

Sustainable Lignin-Based Nano Hybrid Biomaterials with High-Performance Antifungal Activity

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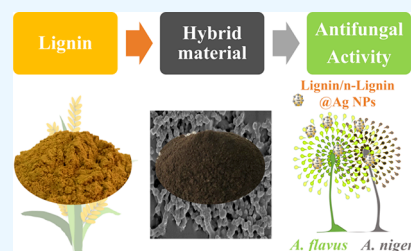
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ABSTRACT: *Aspergillus flavus* (*A. flavus*) and *Aspergillus niger* (*A. niger*) mainly spread through airborne fungal spores. An effective control to impede the dissemination of the spores of *Aspergillus* in the air affecting the environment and food was carried out. This study focuses on the sustainable rice husk-extracted lignin, nanolignin, lignin/n-lignin capped silver nanoparticles used for fungal growth inhibition. These biomaterials inhibit the growth of fungi by altering the permeability of cell membranes and influencing intracellular biosynthesis. The antifungal indexes for *A. flavus* and *A. niger* on day 5 at a concentration of 2000 $\mu\text{g}/100 \mu\text{L}$ are 50.8 and 43.6%, respectively. The results demonstrate that the hybrid biomaterials effectively prevent the growth or generation of fungal spores. The findings of this research hold significant implications for future investigations focused on mitigating the dissemination of *Aspergillus* during the cultivation of agricultural products or in the process of assuring agricultural product management, such as peanuts and onions.



INTRODUCTION

Because of the expansion in storage demands of agricultural commodities, infections of bacteria or fungal can cause a durable effect on the quality of human life. Food safety and health are becoming more important as agriculture improves. As a result, materials having antimicrobial ability that inhibit the growth of fungi have attracted the interest of researchers. The research, development, and manufacture of antimicrobial materials have emerged as a new and promising sector. Silver ions (Ag^+), nanoparticles (AgNPs), and silver-based compounds all exhibit antimicrobial action. Silver ions are commonly employed in medicine.¹ Silver ions, on the other hand, are not stable when exposed to light or kept for a lengthy periods of time. They easily convert to dark brown silver oxide, and the discoloration affects not only the look of the product but also its activity. Moreover, silver ions dissolve quickly in water, resulting in a short-term antimicrobial action.² As a result, substrates are required to stabilize the silver nanoparticles. Recently, researchers have utilized synthetic polymers playing the role as stabilization materials such as polyphenols, polyamide, and polystyrene with promising results.^{3–5} Nevertheless, because synthetic polymers are harmful to consumers' health, they cannot be used directly in food.⁶ Therefore, the use of plant extracts such as chitosan, lignin, and carboxymethyl cellulose are environmentally friendly,^{7–9} nontoxic, capable of acting as a reducing agent, compatible with AgNPs, and suitable for improving the stability through biosynthesis to prepare silver-based antibacterial and fungal agents.

The organic component lignin is an extraordinarily plentiful resource, second only to cellulose (approximately 170 billion tons per year),¹⁰ with far greater potential than what is now being exploited. Although they may be supplied from a number of sources, environmental issues must be prioritized. The concept of employing rice husk (RH) biomass as a byproduct of food processing has been explored as a source of lignin with a comparatively high proportion of around 22.5%.¹¹ Lignin is an aromatic polymer with a strongly cross-linked three-dimensional network structure made of three distinct monolignols, including coniferyl alcohol, sinapyl, and *p*-coumaryl.¹² The aromatic ring structure of lignin makes it harder for microbes to breakdown.¹³ Moreover, lignin with several functional groups such as hydroxyl, carbonyl, and methoxy can act as Ag^+ ion binding sites as well as a natural reducing agent.¹⁴ Additionally, the three-dimensional structure of lignin may efficiently overcome silver nanoparticle aggregation and improve the silver nanoparticle compatibility with macromolecular polymers. Because of lignin's hydroxyl groups, carbonyl groups, and double bonds, it can improve the stability of silver-based antibacterial drugs.¹⁵ Furthermore,

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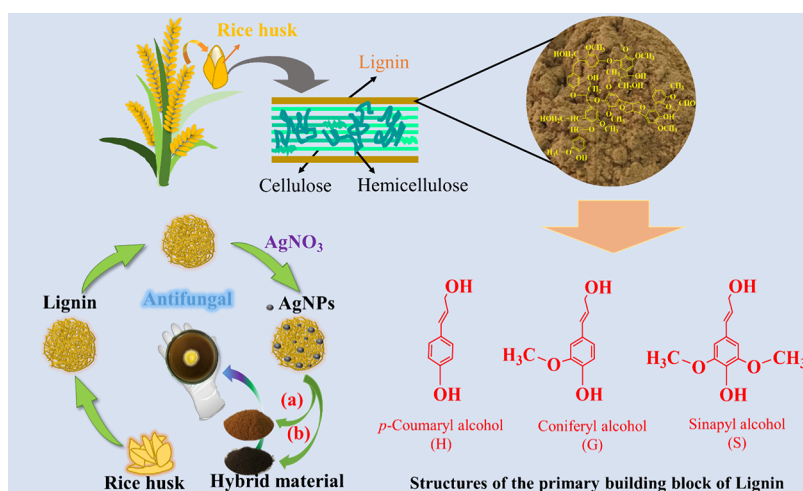


