This is because nanotechnology will have a huge impact on the economy and society in the 21st century and may lead to the next industrial revolution [3]. The continuous conservation of non-renewable resources and energy sources and the reduction of their excessive consumption by humans will lead to the protection of the environment and the preservation of the planet [4].

1.2 Overview of research on zinc ions on water environment pollution

1.2.1 Application of zinc in industrial and agricultural industries

In the dry cell production industry, zinc-nickel and silver-zinc batteries are widely used in various daily lives of human beings. The storage capacity of silver-zinc batteries is five times higher than that of lead batteries and is known as a new type of high energy battery. The manufacture of brass is one of the main uses of zinc. Zinc is also used in the manufacture of rust-proof white iron. Colorless zinc chloride is often used as a de-watering agent in organic chemistry, and in its liquid form as a "clinker" for welding metals. The use of this solution is harmless to the metal surface, and after the water in the solution has evaporated, the melted salt remains on the surface of the metal to prevent oxidation. The surface phosphor material of road signs and warning signs on highways is produced by putting less copper, manganese, and silver as activators in crystalline zinc sulfide, which emits different colors of luminescence when exposed to light [5].

1.2.2 Sources, distribution and behavior of zinc

Elemental zinc is stored in large quantities in nature, reaching 70 mg/kg in the earth's crust. Zinc ores are generally polymetallic, mostly coexisting with lead, silver, cadmium, etc., and lead-zinc ore deposits are abundant in China. Due to its own nature, Zn is mostly stored through compounds, most notably in the form of sulfides [6-7]. Zn in soils is divided into four types according to their compositional structure: insoluble Zn (mineral state Zn), substitutional Zn (adsorbed Zn), water-soluble Zn and organic chelated Zn. The part of the soil that can be absorbed and used by plants only accounts for a relatively small part of the total Zn content, and there is an effective state of Zn. The effective state Zn in soil is influenced by various environmental factors such as soil pH, soil organic matter content, temperature and other climatic conditions [7-8].

1.2.3 Role of zinc in the metabolism of life and the hazards of zinc wastewater

The two main categories of water pollution are domestic sewage and industrial wastewater, and the more difficult to treat of the two comparisons is industrial wastewater. Although a variety of heavy metals are trace elements to ensure the normal metabolism of the organism, excessive levels can also cause greater harm to the environment, and today heavy metal pollution is already an environmental element that prevents socio-economic development. Excess heavy metals in soil affected by water pollution not only inhibit the growth and metabolism of plants, resulting in premature decay and reduced yields, but also pass into the plant through the roots and finally affect the normal metabolism of the human body through the aggregation and enrichment of the food chain [9].

1.3 Treatment of heavy metal wastewater by lignin and its sulfonate

1.3.1 Physicochemical properties of lignin

Lignin itself is a white organic substance with all symmetrical carbon structure [10]. Due to its structural characteristics, lignin has many features such as high calorific value of combustion, non-optical activity, color reactions, and insolubility. Its molecular weight is generally analyzed qualitatively and quantitatively using infrared and ultraviolet spectroscopy and nuclear magnetic resonance spectroscopy, with values mostly in the thousands to tens of thousands.

The complex structure of lignin itself makes it more stable and difficult to degrade, which makes the process of studying lignin difficult, but the biological enzymes extracted from microorganisms can degrade lignin well, which provides very favorable conditions for future research applications of lignin.

1.3.2 Application of lignin-based in the treatment of heavy metal wastewater

Experiments showed that the adsorption of alkaline lignin for zinc, lead, copper and cadmium ions were 7.5 mg/g, 9 mg/g, 137.14 mg/g and 87 mg/g, respectively [11-12]; the adsorption of hydrolyzed lignin and lignin made from paper black liquor was 17.97 mg/g and 1.72 mg/g for lead and cadmium ions, respectively [13-14] ; lignin modified by alkaline glycerol showed adsorption of 7.5 mg/g and 9 mg/g for lead and cadmium ions, respectively; lignin extracted from straw was effective in adsorbing chromium ions in water [15]; lignin modified by methane-based thioethering was effective in adsorbing many harmful heavy metal ions from nitrates [16].

