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Nanotechnology application of natural dyes on wool fibers pretreated with nano chitosan /neem composite to improve their dyeing properties and antimicrobial activity.

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Abstract Nano-particles possess unique properties, such as the large ratio of surface to volume, surface-active multi centers, and high surface activity. Environmentally friendly treatments for wool fibers were enhanced dyeability and antimicrobial activity. Nano chitosan /neem used for pretreatments of wool fibers dyed with natural dye extracted from green algae. The color strength (K/S) and the fastness properties of wool fibers were evaluated.The morphologies structure of the untreated and pretreated wool fibers were shown by scanning electron microscopy. The antimicrobial activity with some species of bacteria and fungi, *Bacillus cereus, Bacillus subtilus*, *Escherichia coli, Pesudomons aerogenosa*, and fungi *Candida albican. Candida tropical*s, and *Aspergillus niger* were presented.The results showed that treatment caused a smoother surface on wool fibers, improved the fastness properties. Scanning electron microscopy (SEM) indicated that the untreated wool fibers showed a rough surface. The treated wool fibers were swelling as compared to the untreated fibers. The results of the antimicrobial activity indicated that the fibers pretreated exhibited a higher reduction percent than the untreated one.

Keywords: Nanotechnology, Antimicrobial activity, Natural dyes, Wool fibers

Introduction

Textiles treated with synthetic antimicrobial compounds Synthetic antimicrobial compounds had a long-lasting impact on fibers and were incredibly effective against bacteria, but they also contaminated water sources and the environment. Thus, there is a great demand for environmentally friendly antimicrobial textiles that not only help to reduce the health concerns associated with microbiological textiles that improve the environment while also assisting in lowering the health hazards associated with microbial growth on textile material (Ali and El-Mohamedy, 2011). According to published research, textile fibers can be treated with natural

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antibacterial finishes using substances like chitosan and natural dyes (Joshi *et al*., 2009; Jothi, 2009; El-Khatib *et al*., 2016). Two other natural herbal medicines that can be used for this are extracts from tea tree and aloe vera plants (Khatib and colleagues, 2016). Many medicinal plants include strong antibacterial properties. Strong antibacterial compounds found in nature have been the subject of very little and poorly documented research. Herba Herbal medications can be utilized as an alternative to current synthetic pharmaceuticals due to their lower cost and comparable effects. textiles that improve the effectiveness of environmentally acceptable alternatives to synthetic antimicrobial compounds in textile applications, while also assisting in reducing health hazards associated with bacteria development on textile material. Novel research avenues have been made possible by recent developments in plant-based bioactive chemicals. Most studies in this area concentrate on the particulars of employing certain natural substances (Malpani, 2013).

Neem extracts have been widely used in the formulation of herbal pesticides because of their ability to inhibit the growth of both Gram-positive and Gram-negative bacteria. Algae have a wide variety of photosynthetic pigments.

Phycobilins, carotenoids, and chlorophylls are the three main types of pigments that are produced during photosynthesis. According to Thilagavathi *et al.* (2007), phycocyanin and phycoerythrin belong to the major class of phycobilins, whereas fucoxanthin and peridinin are members of the carotenoid group of photosynthetic pigment.