Figure 1. Synthesis of LSN: (a) without NaBH₄ and (b) with NaBH₄.

because lignin is biocompatible, it may be used in medical-pharmaceutical and cosmetic applications such as drug delivery, wound dressing material, antidiabetic activity, cytotoxic activity, and antidiabetic activity in the fight against cancer.^{16–18} The remarkable properties of lignin generated from biomass as a reducing agent or stabilizer for antibacterial chemicals not only help protect the environment, but also increase the value of lignin in future research.¹⁹

However, no reports were found concerning the evaluation of antifungal activities of lignin-based nano hybrid materials prepared from RH. *Aspergillus flavus* (*A. flavus*) of peanut and *Aspergillus niger* (*A. niger*) of onion are filamentous saprophytic fungi that are found worldwide and frequently infect agricultural goods. Aflatoxin generated by *A. flavus* causes cancer and liver sickness in people and animals,²⁰ posing a hazard to farmers. In this study, we employ lignin/n-lignin from RH as a reducing agent (lignin's negatively charged carboxyl and phenolic hydroxyl groups may adsorb and reduce silver ions), a stabilizer, or core for metal nanoparticle inlay, and AgNO₃ as a silver supply. The silver nanoparticle-mosaic lignin/n-lignin core hybrid materials were used in this work to suppress the development of the fungus genus *Aspergillus* via reactions occurring inside the cells.

EXPERIMENTAL SECTION

Materials. Raw RHs were collected in southern Vietnam. Acetone, sulfuric acid (H₂SO₄, 98%), hydrochloric acid (HCl, 36.5%), hydrogen peroxide (H₂O₂, 30%), and ethylene glycol (EG) were obtained from Xilong Scientific Co., Ltd. Sodium hydroxide (NaOH, 98%) was purchased from Guangdong Guanghua Sci-Tech Co., Ltd. Potato dextrose agar (PDA) was purchased from TM Media, India. Other chemicals, such as silver nitrate (AgNO₃) and sodium borohydride (NaBH₄) were purchased from Aldrich. Nystatin and cycloheximide used as an active control against the growth of the fungus were purchased from Mekophar Chemical Pharmaceutical Joint-Stock Co., Vietnam, and HiMedia Laboratories Pvt. Ltd., India, respectively. *A. flavus* (in groundnut) and *A. niger* (in onion) were isolated from cultivated soil in Binh Dinh province, Vietnam. Distilled water was used for all of the synthesis processes.

Synthesis of Lignin and Nanolignin (n-Lignin) from RHs. The fabrication of lignin from RH was mentioned in the

previous study.^{19,21} Briefly, the RHs were cleaned with water before being dried at 60 °C to eliminate dirt and other soluble or insoluble impurities. The dry RHs (60 g) were soaked in distilled water for 18 h in a reaction vessel at room temperature. The solution was agitated at 100 °C for 3 h after 500 mL NaOH 1 M was added. The residual material was washed with distilled water to obtain a neutralized sample and then soaked in distilled water for 18 h. This mixture was stirred with a mechanical stirrer, while 1 M NaOH was added to achieve a pH value of 11. Then, 276 mL of H₂O₂ solution (30%) was added to the solution and the mixture reacted at 100 °C for 1 h. After distilled water was added to 1200 mL, the mixture was stirred continuously for 3 h. The mixture was filtered through a stainless-steel mesh to separate the liquid, it was precipitated with H₂SO₄ 0.45 M and kept at 4 °C for 18 h before being centrifuged to obtain lignin from the RHs. Lignin was then dried in an oven at 60 °C for 24 h.

The n-lignin synthesis technique was based on previously published studies.^{19,22,23} Lignin (3 g) in EG (75 mL) was produced and sonicated for 30 min before being agitated for 2 h at room temperature. Then, 0.25 M HCl (6 mL) was added to the reaction vessel for 30 min and stirred continuously for another 2 h. The final solution was centrifuged and washed with distilled water until the n-lignin suspension achieved a pH of 7. The solid n-lignin was obtained by vacuum drying in a 60 °C oven for 24 h.

Synthesis of Hybrid Nanoparticles (LSN, n-LSN). Initially, 1.5 g of lignin dispersed in 150 mL of distilled water was placed in a three-neck round-bottom flask and sonicated at room temperature for 30 min. Afterward, a 100 mL AgNO₃ 15 mM was injected at a rate of 1 drop per min into the reactor fitted with a reflux system with a nitrogen intake. The mixture was then refluxed at 100 °C. The color of the reaction mixture progressively altered from brown to dark brown/gray. The color shift suggested that Ag(I) ions had been reduced to Ag(0) solid, which subsequently agglomerated into silver nanoparticles. The reaction was allowed to continue for 6 h to produce LSN-1. The solid product was washed with distilled water (10 mL, 3 times) and acetone (20 mL, 3 times) before being dried in an oven at 60 °C for 24 h.^{19,24} The same process was utilized to synthesize n-LSN-1; however, n-lignin was employed instead of lignin.

The syntheses of LSN-2 and n-LSN-2, conducted in the presence of NaBH₄, adhered to a similar process (Figure 1).

