Journal of Modern Crop Science (2023)
Clausius Scientific Press, Canada
DOI: 10.23977/jmcs.2023.020106
ISSN 2616-2075 Vol. 2 Num. 1

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

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Keywords: Sodium lignosulfonate nanoparticles; wheat seedling treatment; seedling growth

Abstract: There have been many studies on lignin applications, and from the end of the nineteenth century to the present, as many as 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 nano lignosulfonate preparation on germination rate, root length and seedling length of wheat 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 the enhanced growth and development of plant seedlings treated with sodium nano lignosulfonate preparation, which provides an enlightening exploration for the future development of organic agriculture and green agricultural production with environment-friendly growth regulators.

1. Introduction

Lignin itself is a white organic substance with all symmetrical carbon structure. 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.

2. Literature review

2.1 Preparation of nanoparticles

2.1.1 Vapor phase method

A method to transform 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 can prepare lower and higher melting point metal nanoparticles that are not easy to react [17].

2.1.2 Liquid-phase method

The liquid phase method is a homogeneous solution used as a raw material and a different way of separating the solute and solvent to prepare the pre-reactant of the particles, which is 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].

2.1.3 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].

2.2 Application of nanotechnology in the treatment of atmospheric pollution

Excess sulfur oxides, carbon oxides and nitrogen oxides in the atmosphere are dangerous causes of damage to human health, and nanomaterials and nanotechnology are the current scientific solutions of choice to eliminate these harmful gas states. It was found that the reactive oxygen produced by the reaction using nanoscale titanium dioxide photocatalysts, which interact with natural precipitation can cause the effective removal of nitrogen oxides and sulfur dioxide from the air by producing nitric and sulfuric acids that are soluble in water.

Nanoparticles have the characteristics of large surface area and multiple surface active centers, in the catalytic reaction nanoparticles have greater performance to improve the efficiency of the reaction and control the reaction rate.

(1) Petroleum desulfurization catalyst

(2) Automotive exhaust gas purification catalyst

(3) Nano fuel additives

(4) Combustion catalyst for coal

3. Preparation of nanoformulations

3.1 Main experimental instruments and reagents

3.1.1 Main experimental apparatus

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

Magnetic stirrer: 81-1, power 25W, 0~2000rpm, Jiangsu Jintan Huangyu 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.

3.1.2 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.
Wheat: Purchased from Dalian Pulandian Seed Company.

3.2 Preparation and characterization of sodium lignosulfonate nanopreparations

3.2.1 Preparation of sodium lignosulfonate nanopreparations

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.

3.2.2 Characterization of nano-sodium lignosulfonate formulations

(1) Tindal phenomenon detection

The prepared sodium lignosulfonate nanopreparations 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 lignosulfonate nanopreparations 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.

3.3 Effect of sodium lignosulfonate on the growth of wheat seedlings

3.3.1 Treatment and preparation of materials

Select wheat seeds of uniform size, then rinse the seeds with distilled water; disinfect the seeds with alcohol at a concentration of 75%, soak the wheat seeds for 30s, and finally rinse the seeds with distilled water 1~3 times;

Plant growth regulating effect of sodium lignosulfonate.

The seeds were dipped with 1ppm, 2ppm, 3ppm and 4ppm of sodium nano lignosulfonate 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.

3.4 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 sheets of cut circular filter paper were put on the Petri dishes, and 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.

3.4.1 Index measurement and data processing

The germination rate of each group of seedlings was calculated and counted at the 7th d separately, 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 seedling morphological indicators in wheat

The germination potential of seedlings was observed at 7 d, the number of germination was recorded, and the average germination rate of the three parallel groups was calculated. The root length and seedling length of wheat and mung bean seedlings in each group were measured 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 wheat seedlings

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):

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

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

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

4. Analysis and discussion of the results

4.1 Morphological characterization of sodium lignosulfonate nanopreparations

4.1.1 Direct observation

It can be used as a direct judgment of the success of the nanoformulations 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 produced as the initial detection criteria, the results are shown in Figure 1.