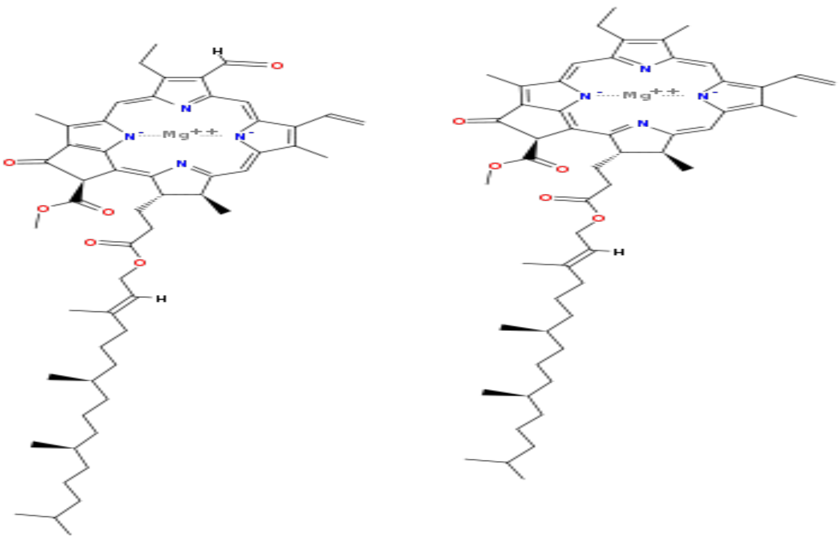
Chlorophyll was created by combining the two molecules, chlorophyll-a and chlorophyll-b. Chlorophyll-a, molecular weight: 893.49. In chlorophyll-b, the methyl group is replaced by an aldehyde (C55H70MgN4O6, mol. wt.: 906.51). Chlorophyta green algae contain chlorophyll (Figure1). Studies had shown a great deal of interest in the usage of chitosan in textiles because of its many unique properties. Because of its large molecular size and high viscosity, the chitosan polymer is unable to penetrate fibers very deeply. Consequently, only surface deposition takes place. When chitosan's particle size is reduced to the nano range, it can penetrate fiber more readily while still preserving the inherent properties of wool fibers (Krifa and Prichard, 2020; Asif and Hasan, 2018; Ramachandran *et al*., 2004). The goal of the study was to enhance dyeing and antimicrobial activity by pretreatment with the composite under investigation.

Materials and methods

Materials

Wool fibers Mill scoured 100% wool fibers used for this study were supplied from Misr Co. (El Mehalla El-Kobra, Egypt for spinning and weaving). The fibers were washed in a bath containing 2g/l non-ionic detergent (Nonidet) at 40°C to remove any impurities and then thoroughly washed with water and then dried by air at room temperature. Wool fibers10/2 fibers supplied by El Mahalla company-Egypt. Neem oil extract was purchased from EL Gmhoria company, Egypt (Asif and Hasan, 2018). Chitosan high molecular weight (210,000), Poly (D-glucosamine), was purchased from ROTH, Germany.

Dyestuff: Algal Pigments Scientific classification is belonging to Kingdom Protista, Division Chlorophyta, Phylum Charophyta, Class Zygnematophyceae, Family Zygnemataceae, Order Zygnematales Genus: Spirogyra.



Structure of Chlorophyll (a) Structure of Chlorophyll (b)

Figure 1. Chemical Structure of Chlorophyll (a) and (b)

Apparatus

The microwave equipment used in this experiment was the Samsung M 245, with an output of 1,550 watts operating at 2450 MHz.

Preparation of neem chitosan nano composite

Neem/Chitosan nanocomposites were prepared using the Multiple Emulsion/ Solvent Evaporation procedure. 3% Chitosan solution was added with 5% (w/v) Tween 80 and placed in a rotary shaker for 5 minutes. Neem extract was added to the emulsion and kept in the rotary shaker for 5 minutes.

Simultaneously, 5% Span 80 was prepared with palm oil and stirred for 10 minutes. Both the solutions were mixed in 9:1 ratio and stirred well for 5 minutes. To this 0.01g of TPP (Tri polyphosphate) was added and kept in the rotary shaker for 5 minutes. The entire suspension was then incubated for ½ hour in a water bath at 50ºC and then cooled. Nanocomposites were segregated from the palm oil by repeated washing with petroleum ether. The pellets were dissolved in Phosphate buffer (Ramachandran *et al.,* 2004).

Pretreatment of wool fibers by neem/ chitosan nanocomposite

Wool fibers were treated by pad-dry-cure techniques. The wool fibers treated by neem/ chitosan at different concentrations (2-10%), then padded to 100% wet pick up, dried at 80 ºC for5 min. and cured at 120 ºC for 3 min.

