

TEMPO-Oxidized Cellulose Nanofibers/Poly(lactic acid)/TiO₂ as Antibacterial Bionanocomposite for Active Packaging

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THE DEVELOPMENT of bionanocomposite films with improved structural morphology of cellulose nanofiber reinforced poly(lactic acid) (PLA) was achieved. The new strategy was attained via grafting of TEMPO oxidized cellulose nanofibers (TOCNF) prepared from corn cobs using wheat gluten protein. The effect of different percent of TOCNF and grafted-CNF (G-CNF) on tensile strength and Young's modulus of the produced films were studied as well as the addition of TiO₂ nanoparticles. The surface morphology and thermal analysis of the films were revealed by scanning electron microscope and thermal gravimetric analysis. The incorporation of G-CNF enhanced the mechanical properties of the films due to the good dispersion and strong interactions with PLA.

Keywords: Biodegradable polyester, Cellulose nanofibers, Mechanical properties, SEM, TGA, Wheat gluten.

Introduction

Recently, considerable research has been performed to manufacture new environmentally plastic materials having high performance. For this purpose thermoplastic polymers were blended with natural fibers to minimize production costs while maintaining their properties [1, 2]. Poly(lactic acid) (PLA) is one of the most important biodegradable polymers which recently gained growing attention from many researchers [3–6]. PLA has a great potential to replace petroleum-based plastics because of its, renewable, high stiffness and strength [7]. Among its many potential uses, PLA already has found applications in textile and medical fields and also in the packaging industry. There are many applications of PLA in food packaging, water and milk bottles, degradable plastic bags as well as in automotive applications. In ideal conditions the products made from PLA are biodegradable and fully disappear in less than 30 days [8-10]. The renewability, low cost and low density of lignocellulosics materials are advantages that contributed to their use as reinforcements in green composites and replacing non-biodegradable reinforcing fillers [11]. The reinforcement of PLA using renewable and biodegradable materials has been studied with the goal of obtaining fully bio-

based composites [12-15]. Cellulose is the most abundant and renewable biomass resource on earth which used to produce new and promising reinforced nanomaterials called cellulose nanofiber (CNF). CNF has attracted attention as potential reinforcement materials in biodegradable packaging due to many of advantages as low cost, low density and their renewability [16]. Recently biodegradable composites of PLA with microcrystalline cellulose, wood fibers, wood flour, cellulose fibers, cellulose nano whiskers were studied [17-21]. Graupner *et al.* found that natural cellulose fibers have a positive influence on mechanical properties of PLA-based composites [20].

In the current study, fully bio-nanocomposite films were prepared from PLA and wheat gluten. The grafting of 2,2,6,6-tetramethyl-piperidine-1-oxyl (TEMPO) oxidized cellulose nanofibers and the effect of grafting of TEMPO oxidized cellulose nanofiber with wheat gluten on the properties of films produced were studied.

Experimental

Materials

Poly (lactic acid) pellets, commercial grade 4042D with 1.24 g/cm³ density, were purchased

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from Nature Works TM LLC (Blair, NE, USA). The polymer was dried for at least 3 h at 50 °C before being used in the blend preparation. Gluten from wheat (~80% protein) was purchased from Sigma Chemical Co. All other reagents and solvents were of analytical grade and were used without further purification.

Preparation of TEMPO-oxidized cellulose nanofiber (TOCNF)

TEMPO oxidized cellulose nanofiber was prepared from alpha cellulose isolated from corn cobs bleached pulp [22]. 10 g alpha cellulose was dispersed in distilled water (3000 ml) with TEMPO (0.16 g) and sodium bromide (1.6 g). Then 300 ml of sodium hypochlorite (Commercial grade 5% active ingredient as sodium hypochlorite) solution was then added with stirring and the pH was adjusted to 10 using NaOH (0.5 N). Finally the pH is adjusted to 7 by using HCl (0.5 N) and the product was centrifuged at 7000 rpm, and then further purified by repeated adding water, dispersion, and centrifugation. The oxidized pulp was disintegrated by a high-shear homogenizer using pulp suspensions of 2% to obtain cellulose nanofiber. The prepared TOCNF was characterized using transmission electron microscopy (JEM-2100 transmission electron microscope, JEOL, Japan) and regarding its carboxylic groups according to TAPPI Test Method T237cm-98 and was found to be 250 $\mu\text{mol/g}$.