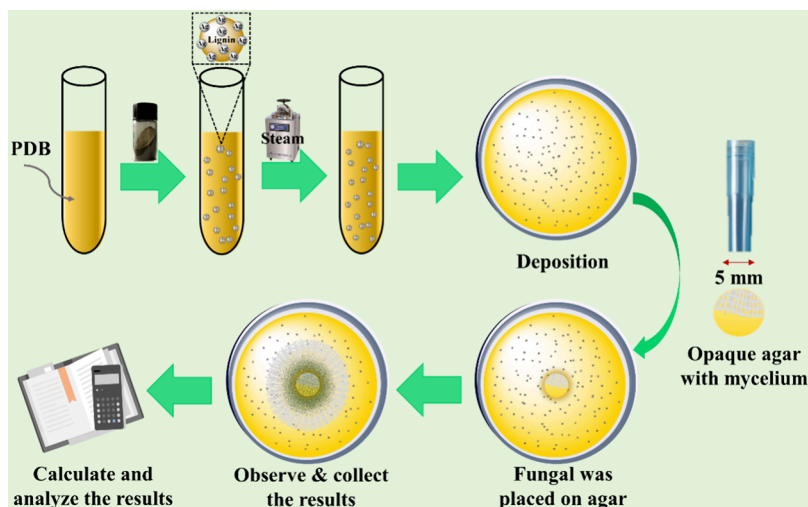


Figure 2. Study of antifungal activity.

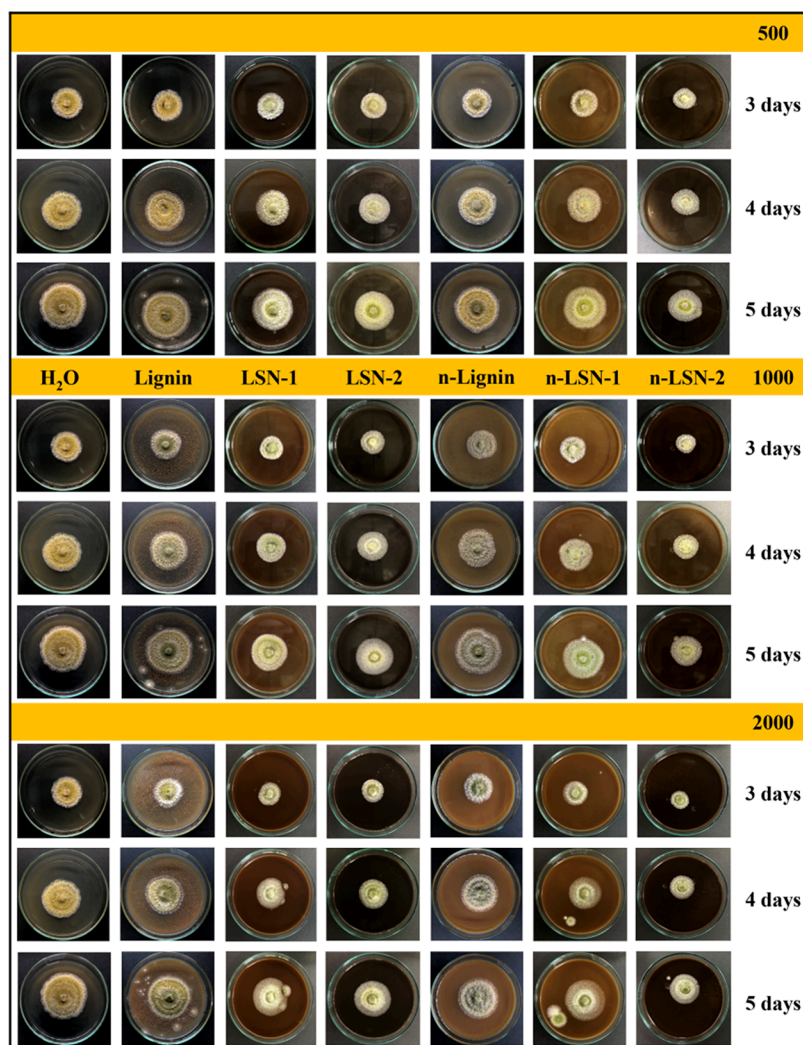


Figure 3. Antifungal properties of materials tested against *A. flavus* on days 3, 4, and 5 at concentrations of 500, 1000, and 2000 $\mu\text{g}/100 \mu\text{L}$.

Initially, 1.5 g of lignin (for LSN-2) or n-lignin (for n-LSN-2) was dispersed in 150 mL of distilled water and subjected to sonication at room temperature for 30 min. Subsequently, 100 mL of AgNO_3 (15 mM) was introduced into the reactor at a rate of one drop per min. Next, 30 mL of 37 mM NaBH_4 was

added dropwise for 30 min and the reaction continuously stirred under a nitrogen atmosphere at 100 °C for 6 h. Following this, the resulting solution was isolated and the solid was then washed several times with distilled water and acetone.

