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Antimicrobial Activity of Some Natural Dyes Extract from Different Plants against Some Human Pathogens

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ABSTRACT

Antimicrobial textiles have gained interest from both academic research and industry because of their potential to provide high-quality life and safety benefits to people. Antimicrobial activities of some natural dyes extracted from different plants such as onion, curcuma, saffron and madder against some species of pathogenic bacteria and fungi as *Escherichia coli* and *Staphylococcus aureus* and fungal strains *Aspergillus* spp. and *Penicillium* spp was studied. The antimicrobial activity of wool fibers pretreated with chitosan and/or dyed with the tested natural dyes was evaluated. The all tested natural dyes showed considerable inhalation against all tested microbes. The results indicated that wool samples pretreated with chitosan and dyed with these natural dyes exhibit higher inhibition percent against all tested pathogens than the untreated samples. It could be suggested that using natural dyes can be successfully used to replace traditional synthetic dyes and avoid environmental pollution.

INTRODUCTION:

Bacteria and fungus, either pathogenic or not, are generally found on human skin, nasal cavities, and other areas, such as in the genital area. Microbial shedding from our body leads to spreading of microorganism into a textile material either directly in clothes or on surrounding textiles. Recent studies showed that contamination of textiles in clinical settings may contribute to the spreading of pathogens to the air then it infects the immediate and non-immediate environment. It is one of the most probable causes of hospital infections. Typically, pathogenic microorganisms as, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Candida albicans* have been found on textiles (Ali, *et al*., 2011).

In addition, microorganism proliferation can cause malodors, stains and damage of mechanical properties of the fibers that could cause a product to be less effective in industrial use. Additionally, may promote skin contamination, inflammation and in sensitive people, atopic dermatitis. Fortunately, the use of antimicrobial textiles may significantly reduce the risk of infections especially when they are used in close contact with the patients or in the immediate and non-immediate surroundings.

Antimicrobial agents are natural or synthetic compounds that inhibit the growth of microorganisms because they can be protein, lipid synthesis or enzyme inhibitors, all of which are essential for cell survival; or kill the microorganisms by damage the cell wall. Almost all antimicrobial synthetic agents in use on textiles are biocides.

Neem (*Azadirachta indica*) is an evergreen tree of India, which belongs to the plant family *Meliaceae*. It is considered as one of the most sources of natural compounds with antimicrobial and medicinal properties (**Rajendran 2012**). The active components of neem are present in all parts of tree. The extract of neem has been widely used in pesticide formulations that due to their pest repellent properties, it has the ability to inhibit the growth of Gram-positive and Gram-negative bacteria (**Kut *et al*., 2005**).

Chitosan is a deacetylated derivate of chitin, nontoxic, resistant to microorganisms, biodegradable and biocompatible. The antimicrobial activity of chitosan is influenced by several factors as the type of chitosan, the degree of deacetylation, molecular weight and other physical and chemical factors such as pH, ionic strength and addition of non-aqueous solvents. Chitosan can be considered an antimicrobial agent for textile finishing (Shinand

JANG, 2010. Dutta, *et al*., 2002). The application of chitosan in textile industry is effective against a wide range of microorganisms only at high concentrations. In recent years antimicrobial textiles have gained interest from both academic research and industry because of their ability to produce high-quality life and safety benefits to people. Textile products are prone to host micro-organisms causing diseases, unpleasant odours, colour degradation and deterioration of textiles. Antimicrobial textiles can be used to produce many products such as sportswear, outdoor apparels, undergarments, shoes, furnishings, hospital linens, wound care wraps, towels and wipes. Several test methods have been developed to determine the efficacy of antimicrobial textiles.The tests to evaluate the antimicrobial properties generally fall into two categories as dynamic shake test (quantitative method such as serial dilution and plate count method) and agar diffusion test (qualitative method such as halo method) (Singh *et al.*, 2005, Shin 2010).