1.3.3 Sodium lignosulfonate and its mechanism of heavy metal ion adsorption

Sodium lignosulfonate itself has more reactive groups, which can form ligand bonds well with metal ions [17-18], forming stable chelates to reduce the ionic concentration of heavy metals and mitigate the pollution of the environment. After sodium lignosulfonate is made into nanoparticles, more sulfonic acid groups and phenolic hydroxyl groups will be released [19], which can better adsorb metal ions and show superior adsorption performance.

2. Preparation of nanoformulations

2.1 Main experimental apparatus and reagents

2.1.1 Main experimental apparatus

Electronic balance: FA2104B, measuring range 0~210g, d=0. 1mg, Shanghai Pingxuan

Scientific Instruments Co.

Magnetic stirrer: 81-1, power 25W, 0~2000 rpm, Jiangsu JintanHuangyu Scientific Instruments Co.

Ultrasonic cleaning machine: FRQ-1030XH, Hangzhou Farrant Ultrasonic Technology Co.

Field emission scanning electron microscope: SU8010, Hitachi.

ICP Inductively Coupled Plasma Emission Spectrometer: SPECTRO BLUE, SPECTRO, Germany.

Scanning electron microscope: ZEISS EVO18, Carl Zeiss, Germany.

Transmission electron microscope: HT7700, Hitachi.

UV spectrophotometer: Changzhou Fipu Experimental Instrument Factory.

Centrifuge: Liaoning Fuyi Machinery Co.

2.1.2 Experimental materials and reagents

Sodium lignin sulfonate: Hubei Xing Yinhe Chemical Co.

Zinc oxide: Tianjin Bodi Chemical Co.

Sodium tripolyphosphate (STTP): Weifang Chenyang Chemical Co.

Sodium hydroxide: Guangzhou Dixindo Scientific Instruments Co.

Hydrochloric acid: Jinzhou Chemical Hydrochloric Acid Factory.

Mung beans: Purchased from Dalian Pulandian Seed Company.

2.2 Preparation and characterization of sodium lignosulfonatenanopreparations

2.2.1 Preparation of sodium lignosulfonatenanopreparations

After adjusting the pH of a certain volume of saturated solution to neutral with alkaline solution, the absorbance of the solution was measured at different pH values (wavelength=420nm) after adding buffer solution to fix the volume. According to the experiment, the absorbance measured at room temperature was A=0.302, and the absorbance system was calculated as 3.02L/g-cm.

To the flask containing 4g of sodium lignosulfonate, deionized water was added to make it fully dissolved. The product was filtered and cleaned and dried in acetone, and then fixed in a volumetric flask with a volume of 500ml and stirred for a period of time at room temperature, and the pH was adjusted to 4.5. After adding an appropriate amount of sodium tripolyphosphate (STPP) to the third group and stirring to fully dissolve, the optimum acidity was adjusted. Finally, the solution was put into the ultrasonicator for a period of ultrasonic shaking to obtain the desired nanoformulations.

2.2.2 Characterization of nano-sodium lignosulfonate formulations

(1) Tindal phenomenon detection

The prepared sodium lignosulfonatenanopreparations were left to stand and observed by the naked eye for the appearance of emulsion color, and then the nanopreparations were irradiated with a laser pointer to observe whether the Tindal phenomenon was produced.

(2) Scanning electron microscope (SEM) detection.

The prepared sodium lignosulfonate solution was added dropwise to the aluminum sheet, dried at room temperature, sprayed with gold and observed under SEM.

(3) Transmission electron microscope detection

The prepared sodium lignosulfonatenanopreparations were diluted to five times, carefully dipped in a small copper mesh, dried to a dry state at room temperature, and then placed under transmission electron microscopy for characterization.