Dyeing procedure

Dyeing wool fibers using the microwave method was done. In a dye bath containing 2 g/l of colorant pigment extracted from green algae (Spirogyra) with a liquor ratio 1:50, the wool fibers were dyed by microwave heating at pH (5) time for 5 minutes. The dyed samples were rinsed by warm water and then cold water, washed in a bath containing 5g/l non-ionic detergent at 50ºC for 30 minutes, then rinsed and dried in air at room temperature.

Dyeing wool fibers using conventional heating

In a dye bath containing different amounts of dye with liquor ratio 30:1, wool fibers were dyed using conventional heating at different pH values (3-9) for different durations (30-60 min.) and at different temperatures(50-90ºC) (Rajendran *et al*., 2012). The dyed samples were rinsed with cold water, washed in a bath of liquor ratio 60:1 using 3 g/Lnon-ionic detergent (Hostapal CV, Clariant) at 50ºCfor 30 min., then rinsed and finally dried at ambient temperature.

Measurements of color strength (K/S value)

An Ultra Scan PRO spectrophotometer was used to measure the reflectance of the samples, and hence, the K/S was measured spectrophotometrically at wavelengths: λ_{max} 385nm). The K/S of untreated and pretreated wool fibers with neem/chitosan dyed with chlorophill natural dye was evaluated. CIELAB coordinates (L* a* b*) of undyed and dyed wool fibers were determined using an Ultra Scan PRO spectrophotometer (Hunter Lab) with a D65 illuminant and 108 standard observers.

Fastness properties

According to ISO standard methods, the specific tests were ISO 105X12 (1987), ISO 106-C06 (1989), ISO 105-E04 (1989), and ISO 105-B02 (1989), corresponding to color fastness to rubbing, washing, perspiration and light, respectively. The color changes of the samples were assessed against an accurate Grayscale.

Scanning electron microscopy (SEM)

The surface morphology of untreated and treated wood fibers were investigated by using scanning electron microscopy (SEM), with a JSMT-20, JEOL-Japan (Kut *et al*., 2005). Before the examination, wool fibers surface was prepared on an appropriate disk and randomly coated with a spray of gold. SEM was carried out at the National Research Centre (Egypt).

Antimicrobial activity

The treated wool and nontreated were tested for their antimicrobial activity. They were tested against bacteria*, Bacillus cereus, B. subtilus*, *E. coli, Pesudomons aerogenosa*, and fungi *Candida albican. Candida tropical*s, and *Aspergillus* sp. The tested samples were added to nutient agar media and incubated at different times of 24,48, and 72 h. for growth. Inhibition zone, the diameter was measured and compared to control wool sample.

The serial dilution blanks were prepared in bottles containing 99 ml distilled water and marked sequentially starting from 10^{-1} to 10^{-5} dilution and autoclave sterilized. 1.0 gm of each fabric sample was added in 99 ml solution i.e., 10^{-1} dilution. 1 ml from this was then transferred to 9 ml of the 10^{-2}labeled test tube i.e., 10^{-2} dilution, using a fresh sterile pipette, and this was repeated for each succeeding step till 10^{-5}. Nutrient peptone Agar media was used for counting of bacterial strains, and for the counting of fungal strains, potato dextrose agar (PDA) media was used. From 10^{-3}, 10^{-4}, and 10^{-5} dilution tubes, 0.1 ml of dilution fluid was then spread on sterilized Petri plates in triplicates using the standard spread plate technique, for both bacterial and fungal strain isolation[17.]The LB agar plates were then incubated at 37 °C for 24 h, and the PDA plates were incubated at 27 °C for 72 h. After the successful growth of microorganisms, characteristics of each distinct colony, e.g., shapes, color, transparency, etc. were determined. A gram stain was performed to observe the cellular morphology and gram reaction of the bacteria. The number of bacterial and fungal colonies in the fiber samples was counted, and the density was expressed as Colony Forming Units (CFU)