Wheat gluten protein grafting TOCNF

Wheat gluten solution was prepared by stirring of 9.0 g wheat gluten and 32.5 ml absolute ethanol and completed to 100 ml of distilled water at pH 10 adjusted by ammonium hydroxide. Then 4.5 gm of the above mentioned TOCNF was immersed in wheat gluten solution with constant stirring for 4 h at 60°C. The grafted CNF (G-CNF) was filtered, washed with deionized water, and dried at room temperature.

Preparation and characterization of PLA/CNF nanocomposite

PLA and PLA bionanocomposite films were prepared using a solvent casting method. 2 g PLA was dissolved in 20 ml chloroform by stirring for 3 h at room temperature (25°C). The solution was then poured into Teflon molds, followed by drying at room temperature for 24 h. CNF was suspended in chloroform under stirring for 4 h using a magnetic stirrer, then homogenized at 4000 rpm for 15 min using an ultra turrax homogenizer followed by sonication for 10 min at room temperature. For the preparation of PLA

nanocomposite films with TOCNF and G-CNF, solutions containing 2.5, 5, 7.5 and 10% based on PLA were added and then stirred for 24 h with a magnetic stirrer. The final film was obtained by the same procedure explained earlier for neat PLA films. After drying at room temperature for 24 h, all PLA/ CNF films were further dried at 50°C in a vacuum dryer to remove the remaining solvent (chloroform). Tensile tests were carried out with a Lloyd instrument (Lloyd Instruments, West Sussex, United Kingdom) with a 100-N load cell. The measurements were performed at a crosshead speed of 2 mm/min at 25°C. PLA nanocomposites films surfaces were examined by FE-SEM (FEI Quanta 200 scanning electron microscope, FEI Company BV, Netherlands) with an acceleration voltage of 20 kV. Thermal analysis was carried out using PerkinElmer thermogravimetric analyzer. Heating rate set at 10°C/ min over temperature range between 50 and 600°C. Measurements were carried out in nitrogen atmosphere; the rate of flow of nitrogen was 50cm³/min. Antimicrobial activity of the tested samples was determined using a modified Kirby-Bauer disc diffusion method (DDM) [23]. Briefly, 100 μl of the test bacteria were grown in 10 ml of fresh media until they reached a bacteria count of approximately 10⁸cells/ml [24]. 100 μl of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Plates inoculated with Gram (+) bacteria as *Staphylococcus aureus*, *Bacillus subtilis*; Gram (-) bacteria as *Escherichia coli*, *Pseudomonas aeruginosa* were incubated at 35-37°C for 24-48 hours and, then the diameters of the inhibition zones were measured in millimeters.

Results and Discussion

Characterization of TEMPO-Oxidized Cellulose nanofiber

Figure 1 shows TEM image of TOCNF isolated from corn cobs, TEM observation revealed that the dispersions consisted of mostly individualized TOCNF of about 4–10 nm in width and a few microns in length. The length of the prepared TOCNF could not be determined due to the intrinsic alignment and formation of continuous network of the fibrils. These dimensions mean that the prepared TOCNF have high aspect ratio (length/width), which explains the high strength properties of CNF and also the main reason for improving tensile strength properties of PLA nanocomposites containing CNF. The prepared

CNF has negative charge due to the presence of carboxylate groups at its surface; the estimated total carboxylic groups' content was about 250 $\mu\text{mol/g}$.