2.3 Effect of sodium lignosulfonate on the growth of wheat seedlings

2.3.1 Treatment and preparation of materials

Mung bean seeds of uniform size were selected and then the seeds were rinsed with distilled water; the seeds were disinfected by soaking the wheat seeds for 30s with alcohol at a concentration of 75%, and finally the seeds were rinsed 1~3 times with distilled water;

Three groups of 10ml, 20ml and 30ml of nano and non-nano preparations of 4mg/ml were used to adsorb 20ml of Zn^{2+} reaction solution with a concentration of 50mg/ml; then distilled water as well as 360ppm of Zn^{2+} standard solution was used as control to soak the seeds for 1d, respectively.

2.4 Experimental method

After the seeds were treated, they were rinsed 3 to 4 times with distilled water and the water remaining on the surface of the seeds was absorbed using filter paper, and two pieces of cut circular filter paper were put on the Petri dishes, 30 seeds were put on each group of filter paper, and the filter paper was always kept moist, and the Petri dishes were put into the incubator with constant light and temperature, and three parallel experimental groups were set up for each experimental condition.

2.4.1 Index measurement and data processing

The germination rate of each group of seedlings was calculated and counted separately at the 7th d, and the average germination rate of the three parallel groups was calculated. Then the root length and seedling length of seedlings were measured at 14d, and the average growing root length and seedling length were calculated, and their dry weight and fresh weight were measured, and finally summarized for significance analysis.

(1) Measurement of morphological indicators of mung bean seedlings

The germination potential of seedlings was observed at 7 d, the number of germination was recorded, and the average germination rate of three parallel groups was calculated. The root length and seedling length of mung bean seedlings in each group were measured separately at 14 d using vernier calipers. And the dry weight was measured separately after killing at 105℃ for 15 min and drying at a constant temperature of 75℃ until constant weight.

(2) Determination of chlorophyll content in mung bean seedlings [20].

0.2g of fresh wheat leaves were weighed separately, cut with scissors and then ground with quartz sand, and then 10mL of 80% acetone was added for chlorophyll extraction. And after centrifuging the extract for five minutes, the supernatant was taken and its OD values were measured at 663 nm and 645 nm, respectively, and the measured OD values were substituted into the following equation to calculate the content of chlorophyll (mg/g Fw).

3. Analysis of results and discussion

3.1 Morphological characterization of sodium lignosulfonatenanopreparations

3.1.1 Direct observation

The success of the nanoformulations can be directly judged by direct observation of the presence of milky white light. Further by irradiation with a laser pointer, the Tindal phenomenon was generated and used as the initial detection standard, and the results are shown in Figure 1.

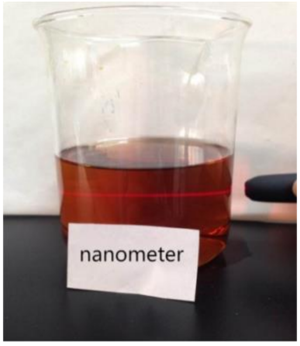
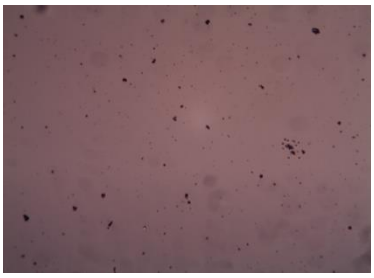


Figure 1: Tindal phenomenon of sodium nanolignosulfonate

3.1.2 Scanning electron microscope characterization

The photos of sodium lignosulfonate under 400x optical microscope in Figure 2, and the photos under field emission electron scanning microscope in Figure 3 show that the original sodium lignosulfonate itself has uneven particle distribution and large particle size.