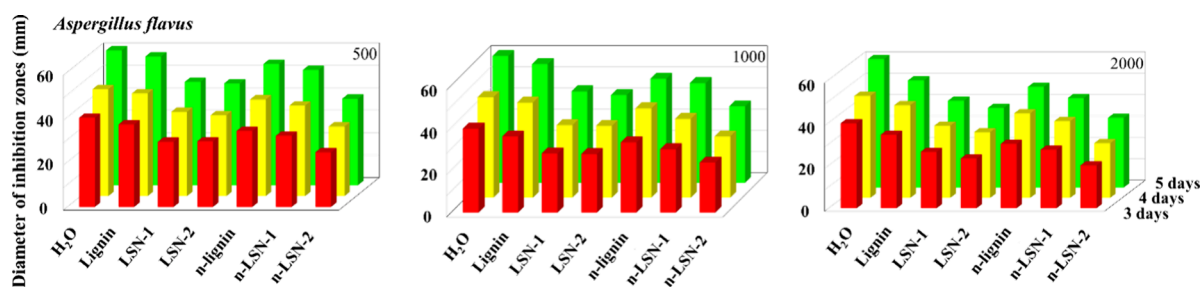


Figure 4. Antifungal activity against *A. flavus* of the samples at concentrations of 500, 1000, and 2000 $\mu\text{g}/100 \mu\text{L}$.

Table 1. Diameter of Inhibition Zones (mm) on 3, 4, and 5 Days of the Samples at Concentrations of 500, 1000, and 2000 $\mu\text{g}/100 \mu\text{L}$ against *A. flavus*

day	sample						
	H ₂ O	Lignin	LSN-1	LSN-2	n-lignin	n-LSN-1	n-LSN-2
500							
3	39.7 ± 0.3	36.7 ± 0.3	29.0 ± 0.7	29.2 ± 0.4	33.8 ± 0.8	31.7 ± 0.2	24.3 ± 0.7
4	47.6 ± 0.6	45.7 ± 0.6	37.5 ± 0.5	36.0 ± 0.5	43.0 ± 0.6	40.3 ± 0.9	31.0 ± 1.0
5	60.0 ± 0.6	57.3 ± 2.0	46.0 ± 1.0	45.3 ± 0.7	54.0 ± 1.0	51.3 ± 0.9	38.5 ± 0.5
1000							
3	39.7 ± 0.3	36.0 ± 0.5	28.0 ± 1.0	27.7 ± 0.6	33.2 ± 0.5	30.0 ± 0.3	23.7 ± 0.7
4	47.6 ± 0.6	45.0 ± 0.9	34.3 ± 0.9	34.0 ± 0.5	42.3 ± 0.9	37.3 ± 0.2	29.0 ± 0.5
5	60.0 ± 0.6	56.2 ± 0.2	43.3 ± 0.6	41.5 ± 0.5	49.2 ± 0.2	47.2 ± 0.2	36.2 ± 0.4
2000							
3	39.7 ± 0.3	34.3 ± 0.7	26.3 ± 0.3	23.2 ± 0.7	30.0 ± 0.6	27.3 ± 0.2	20.0 ± 0.5
4	47.6 ± 0.6	43.3 ± 0.3	33.7 ± 0.9	30.7 ± 0.3	39.5 ± 0.5	35.8 ± 0.2	25.5 ± 0.3
5	60.0 ± 0.6	50.0 ± 0.2	40.5 ± 0.8	37.2 ± 0.2	47.0 ± 0.3	41.8 ± 0.3	32.5 ± 0.3

Finally, the solid product was subjected to drying until a constant weight was achieved.

Study of Antifungal Activity. The ability of the material hybrid to inhibit spore germination of *Aspergillus* was evaluated. The material's antifungal efficacy against *A. flavus* and *A. niger* was studied using the method of pouring agar plate (Figure 2). Different concentrations of the hybrid material were added to test tubes containing potato dextrose broth (PDB), and agar was generated and steam sterilized for fungal culture. At 45 °C, 10 mL of melted PDB medium containing lignin/n-lignin capped silver nanoparticle hybrid samples (500–2000 $\mu\text{g}/100 \mu\text{L}$) was placed into the Petri plate and allowed to harden at room temperature before being utilized as sample plates. Following that, an agar block of uniform size (5 mm diameter) obtained from the edge of a 72-h-old fungal culture was aseptically inserted in the center of the previously prepared sample medium, and the samples were incubated at 25 °C for 3, 4, and 5 days. Subsequently, fungal growth was observed on days 3, 4, and 5 by measuring the diameter of the growth using a ruler and recording the data. The experimental procedures were replicated three times to ensure the consistency and reliability of the results. The acquired data were subsequently subjected to processing through Microsoft Office 365 and are presented as the mean \pm standard error.

The minimum inhibitory concentration (MIC) test of nystatin and cycloheximide was chosen as the positive control which is popular antifungal medicine known to decrease sporulation and thereby effectively restrict the spread of the fungus. The identical PDA preparation procedure was used with the positive and negative controls (H₂O).

Characterization and Measurements. Lignin, n-Lignin, LSN-1, LSN-2, n-LSN-1, and n-LSN-2 synthesized were analyzed by high resolution transmission electron microscopy

(HR-TEM), high-angle annular dark-field scanning TEM (HAADF-STEM), energy-dispersive X-ray spectroscopy (EDX), Fourier transform infrared (FTIR), and X-ray diffraction (XRD). FTIR spectra with KBr pellets were recorded in 400–4000 cm^{-1} range by using a Nicolet Nexus 5700 FTIR, Thermo Electron Corporation, Waltham, MA, USA. HR-TEM, STEM, and the integrated EDX were performed with FEI Titan Themis. XRD were performed by a Rigaku D/Max-2550 V diffractometer with Cu K α radiation ($\lambda = 0.15405 \text{ nm}$, 40 kV, 40 mA) at a scanning speed of 4°/min in the 2θ range from 4 to 80°. Flame atomic absorption spectroscopy was employed for the concentration analysis of silver nanoparticles in materials.

