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Study on the Effect of Sodium Nano Lignin Sulfonate on the Growth and Development of Mung Bean Seedlings

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Abstract: Lignin sulfonate, 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. There are many studies on lignin applications, and from the end of the nineteenth century to the present, more than two hundred lignin products have been developed internationally [1-2], mainly for dye dispersants, concrete admixtures, water treatment agents, animal feed additives, drilling fluid diluents, tertiary oil recovery aids, and various adhesives and complexing agents [2-10].Nanotechnology was combined with low-cost and abundant sodium lignin sulfonate to prepare it into nano-sodium lignin sulfonate formulations by nanotechnology as a way to improve its ability as a growth regulator, and to study and investigate the effects of nano- and non-nano-sodium lignin sulfonate as a growth regulator on the seedling growth and development of wheat by measuring the morphological and physiological and biochemical indices of seedling growth and development.The main research works were (1) the preparation of nano-sodium lignosulfonate formulations; (2) the morphological and structural characterization of nano-sodium lignosulfonate formulations by laser pens, scanning electron microscopy and optical microscopy;The results showed that (1) the prepared nano-adsorbents exhibited homogeneous fine particle size, uniform dispersion, and 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 suitable concentration of adsorbent solution treatment of sodium nanolignosulfonate preparation on germination rate, root length and seedling length of mung bean seedlings was significantly promoted and chlorophyll content was increased compared with the control group. (4) This study found that sodium lignosulfonate has the efficacy of plant growth promoter, and also measured that plant seedlings treated with sodium nanolignosulfonate preparation have enhanced growth and development, which provides an enlightening exploration for future development of organic agriculture and green agricultural production, and environment-friendly growth regulators.

1. Review of the literature

1.1 Preparation of nanoparticles

1.1.1 Vapor phase method

A method that transforms gaseous substances into solid particles under cooling conditions. The vapor phase method can be generally classified into four methods as follows:

(1) Vapor-in-gas method

Vaporization in gas is a method of making nanoparticles by adding inert or reactive gases to the reaction environment, vaporizing the solid reactants and then reacting them with inert gases, and then converting them from the gaseous state to the solid state. Nanoparticles were first produced by Wright H[11] at the end of the 20th century using gas condensation under ultra-high vacuum reaction conditions, and then polycrystals (nanomicrons) were obtained by means of tight pressure densification. Nanoparticles prepared by using this method are generally characterized by low surface impurities, small particle size range, and easily controllable particle size [12].

(2) Chemical vapor phase reaction method

In the presence of a protective gas, the gaseous metal compounds are chemically reacted and rapidly condensed to produce the desired compounds. In this way, nanoparticles of different products are produced. The nanoparticles produced have many advantages such as process continuity, ease of process control and high reactivity. Since the end of the 20th century, chemical vapor phase reaction techniques have been used for the production and preparation of products with different morphological characteristics [13].

(3) Chemical vapor phase condensation method

Most of the chemical vapor phase condensation methods use the heated decomposition process of organic polymers to produce nanoproducts[14]. The specific operation is to first turn the reaction chamber into a vacuum, then add the protective gas helium to the reaction chamber to make the chamber air pressure up to several hundred pascals, then load the reactants and the protective gas helium from the external circulation system, into the front thermal magnetron sputtering system, and then take the pre-reactant, which has gone through the production process to get the product, through the gas convection to the rotary quenchers at the rear position. It is used to collect and cool the synthesized nanoparticles [15-16].

(4) Sputtering method

The sputtering method has the advantage of a wide range of application and allows the preparation of lower and higher melting point metal nanoparticles that are not easily reactive [17].

1.1.2 Liquid-phase method

The liquid phase method is a homogeneous solution used as a raw material, and the solute and solvent are separated in different ways to prepare the pre-reactants of the particles, which are then heated to obtain the nanoparticles [12-15]. Mechanical stirring is not required in the preparation reaction, which makes the product less susceptible to impurities and enables to obtain a higher purity product. Therefore, it has a very promising application [17-19].

