

Original Research Paper

Converting Agricultural Waste into Sustainable and Valuable Antifungal Nanocellulose

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Abstract: To achieve sustainable development goals, due to the limited availability of timber resources and inadequate environmental regulations, studies have turned to consuming agricultural waste as a cost-effective and readily available source for cellulosic resources and simple, eco-friendly fruit preservation technology. The aim of this study was using Three types of agricultural wastes to produce nanocellulose by two methods: acid hydrolysis and grinding and the obtained nanocellulose characterized by Scanning electron microscopy (SEM), Fourier Transform Infra-Red spectroscopy (FTIR) and X-ray diffraction (XRD) analysis and test their antifungal activity against *Aspergillus niger* and *Penicillium* sp. these fungi were obtained from the fungal collection of the biology Department of College of science in Mustansiriyah University. SEM images of the nanocellulose revealed a heterogeneous mixture of irregularly shaped crystals with a size range of 79-93 nm in acid hydrolysis and 60-67 nm in the grinding method. The percentage crystallinity of nanocellulose with high intensity values like 1324.83 and 1421.87. Minimum fungicidal concentration (MFC) of synthesized nanocellulose against Fungi was evaluated by the diffusion in agar method in four concentrations: 0.5%, 1%, 1.5 %, and 2%, respectively. The best inhibition percent of *Aspergillus niger* were 80 % and 90 % for *Penicillium* sp. in 2% of nanocellulose after 7 days of incubation. The increased concentration statistically affected all fungal activity ($P < 0.05$). Nanocellulose was more effective against *Aspergillus niger* and *Penicillium* sp. Accordingly; this study shows that this nanocellulose-based low-cost waste biomass had a potential fungicidal effect.

Keywords: Sustainable biopolymer, Reduce waste generation, Antifungal efficiency

1. Introduction

The increasing demand for healthy diets, coupled with the development of effective solutions to reduce the high waste of fruits and vegetables, and the need for suitable packaging that preserves the nutritional value and freshness of these products, is

particularly important. Recent studies have thus focused on utilizing sustainable, renewable, and green resources to create high-value, ecologically friendly goods [1]. Moreover, cellulose is considered biodegradable, making it a viable substitute for the cellulose-based composites that are produced [2]. High-performance, highly

absorbent hydrogels based on nanocellulose recovered from agricultural waste can be created through chemical or physical processing [3]. Nanocellulose is a molecule that is distinct from cellulose and has at least one dimension in the micro (less than 100 nm) [2]. A plentiful and renewable nanomaterial, nanocellulose combines low density, high strength, adaptability, chemical inertness, and surface chemistry change characteristics [4]. Typically, cellulose nanofibers, cellulose nanocrystals, and bacterial nanocellulose are the three primary forms of nanocellulose [2]. An excellent option for an antimicrobial substance against microorganisms is nanocellulose [5]. This substance is highly recognized for its adaptability and superior qualities, including possessing high mechanical properties, a specific surface area, biodegradability, a renewable nature, and the ability to facilitate surface functionalization. As one of the best substances for filtering a variety of microorganisms, nanocellulose has been demonstrated. Recent years have seen a significant increase in the use of nanocellulose-based antimicrobial materials in many industrial sectors. The antimicrobial substance based on nanocellulose exhibits significant potential for application as a component in various goods, such as food packaging, textiles, paints, protective gear (primarily in healthcare settings), drug carriers, ultrafiltration, adsorbents, and wound dressings [6]. NC possesses highly changeable surface chemistry, a high surface area, and crystallinity. Because of these characteristics, nanocellulose materials have a great deal of potential to constantly increase moisture and oxygen barriers when used as coatings, fillers in composites, and independent thin films [7]. Better packaging performance and a longer food shelf life are achieved when NC is used to package food. One can extract nanocellulose using mechanical means, steam explosion, or acid hydrolysis. Acid hydrolysis is a primary method used to remove nanocellulose from various materials [8]. Over the last few years, focused on using products with appropriate characteristics biodegradable materials and creating antimicrobial packaging. Active packaging is a highly appealing approach because it can regulate the release of antimicrobial and antioxidant chemicals. Active