Results

The pretreatment with 6g/L concentration of neem/ chitosan composite produced the best value of color strength (K/S) for wool fibers microwave-dyed with chlorophyll dye (Figure1). Ionic conduction, a form of resistance heating, is the process by which microwave heating has an effect. The collision between the dye molecules and the fiber molecules happened based on how quickly the ions moved through the dye solution. Microwave dyeing considered the thermal and dielectric characteristics of materials. The term "dielectric property" refers to the inherent electrical characteristics that impacted the dyeing process through the dye's dipolar rotation and the microwave field acting on the dipoles.

Effect of conc. of neem chitosan composite on wool fibers dyed with chlorophyll

Conventional and microwave procedures were shown in Figures 1 and 2. However, the microwave approach was higher with the K/S value at 6g/L when compared to the conventional method.

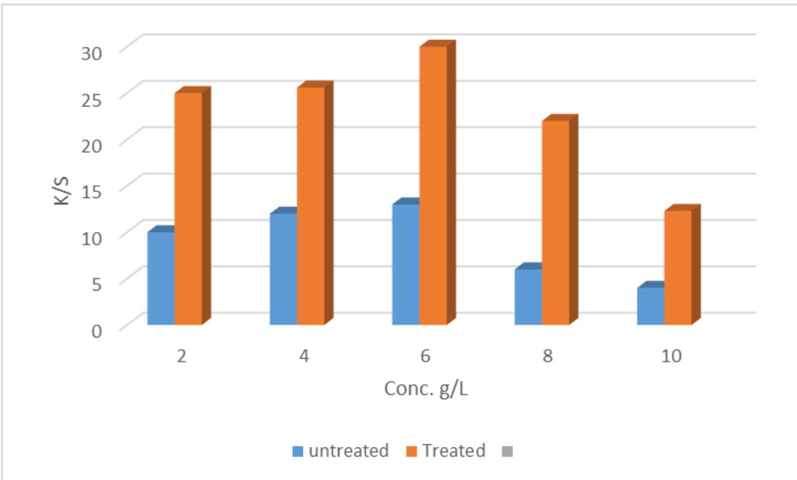


Figure 1. Effect of neem chitosan composite concentrations on wool fibers dyed with chlorophyll by microwave

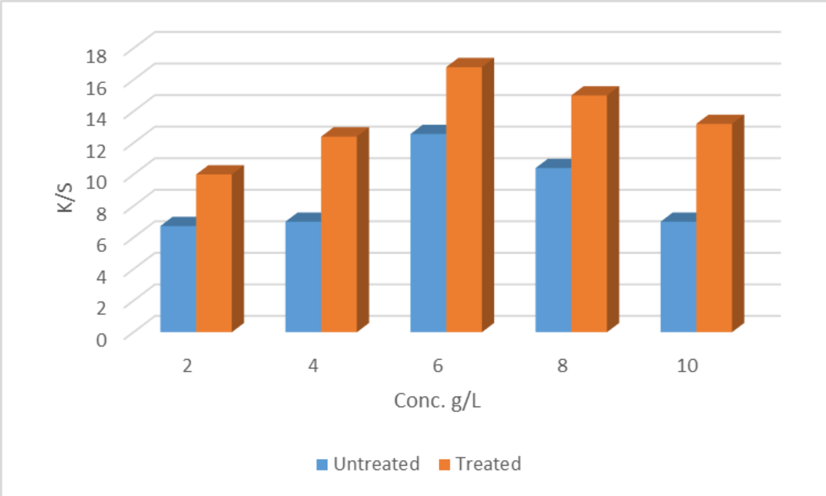


Figure 2. Effect of neem chitosan composite concentrations on wool fibers dyed with chlorophyll by conventional

Effect of time on the color strength for treated and untreated dyed fibers by microwave and conventional methods

The effect of time of dyeing wool fibers pretreated with the nano composite and dyed with chlorophyll using microwave method (Figure 3). The effect of time of dyeing wool fibers were pretreated with the nano composite and dyed with chlorophyll using conventional method (Figure 4). The results showed that the highest values of the color strength at 5 min. with respect to microwave and at 50 min, by conventional method.