1.1.3 Solid phase method

The common method used in the powder metallurgy industry, ceramic industry and mineral industry is the ball milling method. Because the ball milling method has the characteristics of high production capacity and simple production process. Therefore, this method is commonly used in industrial production to prepare nanoproducts[20].

1.2 Application of nanotechnology in sewage treatment

With the development of China's economy, the use of nanotechnology environmental protection industry category to reduce the heavy pollution problems brought about by the petrochemical industry to the environment.

1.2.1 Treatment of inorganic polluted wastewater

Although the heavy metals in wastewater are harmful to human body, in another sense the loss of heavy metals is also a waste of resources. Nanopreparations can generate heavy metal ions in water bodies with extremely strong reducing power substances through photoelectric reaction [21], which adsorb the precious heavy metal ions that are oxidized very stably to the outside, and also reduce it to tiny metal crystals, which not only remove the toxicity of waste water, but also reduce the waste of resources.

1.2.2 Organic wastewater treatment

It is verified through several experiments that nanoscale titanium dioxide as a photocatalyst can catalyze the oxidation of organic pollutants in water bodies under visible light to achieve the need for rapid degradation. The method using titanium dioxide as a catalyst has the advantage of non-pollution and high degree of purification [22].

1.2.3 Nano water purifiers

The new nanoscale water purification substances have a water purification capacity ten to twenty times that of non-nano water purifiers and can completely adsorb and precipitate suspended particles in the water to be treated, and also remove many harmful impurities from it efficiently. After the first two purification treatments, the water is clear and tastes good without odor. After one adsorption can completely remove bacteria and viruses from the water, while minerals and elements are retained. People with liver and kidney failure are recommended to drink this water, which can largely reduce the burden on the liver and kidneys.

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 dissolve it fully. 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 dissolve it fully, the optimum pH 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

The researchers selected uniformly sized mung bean seeds and rinsed them with distilled water; The seeds were disinfected with 75% alcohol, soaked in wheat for 30s, and finally washed with distilled water for 1 to 3 times.

Related personnel use sodium lignosulfonate to regulate plant growth.

The seeds were dipped with 1ppm, 2ppm, 3ppm and 4ppm of sodium nanolignosulfonate and the original agent respectively, and distilled water was used as the control group ck, and three parallel experimental groups were set up and the seeds were dipped for 1d.

2.4 Experimental method

After the seeds were treated, the seeds were rinsed with distilled water 3 to 4 times, and the residual water on the surface of the seeds was absorbed cleanly using filter paper, and two cut circular filter papers were put on the Petri dishes, and 30 seeds were put on each group of filter papers, and the filter papers were 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

0.2g of fresh mung bean 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):

$$Ca=12.7\times OD663-2.69\times OD645 \quad (2.5)$$

$$Cb=22.9\times OD645-4.68\times OD663 \quad (2.6)$$

$$Ca+b= Ca+Cb=8.02\times OD663+20.2\times OD645 \quad (2.7)$$

3. Analysis of results and discussion

3.1 Morphological characterization of sodium lignosulfonatenanopreparations

3.1.1 Direct observation

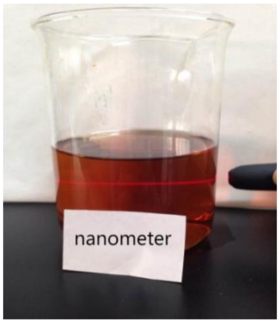

Figure 1: Tindal phenomenon of sodium nanolignosulfonate

The success of the nanoformulations can be directly judged by directly observing whether there is a milky white light or not. Further, by irradiation of the laser pointer, whether the Butyr phenomenon can be generated as the initial detection criteria, the results are shown in Figure 1.