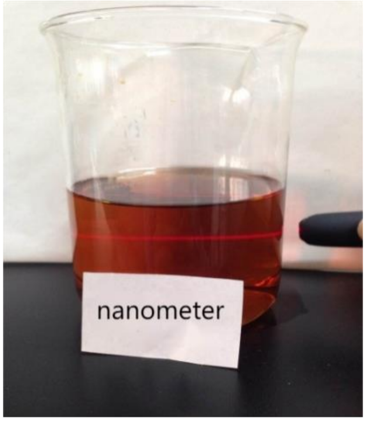

Figure 1: Tindal phenomenon of sodium nanolignosulfonate

4.1.2 Scanning electron microscope characterization

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

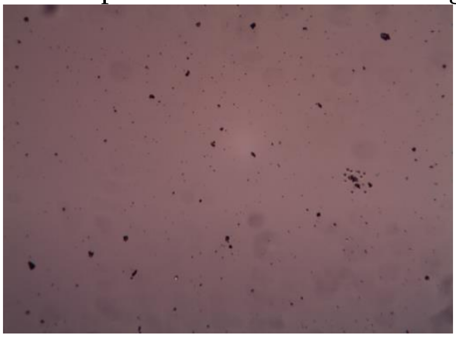

Figure 2: 400x optical microscope image of sodium lignosulfonate

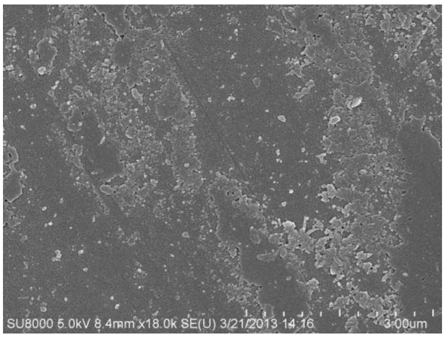

Figure 3: Image of sodium lignosulfonate under electron scanning microscope

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.

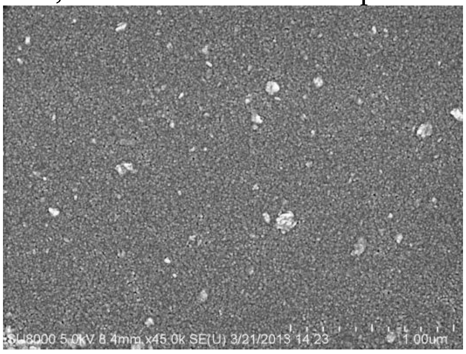

Figure 4: 45k magnification electron scanning microscope image of sodium lignosulfonate nanoparticles

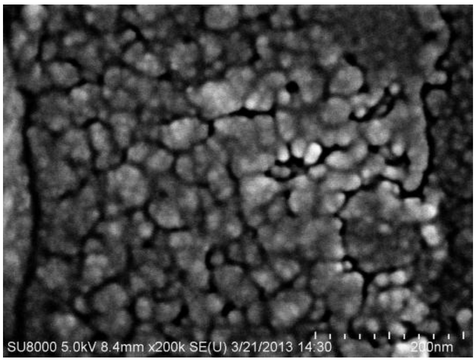

Figure 5: Image of sodium lignosulfonate nanopreparation under electron scanning microscope at 200k magnification

5. Plant growth regulator effects of sodium lignosulfonate and its nano formulations

5.1 Effects on root length, seedling length and germination rate of wheat seedlings at

Table 1: Effects of different concentrations of sodium lignosulfonate on wheat seedlings

	Root length (cm)	Seedling height (cm)	Germination rate (%)
1ppm original formulation	8.01±0.15	8.91±0.12	93.5
1ppm nano formulation	8.65±0.13	9.13±0.22	94.1
2ppm original formulation	8.50±0.19	9.01±0.31	95.5
2ppm nano formulation	9.14±0.19	10.25±0.17	98.3
3ppm original formulation	8.66±0.10	9.45±0.21	96.1
3ppm nano formulation	8.75±0.13	9.85±0.15	97.5
4ppm original formulation	8.11±0.23	9.24±0.24	93.8
4ppm nano formulation	8.36±0.11	8.89±0.14	95.2
Distilled water	7.95±0.20	7.97±0.23	92.5

From Table 1, it can be seen that the seedling growth of wheat was optimal after 2ppm lignosulfonate nanosystem dip, and the increase of root length and seedling height, germination rate were better than the original agent group, when the increase of the nanosolution concentration was 3ppm, the difference of root length and seedling height with the original agent reached its significant level ($P<0.05$), and the effect of the nano group was better than the original agent group at the same dose.