antimicrobial packaging is one of the more innovative packaging techniques that has garnered attention due to its availability, diversity of antimicrobial agent matrices, application methods, and range of products that need to be protected. Antimicrobial wrapping has also been proposed as a way to solve the financial problems brought on by microorganisms causing food to degrade [9]. This study aimed to utilize some agricultural waste to extract and create cellulose nanoparticles and test their antifungal efficiency.

2. Methodology

Fungal isolates

Aspergillus niger and *Penicillium* sp. these fungi were obtained from the fungal collection of the biology Department of College of science in Mustansiriyah University. The fungal species were identified based on their physical characteristics and appearance [20]. Aseptically plated on potato dextrose agar (PDA), and then incubated for five days at 28°C. Sub-culturing each of the distinct colonies that formed on the PDA plates and incubating them for five days at 28°C produced and preserved a pure culture.

Preparation of nanocellulose

Agricultural wastes are utilized to extract and create cellulose nanoparticles.

There are three parts to the experimental project

Part I: Involves collecting samples.

Part II: Consists of (i) the removal of cellulose fibers from cotton, carrots, and sugarcane bagasse, as well as (ii) the methods and procedures utilized in producing nanocellulose.

Part III: Includes the applications of nanocellulose in fungal inhibition

Materials

Cotton, sugarcane bagasse, and carrots were procured from the local market, Baghdad. The samples were cleaned, let to dry in the sun for four days, and then sliced into pieces that were one to three centimeters in size. 60 mesh sieves were used to grind and sieve the chopped leftovers. For a whole day, the ground residues were dried in an oven set at 40°C.

Three additional reagents were used: glacial acetic acid, sodium hydroxide, sodium chlorite, and sulphuric acid.

Isolation of cellulose.

After being mechanically stirred for four hours at 100°C with a 15% (w/v) sodium hydroxide aqueous solution (fiber to liquor ratio of 1:50), the cotton, sugarcane bagasse and carrots were rinsed with distilled water until they were neutral, and they were then dried in a hot air oven for twenty-four hours at 40°C. The fibers were then bleached for six hours at 80°C using an equal parts (v/v) solution of acetate buffer and sodium chlorite aqueous solution of 1.7% (w/v)[11]. (BC) was dried in a hot air oven for 24 hours at 40°C after being cleaned in distilled water until the fibers' pH reached neutral.

Preparation of nanocellulose

1- Acid hydrolysis

The bleached cellulose (BC) was hydrolyzed using a 60% (v/v) sulphuric acid solution (fiber to solution ratio of 1:20). The hydrolysis took place for 90 minutes at 45 °C. In order to avoid heating up, the suspension was placed in an ice bath for ten minutes. (Figure 1 shows a method for making nanocellulose).

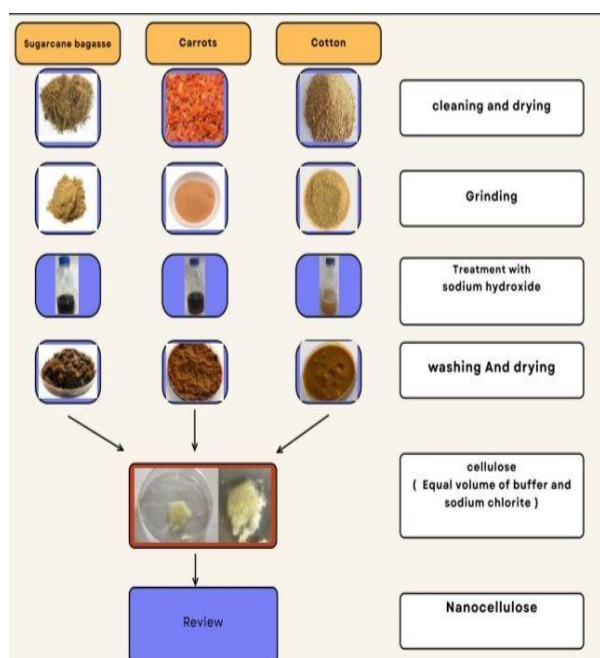


Fig 1: Method for making nanocellulose.