3.1.2 Scanning electron microscope characterization

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

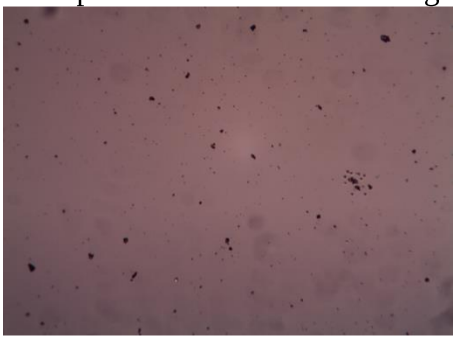

Figure 2: 400x optical microscope image of sodium lignosulfonate

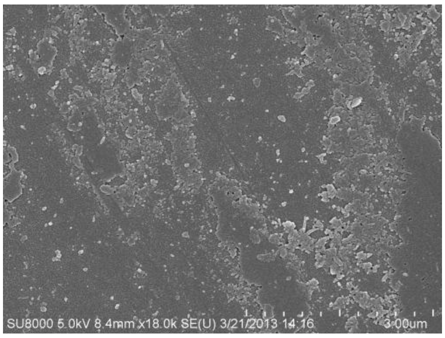

Figure 3: Images of sodium lignosulfonate under electron scanning microscopy

Figures 4 and 5 show the images of the nano-sodium lignosulfonate preparations under field emission electron scanning microscopy at 45k and 200k magnification, 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.

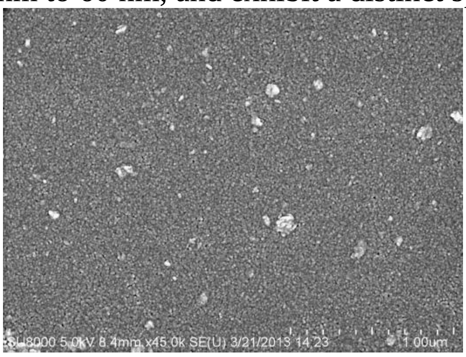

Figure 4: Image of sodium lignosulfonate nanoparticles under 45k magnification electron scanning microscope

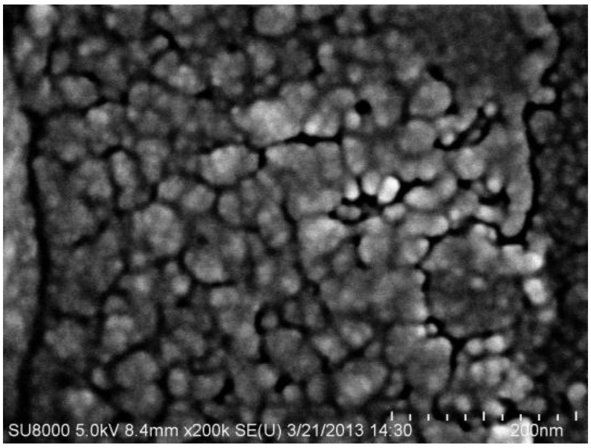

Figure 5: Image of sodium lignosulfonate nanoparticles under electron scanning microscope at 200k magnification

4. Plant growth regulator effects of sodium lignosulfonate and its nanoformulations

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

Table 1: Effect of different concentrations of sodium lignosulfonate on mung bean seedlings

	Root length (cm)	Seedling height (cm)	Germination rate (%)
1ppm original agent	5.01±0.15	5.88±0.19	93.1
1ppmnano formulation	5.14±0.19	5.96±0.22	93.5
2ppm original agent	5.65±0.21	5.91±0.23	95.1
2ppmnano formulation	6.13±0.16	6.78±0.23	97.1
3ppm original agent	5.76±0.23	6.32±0.14	96.5
3ppmnanoformulation	5.98±0.14	6.51±0.16	94.3
4ppm original agent	5.10±0.18	6.10±0.0.11	93.0
4ppmnano formulation	5.22±0.22	6.12±0.18	93.8
Distilled water	4.95±0.21	4.94±0.15	92.5

From Table 1, it can be seen that the seedling growth of mung bean was optimal after 2ppm sodium lignosulfonatenanosystem dip, and the increase in root length as well as seedling height and germination rate were better than the original agent group, and when the increase was 3ppmnanopreparation concentration, the difference between root length and seedling height and the original agent reached and its significant level ($P<0.05$) , and the nanopreparation group was better than the original agent group at the same dose.