5.2 Effects on dry weight and fresh weight of wheat seedlings

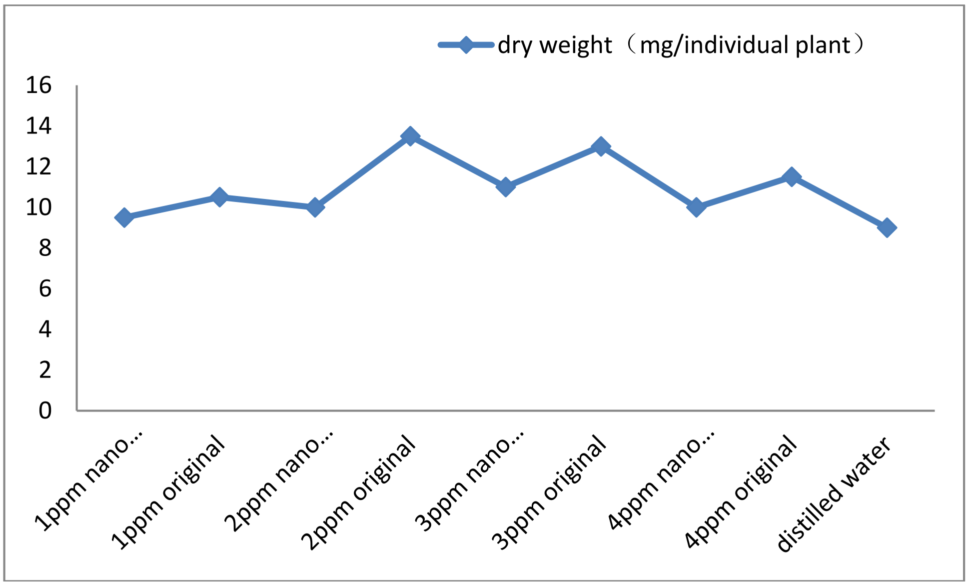

Figure 6: Effect of sodium lignosulfonate on dry weight of wheat seedlings

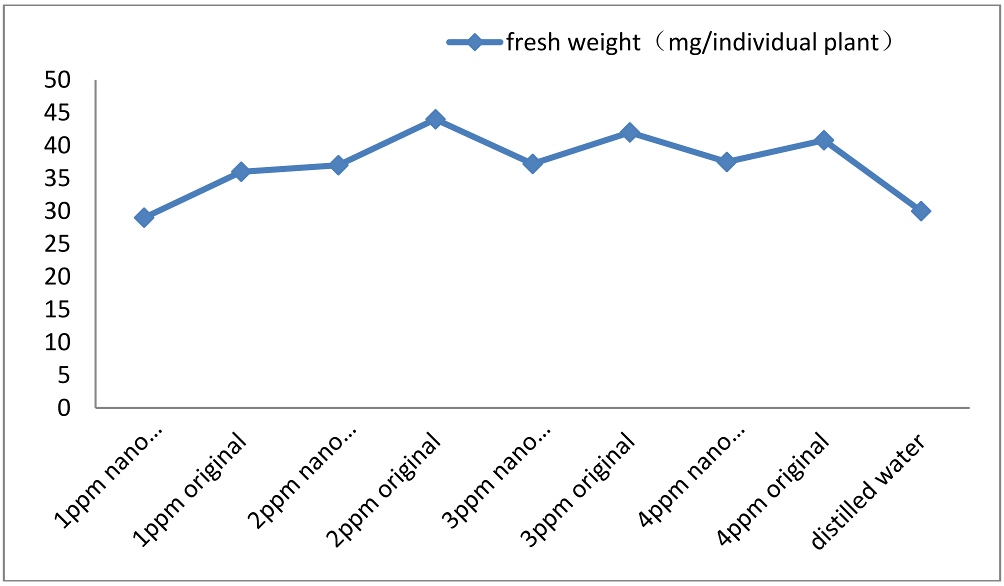

Figure 7: Effect of sodium lignosulfonate on fresh weight of wheat seedlings

According to Figs. 6 and 7, it can be seen that when the seeds were dipped with 2 ppm of sodium nano lignosulfonate solution, it was the most effective for promoting the increase of dry weight and fresh weight content of wheat, and the dry weight and fresh weight increased by 48.42% and 49.3%, 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, and for the control group the dry weight and fresh weight increased by 42.11% and 45.2%. It can be clearly seen that the plant growth regulating effect of sodium nano lignin sulfonate is better, and the effect is also better than the original agent group when the use concentration is lower than the original agent group, so sodium nano lignin sulfonate has the characteristics of high efficiency and low use concentration.

5.3 Effect on chlorophyll in wheat seedlings

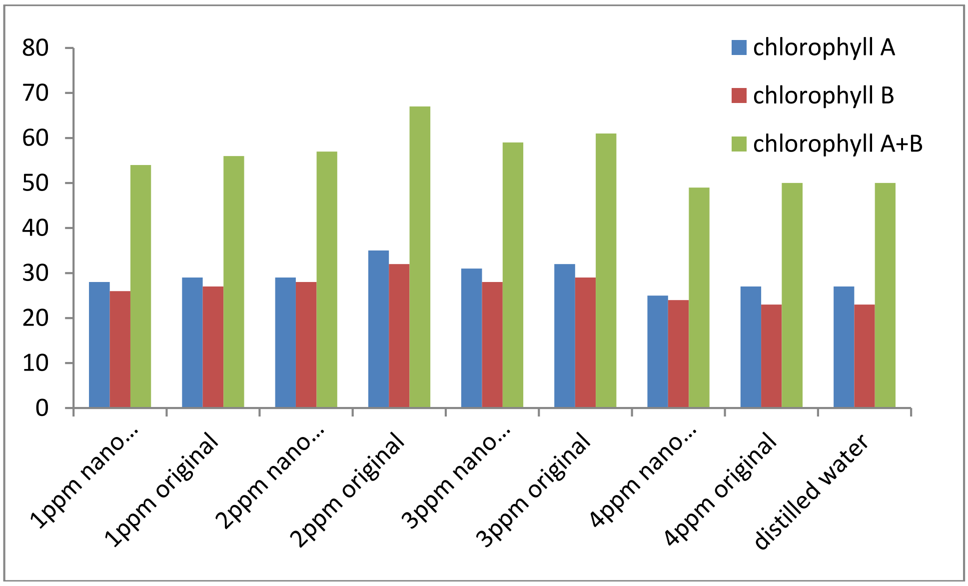

Figure 8: Effect of sodium lignosulfonate on chlorophyll content of wheat seedlings

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 wheat, and chlorophyll a, chlorophyll b, and total chlorophyll increased by 40%, 55%, and 46.67%, 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, and for the control group chlorophyll a, chlorophyll b, and total chlorophyll total amount increased by 20%, 35% and 26.67%, respectively. It can be clearly seen that the plant growth regulating effect of sodium nano lignosulfonate is better, and the effect is also better than the original agent group when using lower concentration than the original agent group, so sodium nano lignosulfonate has the characteristics of high efficiency and low using concentration.

6. Conclusion

(1) The optimal conditions for the preparation of sodium lignosulfonate nanopreparations 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 lignosulfonate itself also has the effect of promoting plant growth, which is a more effective plant growth regulator. Therefore, sodium lignosulfonate nanopreparation, is a green, environmentally compatible, widely sourced, organic adsorbent.

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