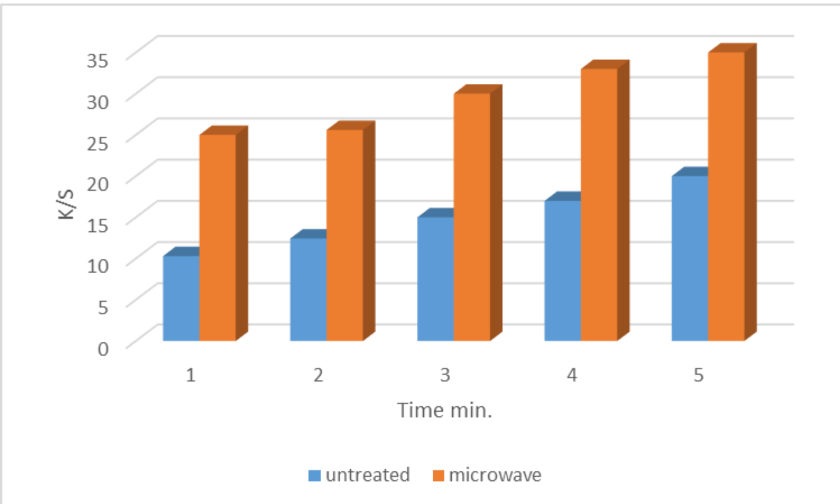


Figure 3. Effect of time for dyeing on wool fibers treated with neem chitosan composite and dyed with chlorophyll by microwave

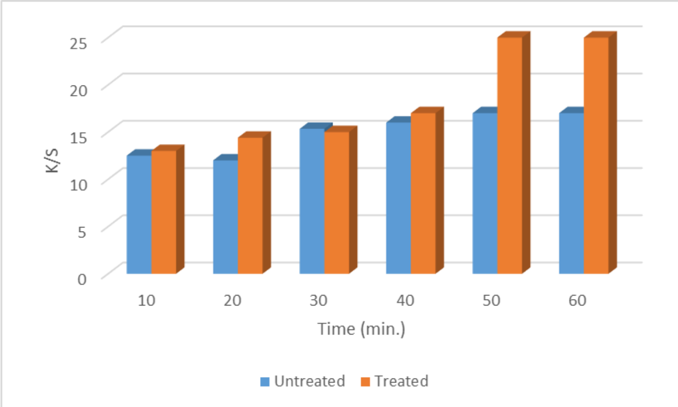


Figure 4. Effect of time for dyeing on wool fibers treated with neem chitosan composite and dyed with chlorophyll by conventional method

Effect of pH on the color strength for treated and untreated dyed fibers by microwave and conventional methods

The effect of pH of the dyeing bath of wool fibers was pretreated with the nano composite and dyed with chlorophyll using microwave and conventional methods (Figure 5). The results showed that the highest values of the color strength at pH 5 with respect to microwave and conventional method.