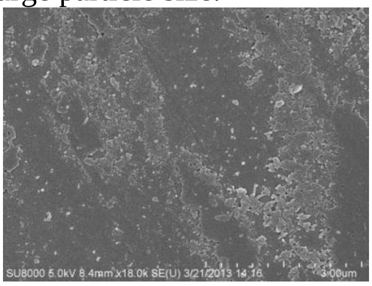
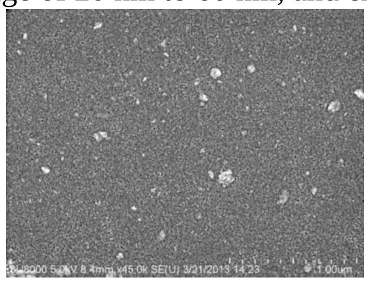


Figure 2: 400x optical microscope image of sodium lignosulfonate Figure 3: electron scanning microscope image of sodium lignosulfonate

Figure 4 and Figure 5 show the images of the nano-sodium lignosulfonate preparation under field emission electron scanning microscopy at 45kx and 200kx, respectively. The photos show that the prepared sodium lignosulfonate reagents are regularly and uniformly distributed, with particle sizes in the range of 20 nm to 60 nm, and exhibit a distinct spherical shape.



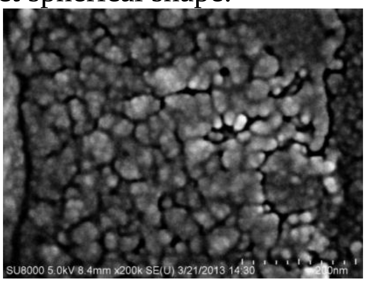


Figure 4: 45k magnification electron scanning microscope image of sodium lignosulfonate nanoparticles Figure 5: Image of sodium nanolignosulfonate under electron scanning microscope at 200k magnification

4. Effect of sodium lignosulfonate and its nanoformulations on morphological indicators of mung bean seedlings

Figure 6: Effects of Zn stress on wheat seedling growth

Figure 6 shows the photos of the growth of mung bean seedlings under zinc stress with sodium lignosulfonate on day 14, from left to right: 360 ppm Zn^{2+} solution, distilled water, nano sodium lignosulfonate solution containing 30 ml of Zn2+ solution added, and mung bean seedling growth under zinc ion stress with 30 ml of original agent sodium lignosulfonate in Zn^{2+} solution.

4.1 Effect on root length, seedling height and germination rate of mung bean seedlings at seedling stage

Table 1: Effect of different adsorbent addition on mung bean seedlings

	Root length (cm)	Seedling height (cm)	Germination rate (%)
10ml original	3.85±0.10	4.07±0.19	79.4
10mlnano formulation	4.03±0.17	4.43±0.20	84.0
20ml original	3.95±0.15	4.32±0.21	83.2
20mlnano formulation	4.21±0.19	4.70±0.23	87.1
30ml original	4.11±0.23	4.55±0.24	85.6
30mlnano formulation	4.75±0.10	4.87±0.16	91.3
Distilled water	4.95±0.20	4.94±0.15	92.5
360ppmZn^{2+}	3.51±0.10	3.32±0.30	70.1

From Table 1, it was shown that the increase in root length and seedling height of mung bean seedlings after 30 ml of Zn^{2+} reaction solution after adsorption of nano-formulation was greater than that of all other treatment groups, and the difference in root length and seedling height reached significant ($P<0.05$) with the original agent, and the nano-group was better than the original agent group at the same dose. Germination rate was also the highest with the addition of 30 ml of sodium nanolignosulfonate preparation adsorbed, which was basically the same as the control group. The worst growth state was in the Zn^{2+} solution at a concentration of 360 ppm.

The principle of action may be due to the fact that the adsorption of Zn^{2+} at lower concentration of sodium lignosulfonate is not complete enough, and the remaining Zn^{2+} will have a stressful effect on the growth metabolism of the plant, thus affecting its root length, seedling height and germination rate, while the adsorption efficiency of the nano preparation is higher than that of the original agent, so the nano preparation of sodium lignosulfonate has a better growth state than the original agent when the same dose is applied. More than 360 ppm Zn^{2+} solution will affect the growth metabolism of plants and produce certain toxic effects, leading to dwarfing of plants and yellowing of leaves.