Figure 2 shows the Fourier transform infrared spectroscopy (FTIR) spectra of TOCNF. The Spectrum shows one peak near 1745 cm^{-1} corresponding to the C=O band of the carboxylic acid of TOCNF, which confirms the oxidation of primary alcohols of native cellulose. Moreover, increasing the width and intensity of peak at 3400 cm^{-1} , reveal the hydrogen bond formation between functional groups in TEMPO oxidized cellulose and wheat gluten protein.

Two series of nanocomposite films were

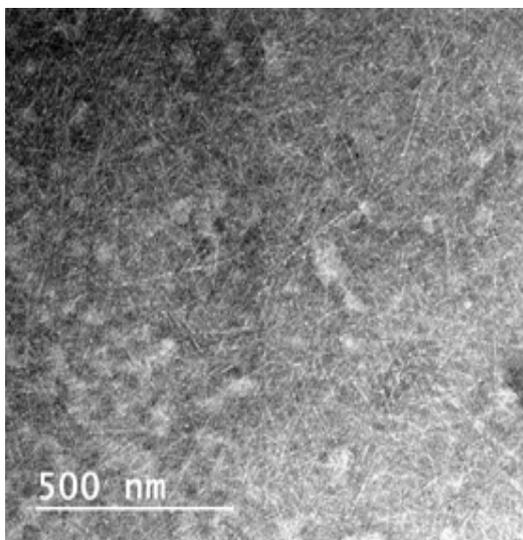


Fig.1. TEM of TOCNF.

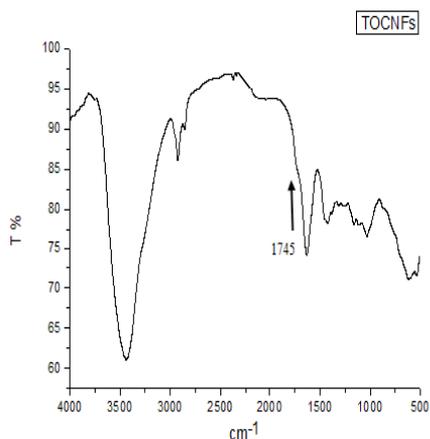


Fig. 2. FT-IR of TOCNF.

prepared as shown in Table 1 using casting technique. The first series was prepared from PLA filled with 2.5, 5.0, 7.5 and 10 wt% of TOCNF. The second series was prepared under the same conditions using G-CNF instead of TOCNF. Also, PLA films filled with 7.5 wt% TOCNF or G-CNF in presence of 0.6% TiO₂ (PLA/ TOCNF /TiO₂ and PLA/G-CNF/TiO₂) were prepared.

Scanning electron microscopy of nanocomposite films (SEM)