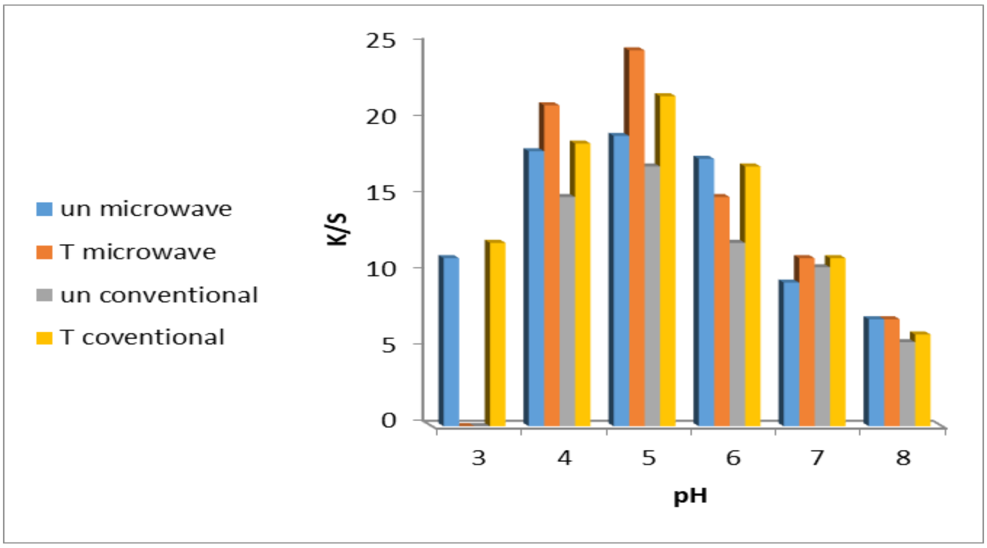


Figure 5. Effect of pH for dyeing bath on wool fibers treated with neem chitosan composite and dyed with chlorophyll by microwave and conventional methods (un: untreated, T: treated)

The outcomes demonstrated that K/S and color data obtained using the microwave approach were good by microwave (Table 1).

Table 1. Effect of neem chitosan concentrations on K/S and color data for wool fibers dyed with chlorophyll dye by microwave method

Conc. g/L.	K/S	L*	a*	b*	C	ΔH	ΔE
0%	20.00	39.44	65.93	27.89	63.39	26.10	74.66
2%	25.35	39.43	57.48	28.83	64.30	26.64	75.43
4%	31.83	37.77	56.46	28.02	63.03	26.39	65.90
6%	29.54	38.15	65.80	27.94	63.30	26.19	73.91
8%	21.84	37.18	50.52	20.19	54.41	21.78	73.48

Fastness properties and the color yield of wool fibers

Fastness properties and the color yield of the dyes under investigation on wool fibers were evaluated. It indicated that color fastness to rubbing, washing, and perspiration of all dyes were excellent which approximately the same in the microwave and conventional methods because the dye was fixed due to the treatment with neem / chitosan composite (Table 2). Also, the light fastness for all dyed fibers was found to be approximately the same for the two methods, but all the fastness for treated samples were higher than the untreated.

Table 2. Fastness properties of dyed wool pretreated with neem chitosan and dyed with Chlorophyll

Dyed samples	Fastness to rubbing		Wash fastness			Fastness to Perspiration						Light
						Alkaline			Acidic			
	Dry	Wet	Alt	SC	SW	Alt	SC	SW	Alt	SC	SW	
Treated	5	5	4-5	4-5	4-5	5	4-5	4-5	5	5	5	7
Untreated	3-4	3-4	4	3	3	4	3-4	3-4	4	4	4	6

Alt = change in color, SC = staining on cotton, SW = staining on wool

Antimicrobial activities of wool fibers

A variety of testing techniques have been created to evaluate the effectiveness of antimicrobial textiles. The tests used to assess the antibacterial capabilities usually fall into two categories: the agar diffusion test (qualitative approach such as the halo method) and the dynamic shake test (quantitative method such as the serial dilution and plate count method).

Two common techniques were used to assess the antibacterial properties of fibers pre-treated with neem-chitosan before being coloured with natural chlorophyll. 1. The plate count and serial dilution method 2. The zone of inhibition, or Halo technique as indicated in Table 3.

The antimicrobial efficacy of wool fibres dyed with chlorophyll and pre-treated with neem-chitosan was expressed as a percentage decrease in the total count (density) of tested bacteria and fungi in the treated fibres.