RESULTS AND DISCUSSION

Antifungal Activity and Mechanism of Action on Fungal Cells. We tested *A. flavus* fungal antibiotic resistance using the antibiotic nystatin at concentrations of 50, 100, 150, 200, and 300 $\mu\text{g}/100 \mu\text{L}$, with experimental findings obtaining a minimum inhibitory concentration (MIC) of 300 $\mu\text{g}/100 \mu\text{L}$. Similarly, for *A. niger*, we applied the fungicide cycloheximide at concentrations of 30, 37, 39, 40, and 50 $\mu\text{g}/100 \mu\text{L}$ and established a minimum inhibitory concentration of 40 $\mu\text{g}/100 \mu\text{L}$. Figures 3 and 4 and Table 1 depict the inhibitory action of lignin, n-lignin, and the hybrid material against *A. flavus* growth. In the case of lignin/n-lignin capped silver nanoparticles hybrid samples at a concentration of 500 $\mu\text{g}/100 \mu\text{L}$, there was a difference in growth diameter or spore-forming ability compared to the negative controls. However, only the n-LSN-2 sample clearly had the ability to inhibit fungi, the other samples did not show too much growth inhibition, and the lignin sample did not differ from the negative control (Table 1, Figure 4). The antifungal drug nystatin was used as positive

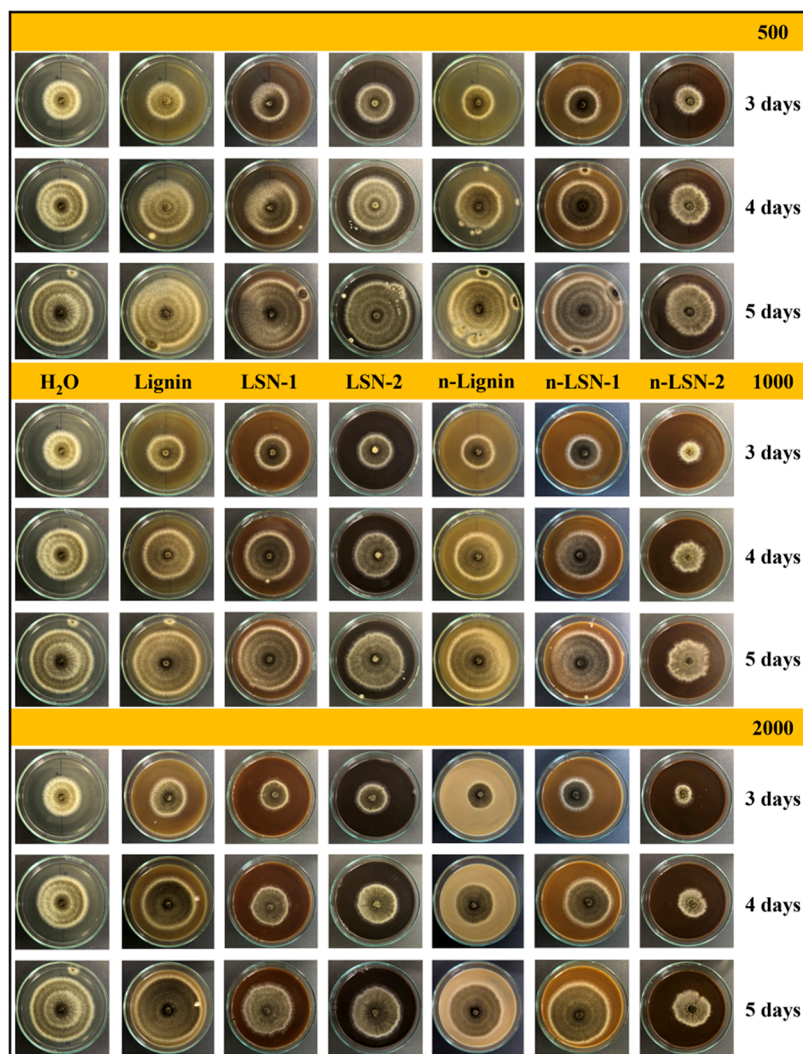


Figure 5. Antifungal properties of materials tested against *A. niger* on 3, 4, and 5 days at concentrations of 500, 1000, and 2000 $\mu\text{g}/100 \mu\text{L}$.