4.2 Effect on fresh and dry weight of mung bean seedlings

The effects of zinc stress on fresh and dry weight of mung bean seedlings at different amounts of adsorbent addition are shown in Figures 7 to 8.

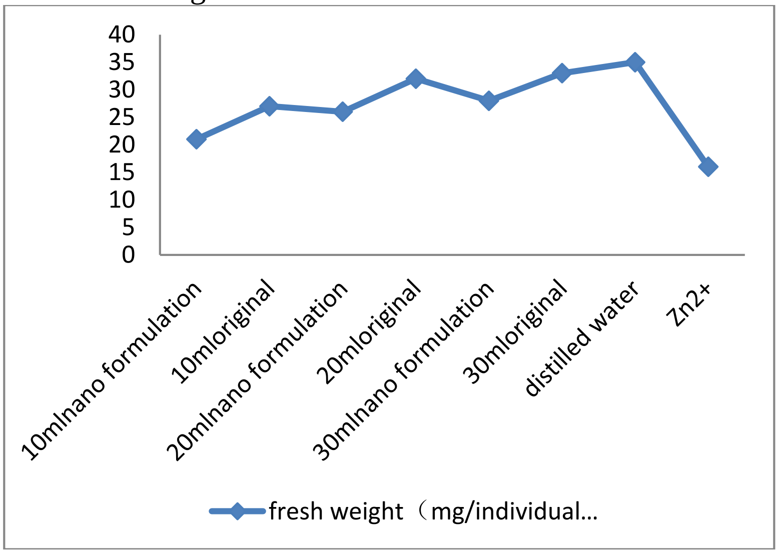


Figure 7: Effect of zinc stress on fresh weight of mung bean seedlings at seedling stage

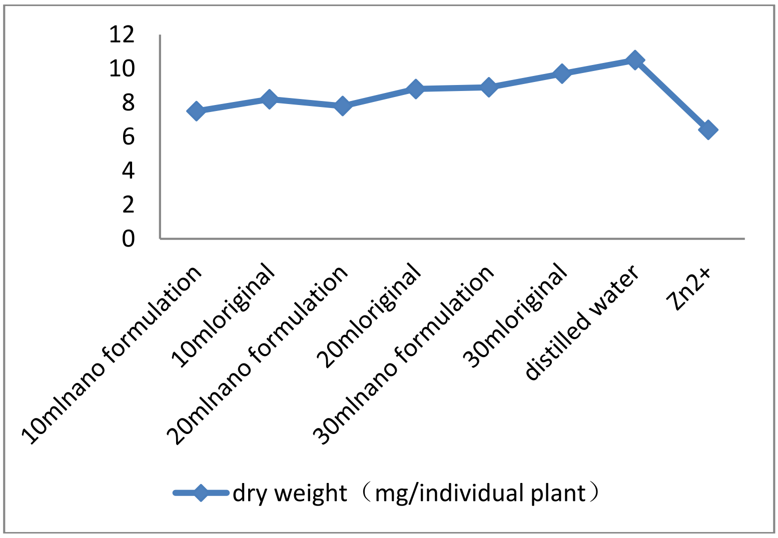


Figure 8: Effect of zinc stress on dry weight of mung bean seedlings at seedling stage

The trend of plant weight increase was consistent with the overall trend of plant height increase. From Figures 7 and 8, it can be concluded that as the amount of sodium lignosulfonate treatment increased, the stress effect of zinc ions on mung bean decreased and the dry weight and fresh weight increased, and the effect of the nano group was better than the original agent group at the same dose. When the increased amount was 30 ml of nano preparation, the dry weight and fresh weight reached the maximum value, and the fresh weight and dry weight were 94.3% and 98% of the control group, and increased by 41.5% and 31% compared with the zinc ion group. It can be seen that the fraction with the addition of sodium nanolignosulfonate, zinc stress was significantly reduced.