The antimicrobial activity demonstrated by growth reduction percent method of the microorganisms under examination. It clarified how the hydroxyl groups in neem chitosan composite structure stack at the cell surface and interact with DNA to prevent the formation of m-RNA, interfering with bacterial metabolism. As the neem/chitosan concentration increased, more of the substance tended to deposit on the fibre's surface, making hydroxyl groups more readily accessible to microbes. Microbe proliferation was greatly reduced in wool fibres treated with neem/chitosan and dyed with a natural chlorophyll dye.

Table 3. Antimicrobial activities of wool fibres treated with neem chitosan and dyed with natural chlorophyll dye

Samples	**Total fungi count cell x 10^4**	**Reduction %**	**Total bacteria count cell x 10^6**	**Reduction %**
Untreated	7.4	7.5	5.4	16.5
Treated with 20%	3.5	53.5	3.6	38
Treated with 30%	1.3	82.5	2.7	67.
Treated with 40%	0.4	95.0	13.4	84
Treated with 50%	0.0	100	0.0	100
Wool fibre undyed (control)	8.0	0.0	6.2	0.0

Table 4. Antimicrobial activity against different gram negative and gram positive, yeast and fungi

Microorganism	**Control Inhibition Zone (cm)**	**Treated with dye only Inhibition (Zone cm)**	**treated with chitosan Inhibition (Zone cm)**	**Nanoform nonochitosanl neem composite Inhibition (Zone cm)**
Bacillus subtilus	1.4	21	2.5	3.1
Bacillus cereus	1,1	2.2	2.4	3.2
Escherichia coli	1,3	2.6	2.6	4.3
Pesudomnas aeroginosa	1,6	2.4	2.7	3.3
Candida albicans	1.7	1,8	2.1	2.8
Candida tropicals	1.5	1.7	2.3	2.9
Aspergillus niger	1.3	1.5	1.7	1.7

The results showed that on the priority of using nanoform composite, it gave the maximum inhibition (4.3 and 3.3cm) against gram negative bacteria (*E. coli* and *Pseudomonas aeruginosa)* as compared to the control. Furthermore, reduced activity was noticed against the tested yeast (Table 4).

Surface morphology

The morphologies of the untreated and treated wool fibers were shown by scanning electron microscopy (SEM). Effect of treatment with 30% conc. of neem /chitosan composite using a scanning electron microscope (SEM) for wool fibers is presented. SEM images of untreated and treated wool fibers are shown in Figures 6 and 7. The untreated samples had a rougher surface indicated that the treated wool fibers swelling, the diameter of the fibers increased and smooth and even surfaces. The surface morphology is changed due to the effect of active ingredients of treatment with neem/chitosan.