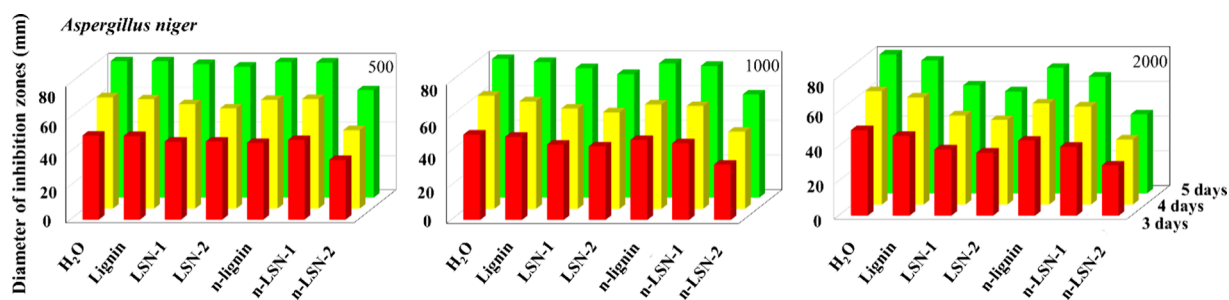


Figure 6. Antifungal activity against *A. niger* of the samples at concentrations of 500, 1000, and 2000 $\mu\text{g}/100 \mu\text{L}$.

control, and it showed a MIC of 300 $\mu\text{g}/100 \mu\text{L}$. These findings suggest that the hybrid materials have antifungal efficacy against *A. flavus*. Nevertheless, a concentration of 500 $\mu\text{g}/100 \mu\text{L}$ was insufficient to kill the fungus.

The growth diameter of *A. flavus* reduced as the concentration of the material samples increased, as shown in Table 1, and the color also varied. The diameter of fungal growth was smaller than that of the negative control sample when the concentration of hybrid materials was 1000–2000 $\mu\text{g}/100 \mu\text{L}$, and the fungal coloration of the plates containing the hybrid materials was whiter than that of the control sample, exhibiting effective inhibition of fungal spore growth.

Based on the results, the lignin demonstrated negligible or minimal inhibitory activity because the particle size was too big to enter the fungal cells. Conversely, n-LSN-2 displayed the most efficacious material, inhibiting both the proliferation and cytogenesis of *A. flavus*. At 2000 $\mu\text{g}/100 \mu\text{L}$, the largest difference in the growth diameter between n-LSN-2 with the control sample is about 27.5 mm on day 5 while these values of n-LSN-1, LSN-2, and LSN-1 are 18–22 mm. Moreover, the usage of hybrid materials including silver nanoparticles impacts on the fungus *A. flavus* manufacturing the carcinogen Aflatoxin B1 for people and animals.²⁵

Table 2. Diameter of Inhibition Zones (mm) on 3, 4, and 5 Days of the Samples at Concentrations of 500, 1000, and 2000 $\mu\text{g}/100 \mu\text{L}$ against *A. niger*

day	sample						
	H ₂ O	Lignin	LSN-1	LSN-2	n-lignin	n-LSN-1	n-LSN-2
500							
3	49.3 \pm 0.7	49.0 \pm 0.6	45.7 \pm 0.8	45.8 \pm 0.9	45.0 \pm 1.0	46.7 \pm 0.3	35.0 \pm 0.4
4	65.3 \pm 0.7	64.2 \pm 0.4	61.3 \pm 0.7	58.7 \pm 0.7	63.8 \pm 0.6	64.3 \pm 0.3	46.0 \pm 0.5
5	80.7 \pm 0.3	80.5 \pm 0.7	78.3 \pm 0.3	76.8 \pm 0.7	79.5 \pm 0.5	79.2 \pm 0.6	63.0 \pm 0.8
1000							
3	49.3 \pm 0.7	48.0 \pm 1.0	43.5 \pm 0.3	42.5 \pm 0.7	46.2 \pm 1.0	44.3 \pm 0.7	32.0 \pm 0.2
4	65.3 \pm 0.7	62.0 \pm 0.6	57.8 \pm 0.6	55.7 \pm 1.3	60.2 \pm 0.3	59.3 \pm 0.6	44.5 \pm 0.5
5	80.7 \pm 0.3	78.3 \pm 0.6	74.7 \pm 0.7	71.3 \pm 0.3	77.5 \pm 0.4	76.0 \pm 0.7	59.5 \pm 0.5
2000							
3	49.3 \pm 0.7	46.0 \pm 0.6	38.2 \pm 0.4	36.3 \pm 0.7	43.3 \pm 0.7	39.7 \pm 0.3	29.0 \pm 1.0
4	65.3 \pm 0.7	61.7 \pm 0.3	51.3 \pm 1.3	48.7 \pm 0.7	58.3 \pm 0.3	56.5 \pm 0.3	37.5 \pm 1.5
5	80.7 \pm 0.3	76.5 \pm 0.5	62.2 \pm 0.7	58.7 \pm 0.7	72.3 \pm 0.5	67.2 \pm 0.4	45.5 \pm 0.5