4.3 Effect on chlorophyll content of mung bean seedling growth

According to Figure 9,the effect of the reaction solution of the same content of Zn2+ adsorbed by the original agent of sodium lignosulfonate and the nano-formulation on the chlorophyll content of mung bean seeds after immersion: it can be concluded from the graph that the reaction solution after adsorption of the nano-formulation has less effect on the zinc stress of mung bean than the reaction solution after adsorption of the original agent, so the effect of chlorophyll content is also weak, and the effect of the nano-group is better than the original agent group at the same dose. In the reaction solution with 30 ml nano formula, the content of chlorophyll a was 99.7% of the control group soaked in distilled water, the content of chlorophyll b was 95.5% of the control group, and the total chlorophyll content was 98% of the control group.Compared with zinc ion group, the adsorption rates were increased by 53.3%, 50% and 52%, respectively, indicating that the adsorption of nano-sodium lignosulfonate was more complete.The effect of zinc stress on the environment and the plant itself is a kind of green organic adsorbent with little effect on the environment and the plant itself.

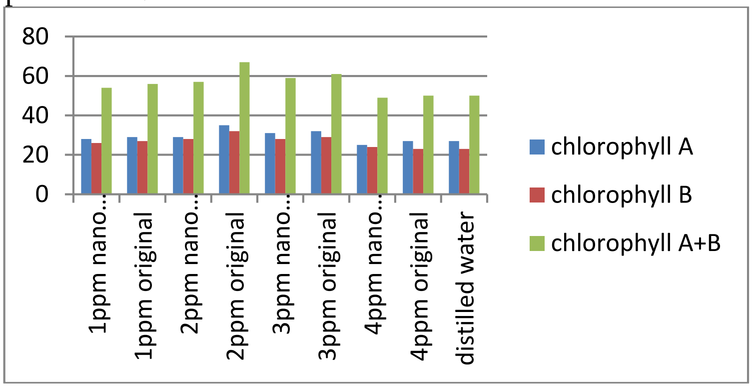


Figure 9: Effect of Zn stress on chlorophyll in mung bean seedlings

5. Conclusion

Sodium lignosulfonatenanopreparations were prepared using sodium lignosulfonate as the object of study. The prepared sodium lignosulfonatenanopreparations were characterized, and the size of the prepared nanopreparations in terms of granularity was determined by optical microscopy and electron scanning microscopy. Then the nanopreparations were used as adsorbent materials for heavy metal effluent containing Zn2+, and the adsorbed reaction solution was used as seeding solution for seedling experiments on mung beans to measure their physiological and biochemical indexes. The experimental results were as follows:

(1) The conditions for the preparation of sodium lignosulfonatenanopreparations were: pH neutral (7 to 8); room temperature (20 to 25℃); sodiumlignosulfonate concentration: 2.4 mg/ml, dispersant (STPP) concentration: 0.8 mg/ml, and the mass ratio of both was 1:3; the optimal magnetic reaction stirring and ultrasonic shaking times were derived from the experiments: 20 min and 30 min, respectively. By SEM characterization, the prepared nanoparticles showed a uniform distribution of regular spherical shape with particle size ranging from 20 nm to 60 nm.

(2) When the same content of Zn^{2+} solution was added to a volume of 30 ml of sodium nanoligninsulfonate with a concentration of 4 mg/ml, the zinc ion content in the solution was lower due to the best adsorption effect on zinc ions at this time, and therefore the zinc stress effect on mung bean seedlings was small.

In summary, the use of sodium lignosulfonatenanopreparation as adsorbent not only has a good adsorption effect on zinc ions, the adsorbed mixture solution has low zinc stress effect on the plant itself, but also sodium lignosulfonate itself has the effect of promoting plant growth, which is a more effective plant growth regulator. Therefore, sodium lignosulfonatenanoformulation, is a green, environmentally compatible, widely sourced, organic adsorbent.

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