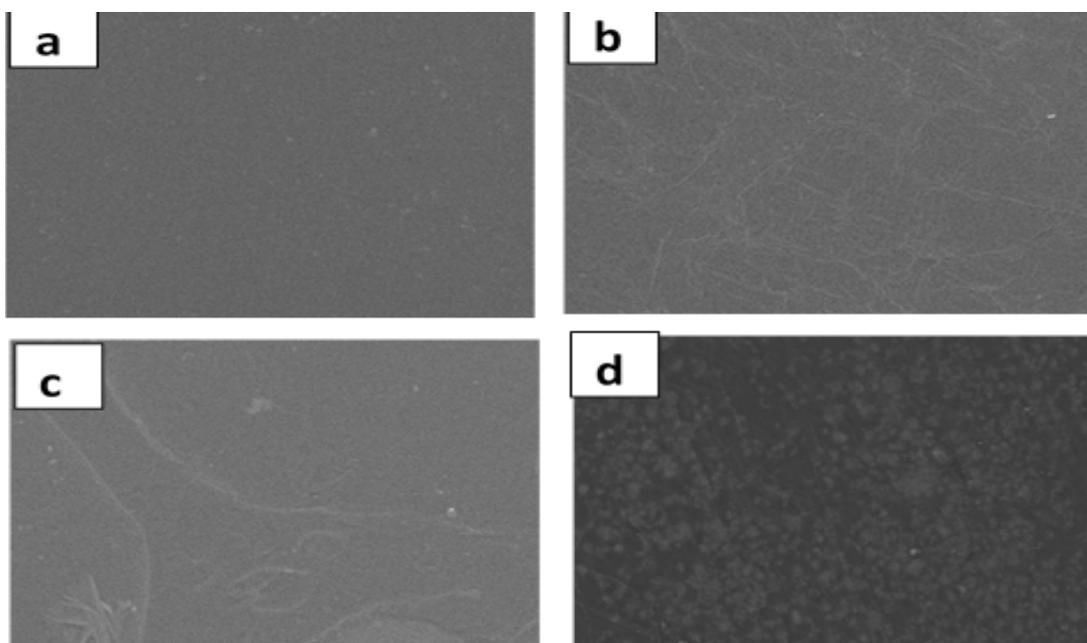
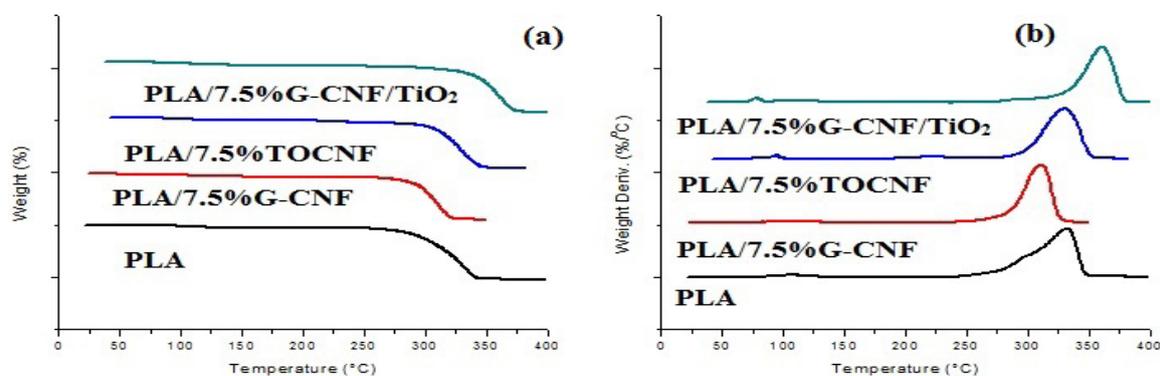
The morphology of the surfaces of the prepared nanocomposite films was studied by means of scanning electron microscope (SEM). Figure 3 shows SEM micrographs of PLA, PLA/7.5% TOCNF, PLA/7.5% G-CNF and PLA/7.5% G-CNF/TiO₂ films. It can be evidenced from SEM micrographs represented in Fig. 3 that a flatter surface of the bionanocomposite film as compared to the neat PLA film and a good dispersion of CNF in the bionanocomposite films was achieved.

Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) was performed on PLA, PLA/7.5% TOCNF, PLA/7.5% G-CNF and PLA/7.5% G-CNF/TiO₂ nanocomposite films. The degradation of these films was compared to the degradation of neat PLA film. Figure 4a shows TGA thermograms, weight loss curves. The first stage of the TGA thermograms is allocated to evaporation of water in the range up to 100°C (2-3%), and then a steady loss (about 15%) was observed up to 300°C . In the second stage, the mass loss is a consequence of thermal decomposition of the film material from 300°C to 390°C . All the film materials started degradation at temperature above 300°C . Also, it can be seen from the thermograms that PLA nanocomposite films containing 7.5 wt% of TOCNF, 7.5 wt% of G-CNF and/or TiO₂ exhibited onset degradation temperature nearly as the same as that of neat PLA film. Figure 4b shows differential thermogravimetric analysis (DTA) curves of the prepared nanocomposite films in comparison with that of neat PLA film. It is clear from figure 4b that temperatures of derivative of weight loss (TD) of nanocomposite film filled with CNF, G-CNF and/or TiO₂ are lower than that of neat PLA. Nanocomposite films filled with 7.5 wt% TOCNF or G-CNF exhibited TD lower by about 20°C than PLA film. Finally, it can be concluded from the figures that there is no significant change in the thermal

TABLE 1. Composition of the prepared films.