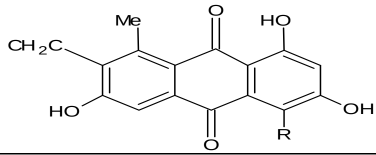
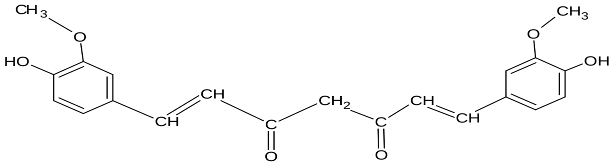
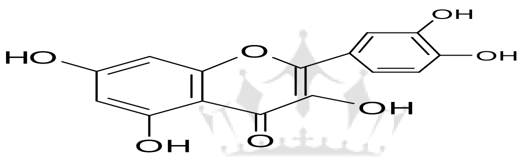
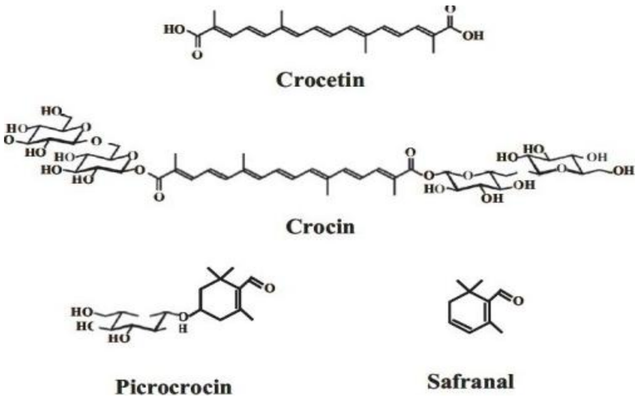
MATERIALS AND METHODS:

MATERIALS:

Wool fibers10/2, and silk fibers supplied by El Mahalla company-Egypt. Neem oil extract was purchased from ELGmhoria Company, Egypt.

Dyestuffs

Table 1: Chemical structure, chemical names and color for natural dyes under investigation

Natural Dye	Chemical structure	Color
powdered madder (roots)	R=H_1 flavokermesic acid R= OH, Kermesic acid 	Red
Turmeric [Curcumin] (*Curcuma longa)*		Brilliant yellow colour
Red onion peels		Yellow colour
Saffron (*Crocus sativus* L)		Red and yellow

Pretreatment wool Fibers with chitosan:

Chitosan high molecular weight solution is freshly prepared by dissolving (1-4 g/l) in distilled water containing acetic acid (1% v/v). The wool fibers were immersed in these solutions at a 50:1 liquor ratio for 5 min using microwave, and then thoroughly washed, and air dried at room temperature.

Pretreatment of wool Fibers by Neem oil:

Wool fibers treated by pad-dry-cure techniques. The wool fibers treated by neem oil extract at concentrations (2-10%), then padded to 100% wet pick up, dried at 80ºC for 5 min. then cured at 120ºC for 3 min.

Scanning Electron Microscopy (SEM):

The surface morphology of untreated and treated wool fibers were investigated by using scanning electron microscopy (SEM), with a JSMT-20, JEOL-Japan. Before 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).

Dyeing processes:

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). The dyed samples were rinsed with cold water, washed in a bath of liquor ratio 60:1 using 3 g/L non-ionic detergent (Hostapal CV, Clariant) at 50ºC for 30 min., then rinsed and finally dried at ambient temperature.

Measurements of antimicrobial activity:

The antimicrobial activities of wool fibers dyed with red onion peals, saffron, curcum, madder and prickly pear natural dyes and pretreated with neem oil was evaluated by using standard methods (serial dilution and plate count method according to Alen, O.N,(1962). Experiment on soil bacteriology Burges Publishing Co. Minneapolis Minnesota USA.