Tumbler mill

Made in the Material Grinding Department of the Ministry of Science and Technology, the tumbler mill produces the best grinding results. Edges were added to the mill's walls to raise the balls as high as possible before they plummeted, and the mill's 200 mm diameter allowed for sufficient area for the balls to descend as shown in (Figs2) and (3)[12]. The diameter of the stainless steel balls ranges from 15 to 35 mm. The revolutions per minute (rpm) ranges from 35 to 40, making the grinding process time-consuming. Because it generates a centrifugal force that accelerates the balls just above the ground, this velocity is ideal because it causes them to ascend as high as possible before hitting, producing the most collision energy [13].

6 Characterization of nanocellulose

1- X-ray diffraction (XRD)

A Rigaku Miniflex X-ray diffractometer fitted with Cu K radiation at 30 kV and 15 mA was used to gather wide-angle X-ray diffraction data in order to examine the cellulosic sample's XRD spectrum. At a speed of 3°/min, scattered radiation was found in the $2\theta = 10 - 80^\circ$ range [14].

2- Scanning electron microscopy (SEM)

Description The University of Technology's Nanotechnology and Advanced Materials Research Centre was the site of the sample surface study. The surface morphology of TR-1 and TR-4 prior to adding Abs, was looked at. One technique that makes it possible to observe the microscopic and nanoscale worlds is SEM. Light microscopy cannot reveal subtleties and complexity that SEM can. The following steps were used to complete the scan: In order to prepare the sample, it is placed in a sample holder.

To prevent charging and improve image quality, a tiny layer of platinum is applied to the sample. Following that, a transfer device compatible with vacuum is used to load the sample holder into the SEM chamber. The SEM chamber is compressed to a high vacuum using a combination of rotary and turbomolecular pumps. adding Abs, was looked at. One technique that makes it possible to observe the

microscopic and nanoscale worlds is SEM. It also eliminates any remaining gas molecules and guarantees that the electron beam can pass through the chamber without being scattered. Heat is applied to a filament to create an electromagnetic lens, and an electron beam to focus it into a tiny beam.

An electron beam scans the sample's surface in a raster pattern, and a detector detects any backscattered or scattered electrons. A signal derived from the measured electrons is converted to produce an image of the sample's surface. The image was displayed on the screen and saved for later research.

3-Fourier Transformer Infrared Spectrometer (FTIR) Analysis:

This test was carried out at the Chemistry Department of Mustansiriyah University's College of Science. Analyses using Fourier transform infrared (FTIR) spectroscopy were performed on the final lyophilized product and Agar-Agar as a control substance. IR absorbance data was obtained for the 400–3600 cm^{-1} wavenumber band during the solid phase study. This made it possible for the initial identification of the materials' chemical functional groups. This apparatus offers useful information about the characteristics of the bands [15].

2.8-In Vitro Antifungal Activity of Nanocellulose

A total of 400 milliliters of the medium were made and divided among glass flasks that had 100 milliliters or 50 milliliters each. In order to sterilize the PDA culture media, cellulose and nanocellulose were added separately at four concentrations 0.5%, 1%, 1.5 % and 2%, respectively. Three duplicates of each concentration were autoclaved for 20 minutes at 121°C and 1.5 kg/cm^2 of pressure. A control treatment consisted of three duplicates that received no additions. Petri dishes measuring 9 cm in diameter were filled with the medium comprising cellulose and nanocellulose, and the plates were then inoculated with a spore suspension of the fungus *A. flavus*. An inoculation needle was placed in the middle of the plate after being dipped in the fungal spore suspension. We incubated the dishes at 25°C.