Films	PLA(g)	TOCNF(g)	G-CNF(g)	0.6 wt%TiO ₂
Neat PLA	1.5	---	---	---
PLA/TOCNF 2.5 wt%	1.46	0.0375	---	---
PLA/TOCNF 5 wt%	1.425	0.750	---	---
PLA/TOCNF 7.5 wt%	1.3875	0.1125	---	---
PLA/TOCNF 10 wt%	1.35	0.15	---	---
PLA/G-CNF 2.5 wt%	1.4625	---	0.0375	---
PLA/G-CNF 5 wt%	1.425	---	0.750	---
PLA/G-CNF 7.5 wt%	1.3875	---	0.1125	---
PLA/G-CNF 10 wt%	1.35	---	0.15	---
PLA/CNF TiO ₂	1.3785	0.1125	---	0.009
PLA/G-CNF / TiO ₂	1.3785	---	0.1125	0.009

**Fig. 3.** SEM micrographs of nanocomposite films prepared from a) PLA, b) PLA/TOCNF, c) PLA/G-CNF and d) PLA/G-CNF/TiO₂.**Fig. 4.** Thermogravimetric analysis (a-TGA) and (b-DTA) of PLA Film, PLA/7.5 wt% G-CNF film, PLA/7.5 wt% TOCNF film and PLA/G-CNF/TiO₂ film

stability the PLA films on addition of CNF or G-CNF.

Mechanical properties of PLA films loaded with CNF

Table 2 and 3 illustrate the tensile strength and Young's modulus of PLA/ TOCNF film and PLA/ G-CNF respectively. It is clear from the tables that the nanocomposites show improvement in tensile strength and Young's modulus of PLA/ TOCNF film and PLA/G-CNF films compared to pure PLA film. The tensile strength of pure PLA film and PLA films containing 2.5 and 10 wt % TOCNF were measured as 15, 27.43 and 38.65 MPa respectively as well as in the case of addition of 2.5 and 10 wt % G-CNF were measured as 30.54 and 45.5 MPa respectively. Thus TOCNF increased the tensile strength of PLA by 83-158%, while G-CNF increased it by 104-203% with the addition of 2.5-10.0 wt%. The Young's modulus is measured as 1508 MPa for

pure PLA film, while it was 1755 and 3500 MPa for PLA films with 2.5 and 10% TOCNF addition, respectively. In the case of addition of 2.5 and 10 wt% G-CNFs Young's modulus were measured as 2140 and 3700 MPa respectively. Thus CNF and G-CNF increased the Young's modulus by 40-132 and 42-145% with the addition of 2.5-10 wt% respectively. The positive impact of TOCNF in the nanocomposite strength indicated that CNF has a good mechanical property and there is an interaction between the PLA and the TOCNF. The modification of cellulose nanofiber using wheat gluten protein increases the compatibility between TOCNF and PLA. Based on this results, PLA film loaded with 7.5% G-CNF was chosen for the addition of 0.6% of TiO₂ nanoparticles, where the tensile strength for this nanocomposite is 45.32 MPa while that for nanocomposite loaded with 10% was 45.5 MPa. This slight enhancement is not imperceptible when 33% of modified CNF are saved [25]. It clear from the Tables 2 and 3 that

TABLE 2. Effect of addition of TOCNF on mechanical properties of PLA/ TOCNF nanocomposite.