4.2 Effect on dry weight and fresh weight of mung bean seedlings

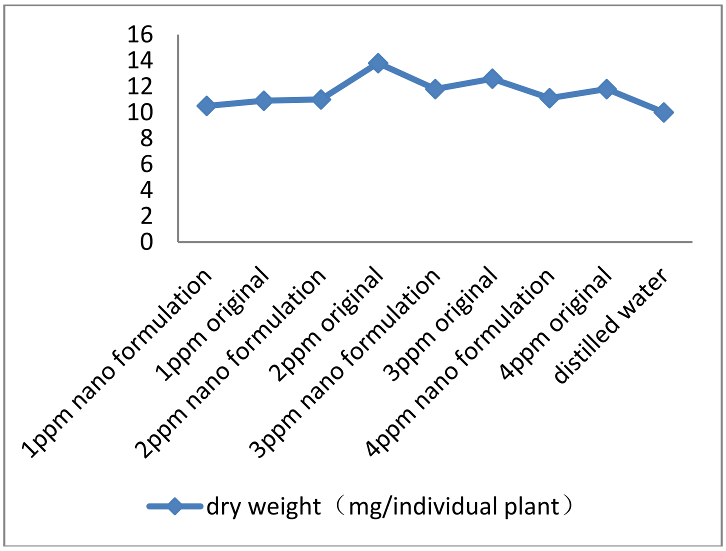

Figure 6: Effect of sodium lignosulfonate on dry weight of mung bean seedlings

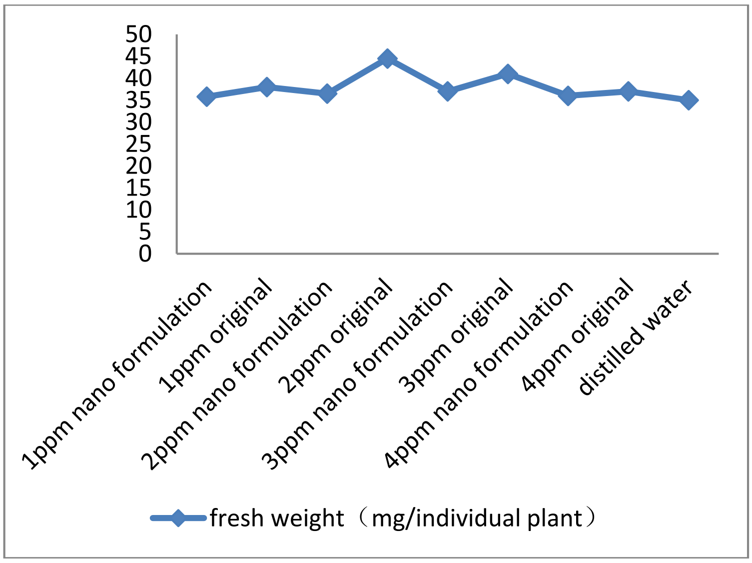

Figure 7: Effect of sodium lignosulfonate on fresh weight of mung bean seedlings

According to Figs. 6 and 7, it can be seen that when the seeds were dipped with 2 ppm of sodium nanolignosulfonate solution, it had the best effect on promoting the increase of dry weight and fresh weight content of mung bean seedlings, and the dry weight and fresh weight increased by 29% and 23.4%, respectively, compared with the control group; the effect was better when the original agent group was 3 ppm, but the increment was not as good as that of the nano formulation group, and the dry weight and fresh weight increased by 29% and 26%, respectively, for the control group. It can be clearly seen that the plant growth regulating effect of sodium nanolignosulfonate is better, and the effect is also better than that of the original agent group when the use concentration is lower than that of the original agent group, so sodium nanolignosulfonate has the characteristics of high efficiency and low use concentration.