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. 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 successful growth of microorganisms, characteristics of each distinct colony, e.g., shapes, color, transparency, *etc*. were determined. 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). The antimicrobial activity was estimated and expressed as reduction in total count of fungi and bacteria in each treatment.

RESULTS AND DISCUSSION

Antimicrobial Activity:

Antimicrobial activities of chitosan may be attributed to the chelation of metals, suppression of spore elements and binding to essential nutrients to microbial growth. Chitosan oligomers diffuse inside hyphae interfering on the enzymes activity responsible for the fungus growth. Chitosan molecules in bacteria surrounds might complex metals and blockage some essential nutrients to flow, contributing to cell death (Rejane *et al*., 2009). The positive charge on the N atom of, chitosan below pH 6.0 is more soluble and has a better antimicrobial activity by interfering with the negatively charged residues of macromolecules exposed on the fungal cell surface, and thereby changes the permeability of the plasma membrane (Rabea *et al.*, 2003, Avadi *et al*., 2004). Rabea *et al*., (2009) and Badawy *et al*., (2012). It is found that the pretreatment with chitosan, effectively enhanced the antifungal activity of chitosan against many pathogens (Hebeish *et al*., 2012) for wool fibers as shown in figures.

The effect of the antimicrobial activity on the concentration of chitosan and neem oil:

Figure 1, 3, 5, 7 shows the dependence of the antimicrobial activity of wool fibers on concentration for curcum, onion, madder and saffron of chitosan and neem oil. Four strains of bacteria and fungi were used to assess the antimicrobial activity under the combined effect of chitosan and neem oil with the dye. Antimicrobial activity varies by varying the strains of bacteria and fungi, the bacterial reduction rate increases as the molecular weight and concentration of chitosan increases. The magnitude of such increase depends on the strain of bacteria and fungi. For instance, 100% reduction with dyed wool fibers with 2% chitosan. Antimicrobial activity expressed as growth reduction of the bacteria, (El-khatib and Ali 2011) could be explained.

The microbial reduction rate increases as the concentration of neem oil increases. The magnitude of such increase relies on the strain of bacteria and fungi.

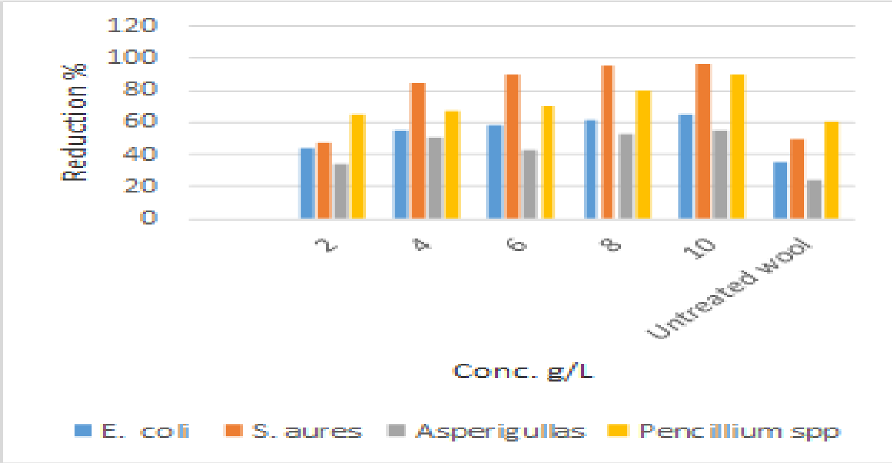


Figure 1: Antimicrobial activities of wool fibres treated with chitosan and dyed with curcum natural dyes.

The effect of the antimicrobial activity on the pH of the dye bath.

Figure 2, 6, 8: show the dependence of the antimicrobial activity of dyed wool fibers with curcum, madder, saffron natural dyes pretreated with neem oil on the pH of the dye bath. Antimicrobial activity varies by varying the strains of bacteria. It is noticed from the figures that acidic pH is most suitable for antimicrobial activity. The highest reduction % is exhibited at pH 3 for both treated and untreated fibers. It is also observed that the fungi exhibited higher reduction % than bacteria.