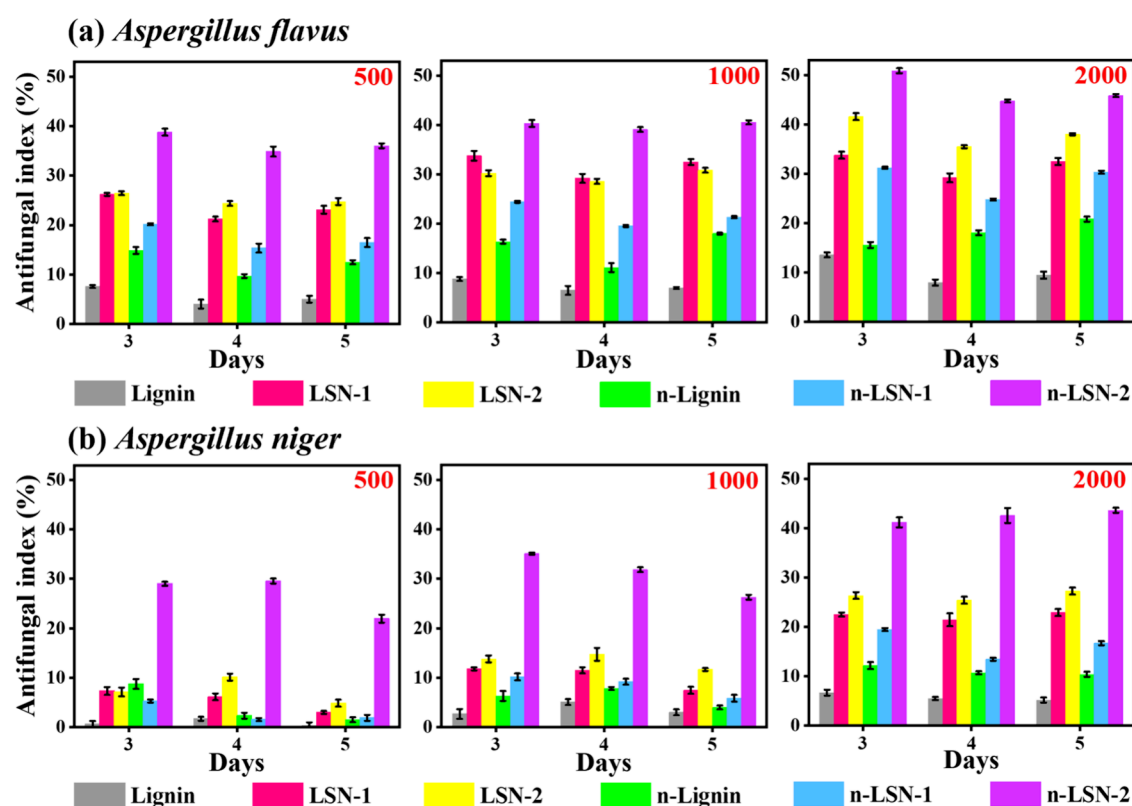


Figure 7. Antifungal index (%) on 3, 4, and 5 days of the samples at concentrations of 500, 1000, and 2000 $\mu\text{g}/100 \mu\text{L}$ against: (a) *A. flavus* and (b) *A. niger*.

Figures 5 and 6 and Table 2 show the antifungal properties of materials tested against *A. niger* on 3, 4, and 5 days at concentrations of 500, 1000, and 2000 $\mu\text{g}/100 \mu\text{L}$. The results revealed that most of the samples did not limit the fungal growth at a 500 $\mu\text{g}/100 \mu\text{L}$ concentration. The concentration of 500 $\mu\text{g}/100 \mu\text{L}$ was not too powerful enough to block *A. niger*; thus, the concentration of hybrid materials was raised to 1000 and 2000 $\mu\text{g}/100 \mu\text{L}$ for further study. At a concentration of 1000 $\mu\text{g}/100 \mu\text{L}$, only two samples n-LSN-2 and LSN-2 showed the potential to decrease spore development or formation. In four hybrid samples, increasing the concentration to 2000 $\mu\text{g}/100 \mu\text{L}$ had the intended result. The strongest inhibitory effect was seen in the n-LSN-2 sample at 2000 $\mu\text{g}/100 \mu\text{L}$, the growth diameter at day 5 was 45.5 ± 0.5 mm,

which is rather smaller than that of the negative control sample (80.7 ± 0.3 mm), and the quantity of spores generated was much lower than that of the negative control, as shown by color. The three hybrid material samples n-LSN-1, LSN-1, and LSN-2 exhibit variations at day 5 sequence 13, 18, and 22 mm, respectively. In summary, based on the testing results, it was discovered that hybrid material samples had a stronger antifungal ability than lignin and n-lignin to prevent the growth or minimize the release of spores into the environment, causing harm to humans and plants. The n-LSN-2 material has the best result in antifungal ability, exhibiting the feasibility at all three doses for both studied fungal strains.

The antifungal tests were carried out in accordance with the growth rate approach reported by Guo. et al.²⁶ The antifungal

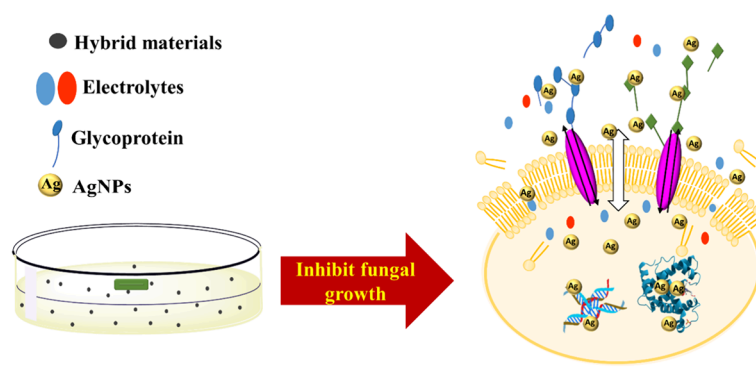


Figure 8. Effect of nano silver is toxic to fungal cells.

index was measured when the fungus developed in the negative control and hybrid samples after 3, 4, and 5 days:

Antifungal index (%) = $\left[1 - \frac{D_a}{D_b}\right] \times 100$ Where: D_a denotes the diameter of the growth zone containing the test sample, and D_b is the diameter of the growth zone in the control plate. Each experiment was carried out three times.