3. Results and discussion

Extraction of cellulose

Using a combination of mechanical and chemical techniques, the study's goal was to separate cellulose from agricultural waste and then transform it into nanocellulose. One of the crucial elements that influence cellulose's mechanical and physical characteristics is its crystallinity. The crystallinity value of cellulose will drop as its amorphous content increases. The amorphous areas of cellulose are removed during extraction, resulting in high crystallinity later on [16]. Prepared cellulose by grinding bagasse, cotton, and carrots, washing them with sodium hydroxide, then bleaching them with acetate buffer and sodium chlorite solution. The bleached cellulose produced by this technique is extremely pure. The primary application for this cellulose is acid hydrolysis, which yields stable nanocellulose suspension.

Characterization of nanocellulose

XRD analysis

The X-ray diffraction (XRD) pattern's strong peaks and high intensity demonstrate the chemically produced sample's high crystalline order. A large number of distinct crystallographic planes are present when there are abrupt peaks at different 2 θ angles, especially those with high intensity values like 1324.83 and 1421.87. Additionally, the tiny Full Width at Half Maximum (FWHM) values for a few peaks, which range from 0.0984 to 0.5904, indicate that the sample's crystallite size is rather high. This observation is further corroborated by the high peak intensity, which verifies a high degree of crystallinity and good structural regularity.

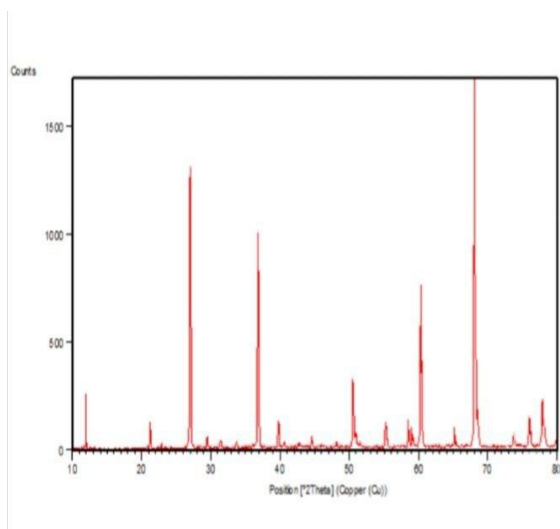


Fig 3.1: XRD pattern of cellulose hydrolysis using H2SO4

Grinding-Prepared Sample: In contrast to the chemically prepared sample, the grinding-prepared sample exhibits somewhat broader diffraction peaks and somewhat lower peak intensity, as seen at height 493.08. A lesser degree of crystallinity or a smaller crystallite size in this sample is indicated by the higher FWHM values, which for certain peaks reach up to 0.5904. Because grinding can break down the crystalline structure and introduce flaws, the fall in relative intensity may also indicate the existence of more amorphous material.

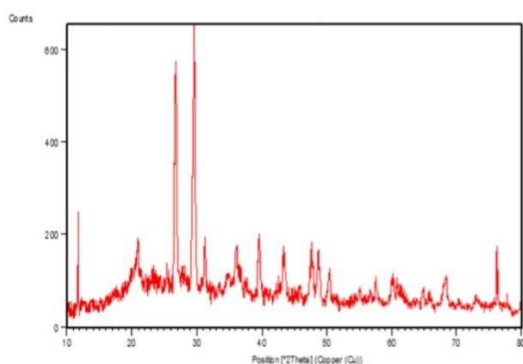


Figure 3.2: XRD pattern of milled cellulose

Crystallites in the chemically produced sample are typically larger and more organized, whereas the ground sample has smaller particles and/or less crystallinity [17].