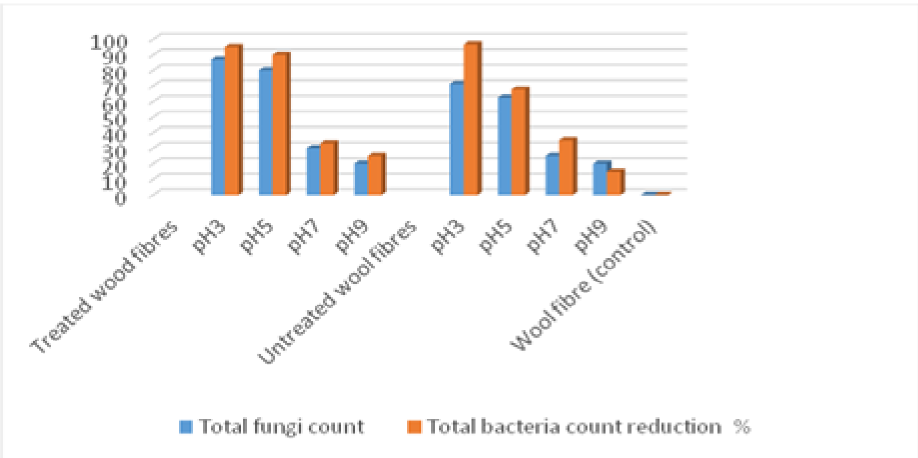


Figure 2: Antimicrobial activities of wool fibres treated with neem oil extract and dyed with curcum natural dyes.

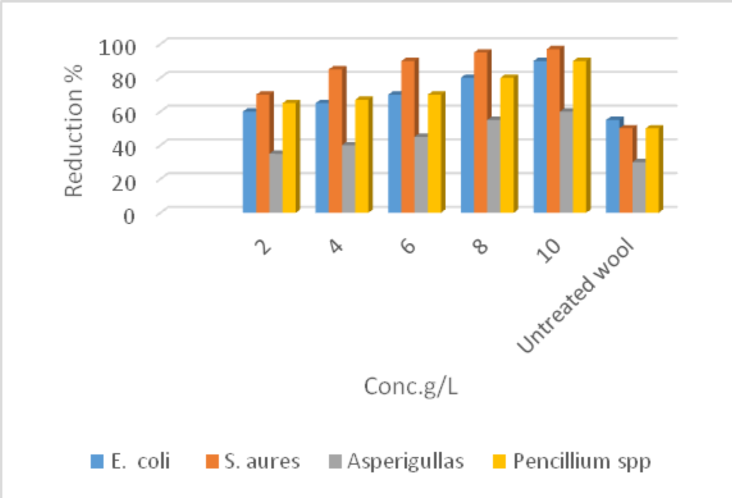


Figure 3: Effect of concentration of neem on the reduction % for different species of fungi and bacteria for onion natural dye.

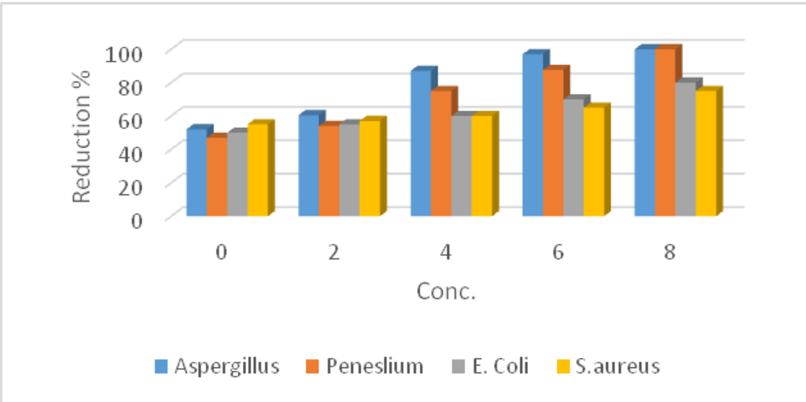


Figure 4: Effect of concentration of chitosan on the reduction % for different species of fungi and bacteria for onion natural dye.