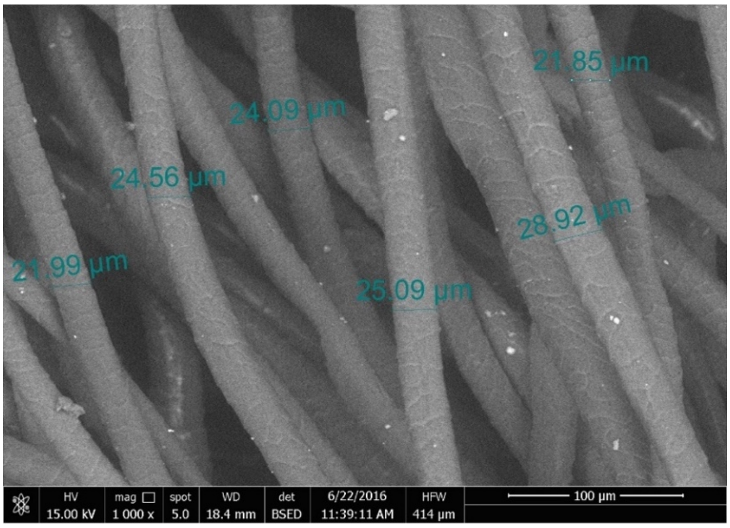


Figure 6. SEM for untreated wool fibers

The uniform coating of neem chitosan nanocomposites on the fibers with a particle size ranging 30 nm is shown in Figure 6. It accounted for the improvement of antibacterial activity of these nanocomposites when compared to other antimicrobial finished fibers, which resulted of the AATCC Standard procedures. The surface properties of the nanoparticle that is allowed for the sustained and slow released from the washing fibers. The increasing of concentration of neem /chitosan composite showed more tendencies to deposit on the surface of the fibers resulting in hydroxyl groups which had more easily accessible to microorganisms. Wool fibers treated with neem /chitosan composite and dyed with the chlorophyll natural dye displayed high growth reduction of microbes.

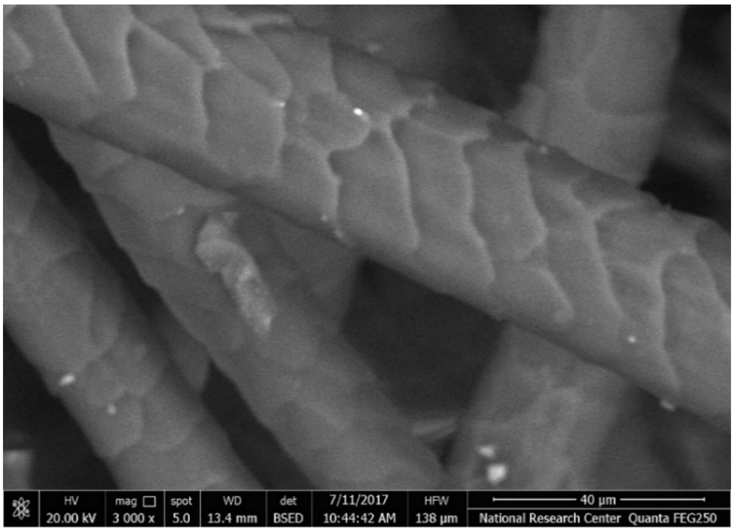


Figure 7. SEM for treated wool fibers

Discussion

An impact on the dipoles' microwave field was shown in the high-frequency microwave field, there are two polar components in the dye aqueous solution. It affects the dye molecules' and water molecules' vibration energy (Imam *et al*., 2012). All dyed fibers were found to be roughly the same light fastness for both procedures; however, all treated samples had better light fastness than untreated samples. The treated and untreated wool fibers SEM pictures.

The wool fibers were treated, causing swelling, an increase in fiber diameter, and smoother, more uniform surfaces than the untreated samples. The active components of the neem/chitosan treatment caused the alterations in the surface morphology. On the studied fungus, the lowered inhibition was observed. When dyeing in the microwave, the dielectric and the thermal characteristics of materials affected on the dyeing process.

The investigated microorganisms' antimicrobial activity varied, which led to variations in their cell wall architectures. The studied fungus with phospholipid cell membranes, and the peptidoglycan layer were impacted which inhibited the lipophobicity-dependent color formation (Ali and Abd-Elsalam, 2020). The neem/chitosan composite pretreatments for wool overcame its hydrophilicity and shrinkage issues was found while it enhanced the coloring and antibacterial activity of the material. It became evident to prevent cross-infection by harmful microbes. However, natural dyes are used to color fabrics in order to save the environment and stop industrial pollution.

Microwave heating is economical technique which used to complete the dyeing. However, the textile industry is being forced to replace chemical treatments with ecological techniques like biopolymers due to mounting environmental pressure. This treatment can protect the bulk characteristics of fibers. Numerous studies have focused a significant deal of emphasis on the applications of chitosan in textiles because of its many special qualities. The chitosan polymer's enormous molecular size and high viscosity indicated a limit to its penetration into the fiber (Kieken, 2019). As a result, only surface deposition occurred which is deteriorated the fibers' attractiveness and handling. The degree of chitosan penetration into the fiber structure is enhanced and the intrinsic qualities of wool fibers which are preserved when the particle size is reduced to the nanoscale.

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