3-3-2(SEM) analysis

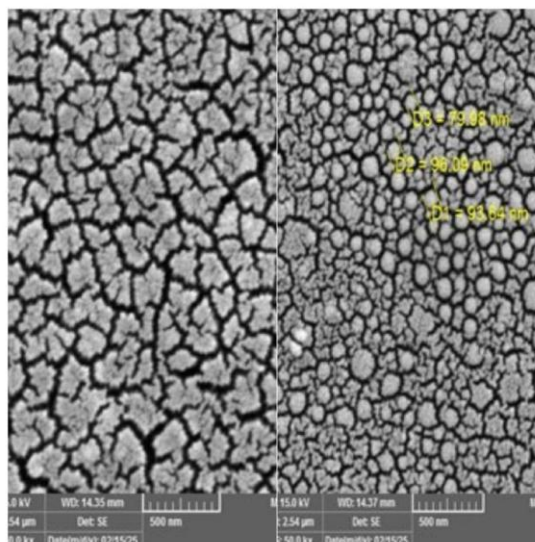


Fig 3.3: SEM chemical analysis of nanocellulose.

The nanocellulose dimensions acquired using various processing techniques show significant variances, according to the SEM examination. The chemical approach produced nanostructures with a comparatively smaller size distribution, with D2 = 67.56 nm and D3 = 60.20 nm. Larger dimensions, D2 = 96.09 nm and D3 = 79.98 nm, were obtained using the mechanical approach, in contrast. According to this disparity, chemical processing, as opposed to mechanical procedures, enables cellulose fibers to break down more efficiently into nanoscale components. Targeting cellulose's amorphous areas through chemical treatments—often acid hydrolysis—individual cellulose nanocrystals (CNCs) with high crystallinity and small particle sizes are released.

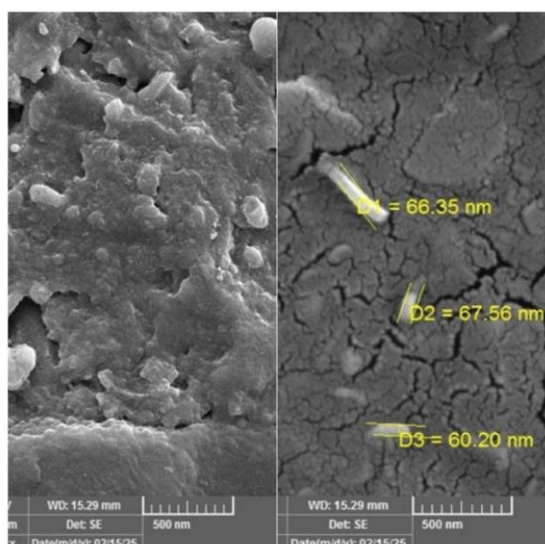


Fig 3.4: SEM mechanical analysis of nanocellulose

On the other hand, mechanical techniques that depend on physical forces like grinding or homogenization might retain more of the fibrous structure, which could lead to a wider range of sizes and possibly larger cellulose nanofibrils (CNFs). These results are in line with other studies that emphasize how the extraction process affects the characteristics of nanocellulose.

According to studies, mechanical processes can result in CNFs with widths of 5 to 60 nm but lengths of several micrometers, whereas chemical processes usually create CNCs with diameters between 5 and 70 nm and lengths between 100 and 250 nm[18]. CNCs are characterized by the smaller dimensions found in the chemically processed sample, while CNFs are characterized by the bigger dimensions obtained by the mechanical approach. Therefore, the selection of the extraction technique is essential in establishing the final shape and dimensions of nanocellulose, which in turn determines its appropriateness for particular uses, including papermaking, biomedical materials, and nanocomposites[19].