4.3 Effect on chlorophyll in mung bean seedlings

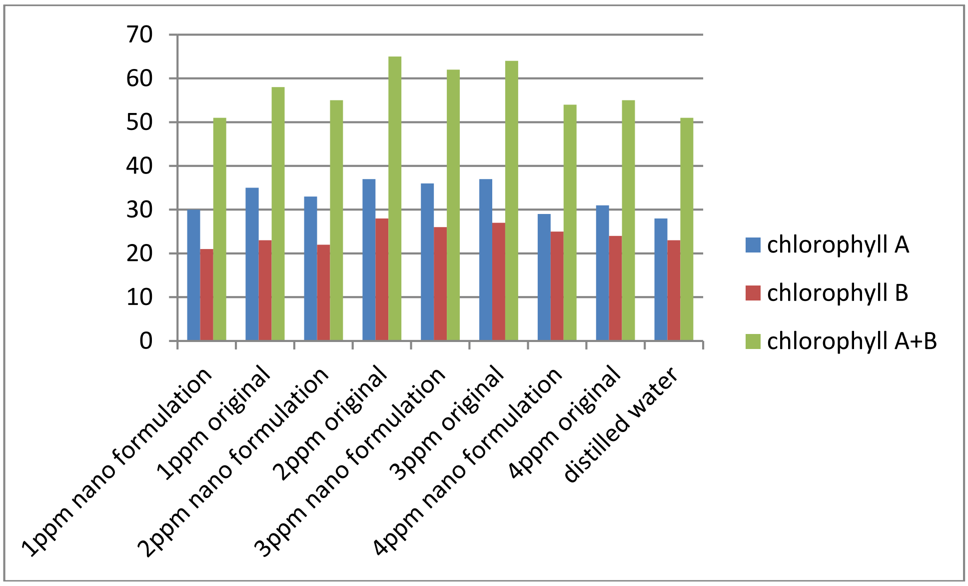

Figure 8: Effect of sodium lignosulfonate on chlorophyll content of wheat seedlings

According to Figure 8, it can be seen that when the seeds were dipped with 2 ppm of nano sodium lignosulfonate solution, it was the most effective for promoting the increase of chlorophyll content in mung bean seedlings, with chlorophyll a, chlorophyll b, and total chlorophyll increasing by 32.15%, 31.82%, and 32%, respectively, compared with the control group; the original agent group was more effective at 3 ppm, but the increment was not as good as the nano formulation group, for the control group chlorophyll a, chlorophyll b, and total chlorophyll and total chlorophyll increased by 25%, 13.64% and 26%, respectively. The increase of chlorophyll a was greater than that of chlorophyll b in both the nano-formulation group and the original agent group, indicating that the promotion effect of sodium lignosulfonate on chlorophyll a was greater than that of chlorophyll b. It was also obvious that the plant growth regulating effect of sodium nano-lignosulfonate was better, and the effect might be better than that of the original agent group when the use concentration was lower than that of the original agent group, so sodium nano-lignosulfonate had the advantages of good promotion effect and low use concentration. Therefore, sodium nanolignosulfonate has the advantages of good promotion effect and low concentration.

5. Conclusion

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

(2) Sodium lignin sulfonate itself also has the effect of promoting plant growth, which is a more effective plant growth regulator. When the seeds were dipped with 2 ppm of sodium lignosulfonatenano solution, it was the most effective in promoting the increase of chlorophyll content in mung bean seedlings, and the effect was better when the original agent group was 3 ppm, but the increment was not as good as the nano formulation group. Thus sodium

lignosulfonatenanopreparation, is a green, environmentally compatible, widely available, organic adsorbent.

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