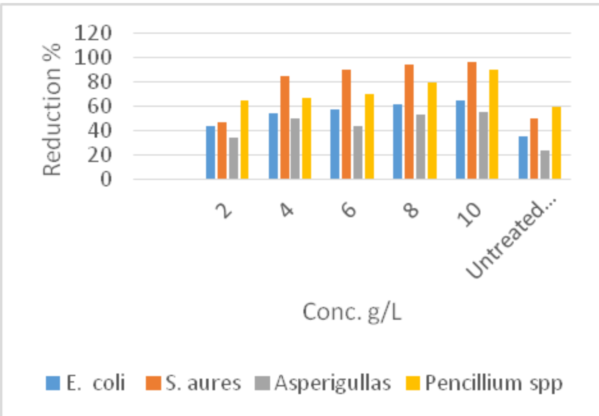


Figure 5: Effect of concentration of chitosan on the reduction % for different species of fungi and bacteria for madder natural dye.

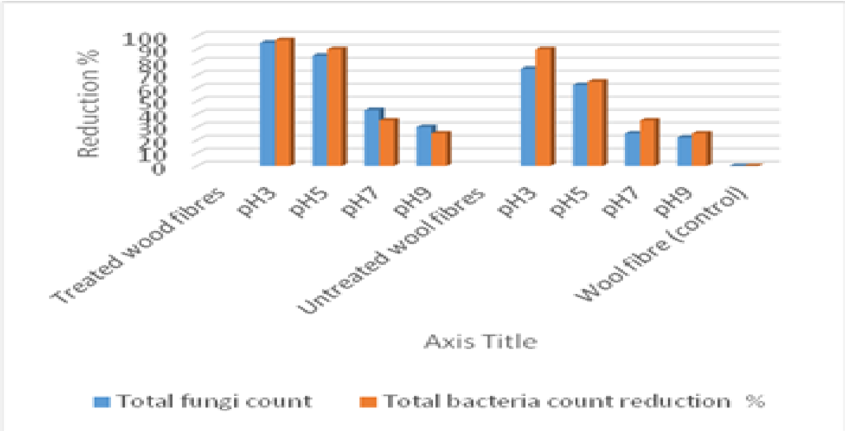


Figure 6: Effect of pH on the reduction % for different species of fungi and bacteria for wool fibers treated with neem oil and dyed with madder natural dye.

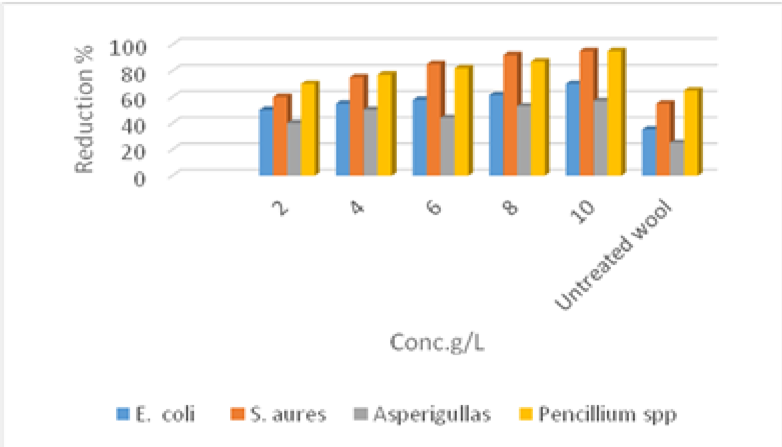


Figure 7: Effect of concentration of chitosan on the reduction % for different species of fungi and bacteria for saffron natural dye.