The antifungal index presented in Figure 7 concluded the antifungal activity of the materials. Observing the *A. flavus*-graphs, it is found that the n-LSN-2 sample has the highest antifungal index at the concentration of 2000 $\mu\text{g}/100 \mu\text{L}$, around 50% on day 3. It can be observed that the samples in both fungal strains follow the pattern throughout all concentrations and days. In the *A. niger* charts, the antifungal index in lignin samples was nearly nonexistent at 500 $\mu\text{g}/100 \mu\text{L}$ and then grew to a greater at 2000 $\mu\text{g}/100 \mu\text{L}$ concentration, and the antifungal index was less than 10%, which can be deemed a small unfavorable effect on *A. niger* growth. In all three concentrations, the n-LSN-2 sample had the best antifungal activity. The obtained results align consistently with our initial hypotheses. The antifungal properties of hybrid materials depend on two factors: the size of material and the amount of silver nanoparticles in the material. In fact, n-LSN-2 was synthesized from *n*-lignin, so the size of n-LSN-2 tends to be smaller than that of other materials. The crystallite sizes of AgNPs calculated by Scherrer's equation are 4.2, 4.9, 4.3, and 2.9 nm for LSN-1, LSN-2, n-LSN-1, and n-LSN-2, respectively [19]. In addition, n-LSN-2 formed in the presence of both *n*-lignin and an additional reducing agent, sodium borohydride, n-LSN-2 exhibited the higher silver content compared to n-LSN-1. Specifically, as determined by flame atomic absorption spectroscopy, n-LSN-2 exhibited a silver content of 7.39 wt %, whereas n-LSN-1 displayed at 0.98 wt %. Therefore, n-LSN-2 demonstrated superior efficacy in inhibiting the growth of both fungal strains. When the antifungal activity of the material samples for the two strains was evaluated, *A. flavus* had a higher index than *A. niger*.

Figure 8 depicts research into the antifungal capability of AgNPs against plant pathogenic fungus, namely, cell membrane breakdown, and the discovery that they have good antifungal efficacy. AgNPs greatly reduce plant pathogenic mycelium growth and development, damage the cell membrane, modify the structure and permeability of the cell membrane, inhibit protein synthesis, degrade DNA structure, and impede DNA replication. AgNPs, on the other

hand, had no effect on plant seeds.²⁷ Particle size reduction is a crucial component in entry and cytotoxicity. As the size of the nanoparticles decreases, the corresponding increase in the surface area leads to a higher density of adsorption onto the surface of fungal cells. This, in turn, compromises the integrity of the cell membrane and facilitates the penetration of the nanoparticles into fungal cells through membrane proteins or metabolic channels.²⁸

Toxicity and Environmental Impact. The use of nanoparticles or hybrid materials for agricultural purposes must be balanced with hazardous effects and environmental consequences. Lignin derived from rice husk is soluble in alkaline solutions but insoluble in neutral and acidic solutions, and it has a high capacity for heavy metal ion absorption. It serves as a transporter, as well as a reducing agent, in the production of ecologically friendly lignin-core silver nanoparticles. Nevertheless, this does not necessarily ensure that broad usage of items already used in agriculture or food would not have a harmful influence on the environment, as is the case with silver nanoparticles.^{29,30} Toxicity is another consideration to consider before the employment of nanoproducts in agricultural applications. Nanoparticles' direct harmful effects are frequently linked to their chemical composition and high specific surface area, which renders them physiologically reactive.³¹ Therefore, it is critical to distinguish between cytotoxic substances and those that are harmful to the entire organism. As an alternative to underused agricultural biochemical products, antifungal hybrid materials based on lignin as a biomass feedstock are widely accessible for the production of nanoparticles. Certainly, the utilization of a green and biodegradable polymer, such as lignin/*n*-lignin, as a stabilizer and reducing agent showcases its minimal environmental impact, as evidenced by a much lower silver ion release or even the absence of silver ion release after the implementation ability to inhibit microbial growth.^{32,33} The targeted biosubstrate nanoparticles will rapidly lose their postutilization activity and biodegrade in the environment after disposal.³²

CONCLUSIONS

In recent years, there has been research into the production of hybrid materials by using a mix of metals and biopolymers. A special focus has been placed on lignin mediated AgNPs synthesis in order to produce a hybrid material with nano particle size that has the ability to limit the development of the fungus *A. flavus* and *A. niger*. The capacity to limit the growth

of *A. flavus* and *A. niger* mycelium as well as the spore production process was determined. Experiments on the fungus's growth diameter have shown the potential utility of the hybrid materials in agriculture in general and as a substitute for harmful products in particular. Utilizing green nanomaterials plays an essential role in creating sustainable materials in environmental applications.

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Notes

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