(FTIR) analysis

The FTIR examination of chemically treated nanocellulose shows a wider range of transmittance values, suggesting a more complicated interaction of functional groups as a result of the chemical

alterations the samples underwent (figure 1). Changes in hydrogen bonding and possibly the addition of additional functional groups are suggested by the existence of large and comparatively ill-defined peaks, especially in the 3600–3200 cm^{-1} range representing the O–H stretching vibrations typical of cellulose. These alterations may be the result of cellulose's chemical makeup changing, which could improve some characteristics like its reactivity or hydrophilicity. A possible disturbance of the crystalline structure of cellulose, leading to a less ordered structure, is further suggested by the peaks' broadness and lack of definition. These results are consistent with research by [20]. Research suggests that chemical treatments may cause cellulose chains to partially degrade and have an adverse effect on the degree of crystallinity and mechanical qualities.

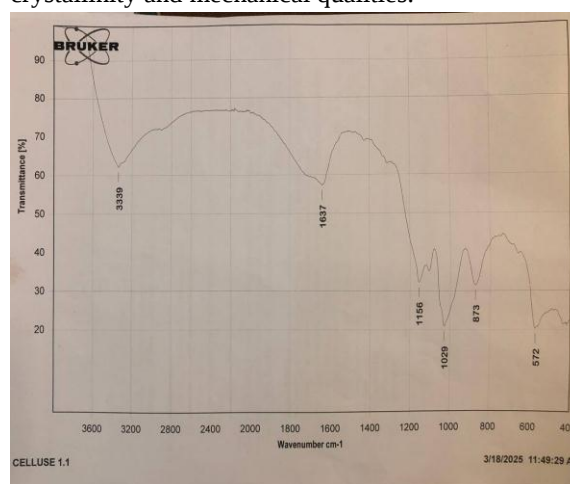


Fig 3.3: FTIR Analysis of Chemically Treated Nanocellulose.

The FTIR measurement of the mechanically ground samples (figure 2) shows sharper and more defined peaks, particularly in the 1000 cm^{-1} area, which indicates C–O stretching vibrations, a characteristic of the fundamental cellulose structure. These more pronounced peaks show how well the mechanical treatment maintains cellulose's basic chemical structure. Additionally, in contrast to the chemically treated samples, the sharpness of the peaks in this region indicates the maintenance of a higher degree of crystallinity. This result illustrates how mechanical treatment mostly concentrates on physical changes, including decreasing particle size

and boosting surface area, without significantly changing the chemical makeup. These results are consistent with research by [8] . Who observed that mechanical processes enhance cellulose's dispersion and compatibility in composite materials while generally preserving its original properties.

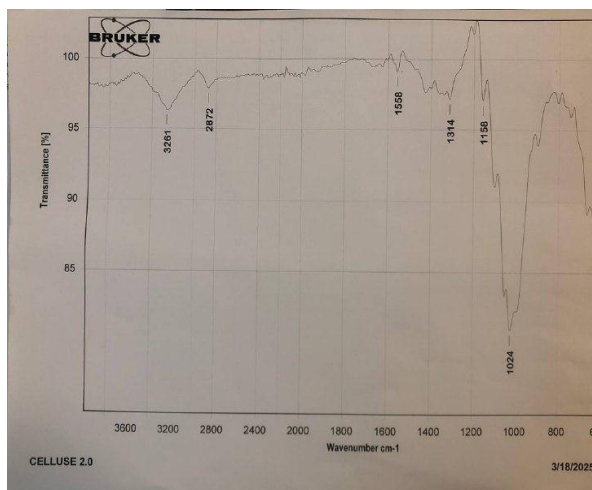


Fig 3.4: FTIR Analysis of Mechanically Treated Nanocellulose.

The differing impacts of mechanical and chemical treatments on the structure of nanocellulose are shown by the FTIR analysis. While mechanical treatment mainly maintains the chemical composition and degree of crystallinity while causing physical changes, chemical treatment results in changes to functional groups and possibly a breakdown of the crystalline structure. These findings lend credence to the notion that the method of treatment should be chosen based on the intended use, with mechanical treatment being the best option for preserving the natural properties of cellulose and improving its physical attributes and chemical treatment being the preferred option when altering chemical and surface properties are require.