Samples	Tensile Strength (MPa)	Young's Modulus (MPa)	Elongation at max. load (mm)
PLA	15.00 ± 2.2	1508 ± 120	0.86 ± 0.21
PLA/2.5% TOCNF	27.43 ± 3.1	1755 ± 145	1.70 ± 0.34
PLA/5% TOCNF	33.35 ± 1.5	2050 ± 153	1.64 ± 0.42
PLA/7.5% TOCNF	37.54 ± 3.7	3200 ± 126	1.62 ± 0.63
PLA/10% TOCNF	38.65 ± 3.2	3500 ± 164	1.50 ± 0.24
PLA/7.5%TOCNF /0.6% TiO ₂	36.78 ± 2.5	3100 ± 187	1.35 ± 0.53

TABLE 3. Effect of modification of TOCNF on mechanical properties of PLA/ G-CNF nanocomposite.

Samples	Tensile Strength (MPa)	Young's Modulus (MPa)	Elongation at max. load (mm)
PLA	15.00 ± 2.12	1508 ± 89	0.86 ± 0.20
PLA/2.5 % G-CNF	30.54 ± 3.6	2140 ± 80	2.10 ± 0.78
PLA/5 % G-CNF	40.23 ± 0.9	3570 ± 105	2.21 ± 0.56
PLA/7.5% G-CNF	45.32 ± 0.87	3500 ± 145	1.89 ± 0.87
PLA/10% G-CNF	45.50 ± 4.1	3700 ± 125	1.68 ± 0.12
PLA/7.5% G-CNF /0.6 % TiO ₂	42.91 ± 2.7	3250 ± 134	1.51 ± 0.41

there is slight decrease in the tensile strength and Young's modulus by the addition of 0.6% TiO₂.

Antibacterial activity of the PLA nanocomposite films

It is expected that PLA film is an important bio based packaging material due to its good mechanical properties but the main drawback is the good media for microbial growth. In this work, G-CNF and 0.6 % TiO₂ reinforced of PLA

films were prepared.

The antibacterial activity of PLA/TOCNF, PLA/G-CNFs as well as PLA/G-CNF/TiO₂ was carried out according to the standard method by Bauer et al. [23]. The effectiveness of TiO₂ was evaluated for its antibacterial properties in PLA nanocomposite films, using standard protocol of disc diffusion method (DDM). The experimental

results of the antimicrobial activity are presented in Table 4. The disc diffusion method used to determine the mean diameter of inhibition for the evaluation of antibacterial activity of the nanocomposite film can be seen in Fig. 5. Generally as expected the films prepared from PLA, and PLA filled with TOCNF and G-CNF have no antibacterial activity, while film prepared from PLA/G-CNF filled with 0.6 wt% TiO₂ earned antibacterial. The addition of TiO₂ to the tested nanocomposite films severely increased the antibacterial effect against Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) [25].

Conclusions

The mechanical properties of PLA/CNF nanocomposite films were studied. Tensile strength and Young's modulus measurements revealed that TOCNF can act as strengthening agent for PLA. The addition of TOCNF, modified with wheat gluten, increased the tensile strength and Young's modulus of PLA by 203% and 145% respectively at G-CNF content of 7.5 wt%. Also, experimental results indicated that PLA/G-CNF/TiO₂ nanocomposites films have a considerable antibacterial activity against the tested Gram-positive and Gram-negative bacteria. These results demonstrated that CNF could extend the application of PLA as a packaging material.

Acknowledgement

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TABLE 4. The diameters of the inhibition zones measured in millimeters.