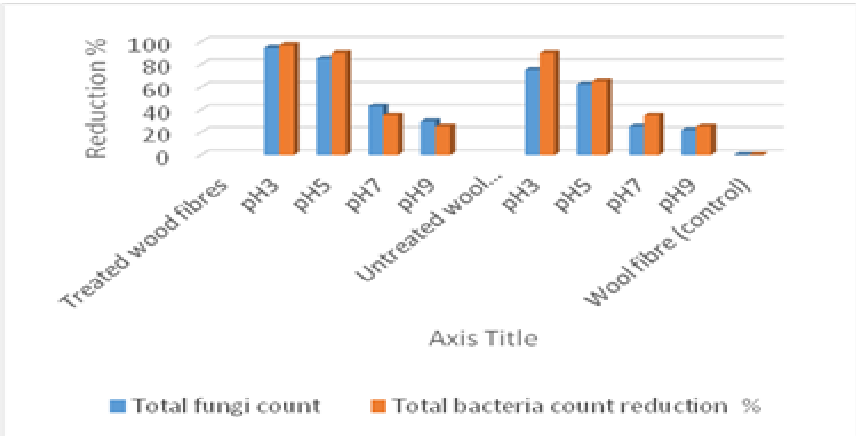


Figure 8: Effect of pH on the reduction % for different species of fungi and bacteria for wool fibers treated with neem oil and dyed with saffron natural dye.

Surface Morphology

The morphologies of the untreated and pretreated wool fibers were examined by scanning electron microscopy (SEM). Effect of pretreatment with chitosan and neem oil chitosan using scanning electron microscope (SEM) for wool fibers. Figs 11a, b, c represent the SEM images of untreated, pretreated wool fibers with chitosan and nano chitosan respectively. The untreated samples have a rough surface as shown in Fig. 8a, the treated samples as shown in Figs.11 a, b and c indicate that the treated wool fibers were swelling compared to the untreated fibers, the diameter of the fibers increase and have smooth and even surfaces. The changes in the surface morphology due to the effect of pretreatment with chitosan and neem oil. As can be seen from the images, chitosan treatments covers wool surface. Chitosan and created a smooth surface which is a significant improvement for wool fibers in terms of their hydrophilicity and dye ability properties.