Mechanical Versus Acidic Methods for Nanocellulose Extraction Comparison

The extraction of cellulose nanocrystals (CNCs) using mechanical (ultrasonic) and acid hydrolysis techniques was compared. It was observed that the particle size of acid hydrolysis spherical CNCs ranged from 160 to 320 nm.

Similarly, [20] produced cellulose nanocrystals with diameters of 3–10 nm and lengths of 100–300 nm by employing 60% sulphuric acid at 45°C to extract the nanocrystals from waste paper. These results demonstrate how the extraction procedure significantly affects the size and shape of the final cellulose nanocrystals, with mechanical processes producing larger and less homogeneous nanocellulose structures and acid hydrolysis typically producing smaller and more uniform particles.

Antifungal Activity of Nanocellulose

The results of the antifungal effect of natamycin and amphotericin are given in Table 1. The antifungal effects in the form of inhibition of the growth of three types of *Aspergillus flavus* against B carriers containing natamycin and amphotericin

Table 1: Inhibition percentage % of nanocellulose against fungi with different concentrations after 7 days of incubation at 25 C°.

Treatments	<i>Aspergillus niger</i>	<i>Penicillium sp.</i>
Control	0 c	0 c
0.5% NC	4 ±0.94 c	5±0.94 c
1% NC	5.5 ±0.47 c	5 ±0.4 c
1.5% NC	12±0.23 b	15±0.4 b
2 % NC	84±0.47 a	90±0.2 a

Different letters mean there are significant differences between the two Colom.

Lyophilized nanocellulose showed the strongest antifungal effects against both fungi studied. Increasing the concentration of natamycin showed statistically significant effects on the antifungal properties of all films ($P<0.05$). Meanwhile, the antifungal effect was higher (Figure 1). *Aspergillus flavus*. The results of the investigation of the antifungal effects of nanocellulose without natamycin and amphotericin B showed that the synthesized film had no antifungal effect against the fungi studied.

In the present study, the effectiveness of nanocellulose films containing two antifungal compounds against *Aspergillus flavus* was determined to be greater than that of *Penicillium citrinum*. Overall, the effects of natamycin were

greater than those of amphotericin B, regardless of the type of film and the type of fungus. Increasing the concentration of both antifungal compounds resulted in a statistically significant increase in the antifungal effect. The antifungal drugs natamycin and amphotericin B in this study are drugs that are generally used in most developing countries, singly or in combination, and in a topical form for the treatment of keratitis caused by

Discussion In the present study, while producing bacterial nanocellulose films, the binding and transport capacity of two widely used antifungal compounds against two important and prevalent fungi was investigated. The results of this study showed that natamycin has a higher potency than amphotericin B in inhibiting the growth of both fungi. Also, both natamycin and amphotericin B were chemically bound to nanocellulose based on Fourier transform infrared spectroscopy characteristics, and the release rate of the bound drug and the subsequent effect of the bound drug on the lyophilized form of nanocellulose were higher than its wet form and both were higher than on parchment paper. The researchers showed that the antimicrobial properties of lysozyme increased when added to the nanocellulose film. 6 Similar results were reported in the present study. The effects of antimicrobial films against microbial agents depend on the type of film, the type and amount of antimicrobial compound, and the type of microorganism.

Conclusion

Research into agricultural waste-derived nanocellulose shows promise in tackling the buildup of agricultural waste and the demand for sustainable products. Because of its exceptional qualities such as strength, biodegradability and adaptability, it may be used in a wide range of applications. It has a good success rate in keeping and maintaining food safety when used as an antifungal & in food packaging. According to research, nanocellulose is typically safe for use in packaging & is thought to be ecologically benign, which will help create a more sustainable future.

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Author's Contribution

The first author conducted this study, and the second author suggested and supervised the stages to achieve the study.

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