Sample	Inhibition zone diameter (mm / 1 cm Sample)			
	G ⁺		G ⁻	
	<i>Bacillus</i>	<i>Pseudomonas</i>	<i>Escherichia</i>	<i>Staphylococcus</i>
	<i>Subtilis</i>	<i>aeruginosa</i>	<i>coli</i>	<i>aureus</i>
PLA	0.0	0.0	0.0	0.0
PLA / TOCNF	0.0	0.0	0.0	0.0
PLA/ G-CNF	0.0	0.0	0.0	0.0
PLA /GCNF/ 0.6%TiO ₂	12.0	14.0	12.0	13.0

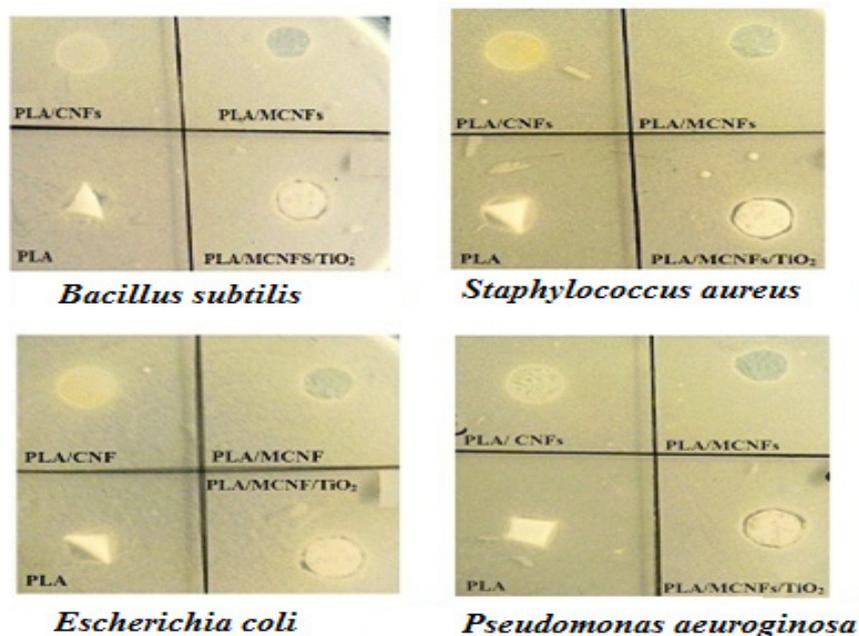


Fig.5. Antibacterial activity of the PLA nanocomposite films.

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ألياف السليلوز النانومترية المؤكسدة بطريقة ال TEMPO و حمض البولي لاكتيك و ثاني أكسيد التيتانيوم كمتراكبات نانومترية حيوية مضادة للبكتريا لاستخدامها لتعبئة المواد الغذائية

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تناول البحث استراتيجيات جديدة لتحضير أفلام ذات خصائص مورفولوجية محسنة من متراكبات نانومترية حيوية تم تحضيرها من حمض البولي لاكتيك المعزز بألياف السليلوز النانومترية. في البداية تم توصيف الألياف السليلوز النانومترية المؤكسدة بواسطة الميكروسكوب الإلكتروني النافذ (TEM) وكذلك باستخدام جهاز الأشعة تحت الحمراء (FTIR).

تم تحقيق ذلك من خلال تطعيم ألياف السليلوز النانوية المؤكسدة بطريقة ال TEMPO باستخدام بروتين القمح. تم دراسة تأثير نسب مختلفة من ألياف السليلوز النانومترية المؤكسدة بطريقة ال TEMPO وكذلك المطعمة بالبروتين على قوة الشد ومعامل المرونة للأفلام المنتجة، فضلا عن دراسة تأثير إضافة جزيئات ثاني أكسيد التيتانيوم على مقاومة الأفلام المحضرة لكل من البكتريا سالبة وموجبة الجرام. وتناول البحث دراسة شكل سطح الأفلام بواسطة المجهر الإلكتروني الماسح (SEM) وكذلك دراسة التحليل الحراري للأفلام. وتوصلت النتائج الي ان تطعيم الياف السليلوز النانومترية و اضافتها لحمض البولي لاكتيك يعزز الخصائص الميكانيكية للأفلام. كما ان اضافة ثاني اكسيد التيتانيوم يكسب الأفلام الناتجة نشاطا حيويا مضادا للبكتريا مما يعطي فرصة لهذا النوع من الأفلام للاستخدام في مجالات تغليف المواد الغذائية.