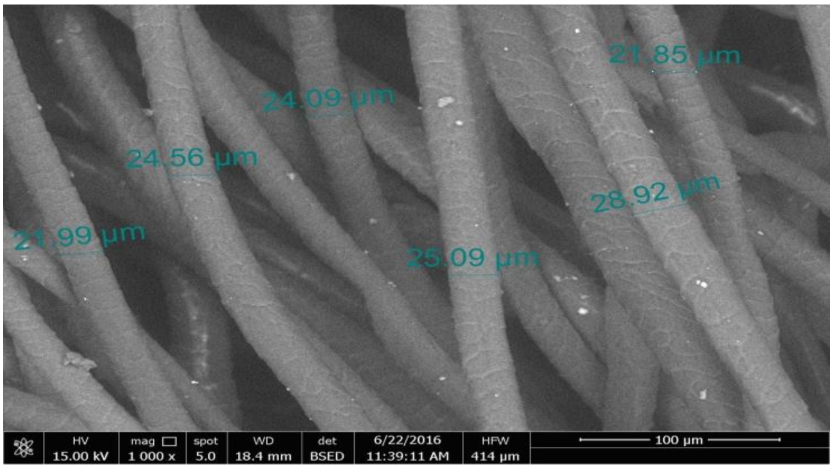


Figure 9 a: SEM for wool fibers treated with 30% conc. of neem oil

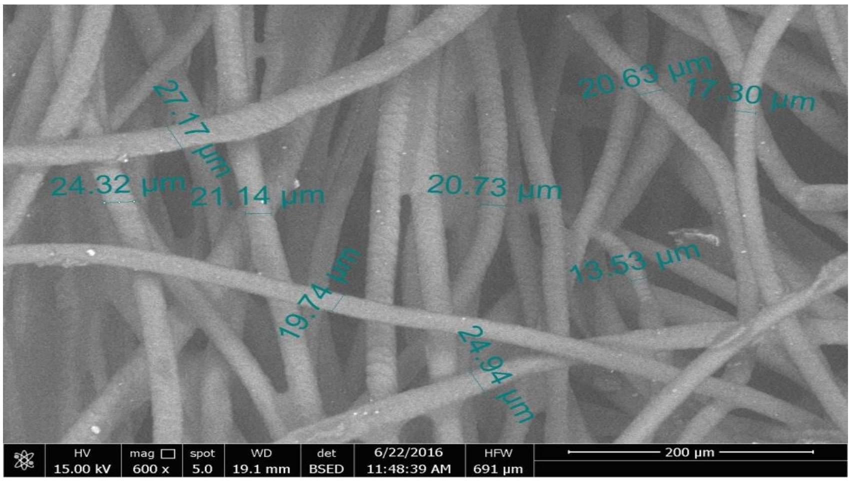


Figure 9 b: SEM for untreated wool fibers

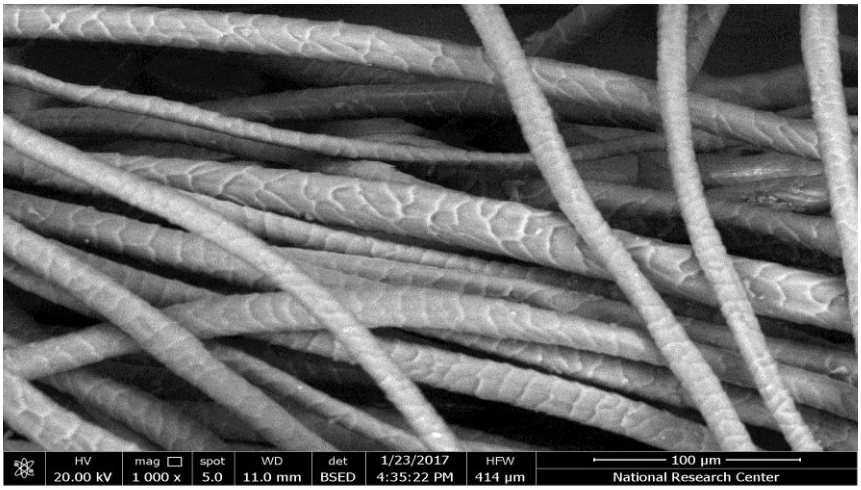


Figure 9 c: SEM for pretreated wool fibers with chitosan

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CONCLUSION:

Natural dyes are extracted from red Onion peals, prickly pear, curcum and saffron are used for dyeing wool fibers that are pretreated with chitosan and neem were monitored for antimicrobial activity. Results signified that the chitosan and neem concentration determine the magnitudes of the antimicrobial activity. Higher concentrations of both chitosan and neem are accompanied by higher reduction % values. Utilization of high molecular weight chitosan at a concentration of 8 g/L in the treating solution inhibits completely the growth of *E. coli*. The bacterial reduction rate increases as the concentration of chitosan increases. For instance, higher growth reduction of *E. coli* and *S. aureus* could be achieved with dyed wool fibers with 10g/L chitosan wool fibers treated with chitosan and neem and dyed with the natural dyes under investigation display high growth reduction of microbes because the amino groups of chitosan afford dyeing sites for the dye. Dye adsorption increases of chitosan which, in turn, increases the amino groups which are accessible for the dye molecules. Results clearly show that inhabitation reduction of the growth of *A. niger* is low correlated with other species.The growth reduction of these pathogens reach up to 70 % and 95% of wool fibers pre-treated with neem oil.

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