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ORIGINAL ARTICLE

Studying Anti-bacterial effect of Silk in presence of several nicks with Chitosan and Annatto dye

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ABSTRACT
Silk yarns are the most beautiful, soft, and worthy yarns used by human beings; thus, its antibacterial effect has a specific importance. One of natural colors is annatto. Some benefits of natural colors such as environmental compatibility, stabilized features, color foams, economic issues and tendency of natural yarns to absorb them highlights the importance of using natural colors despite producing diverse chemical ones. Nicks are considered as one of the main elements of dying process with natural dyes with purpose of increasing stability and improving absorption. In this article, silk fabric was dyed with annatto dye and two pre-nick and post-nick methods in presence of iron sulfate, potassium de chromate, copper sulfate, aluminum sulfate, and also chitosan (0.1 and 0.6) as a biologicrack. The anti-bacterial effect of nicks and chitosan were examined with different densities on Escherichia coli and Staphylococcus aureus microorganisms. Infrared spectrometry test (FTIR) was used to measure mechanical and morphologic features offabrics consumed and to identify chemical changes on them. The results obtained imply that increasing density of consumed chitosan up to 0.6 % on the silk fabric causes growth prevention on both types of microorganism, especially in racked copper sulfate sample which has the highest anti-bacterial effect. Also results of FTIR shows that the peak related to NH2, NH, C-O-C and CN completed with chitosan is strengthened with 0.1 and 0.6% densities; but there is no change observed in OH peak.
Keywords: anti-bacterial completion, silk fabric, chitosan, racks, microorganism.

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INTRODUCTION

Textiles are good environments for bacteria growth; thus, its antibacterial effect has a specific importance. One of textiles is silk fabrics which has specific importance among natural yarns due to its elegance, transparency, and other desirable features. In the recent years, awareness of consumers about hygiene levels of textiles and increase in demand for using anti-bacterial materials with environmental friendly features has attracted manufactures of chemical products to anti-bacterial materials with highest durability effect and lowest toxicity. [1]Today, several herbal anti-bacterial materials are used for textile products including natural dyes (e.g. annatto). [2] Figure 1 shows chemical structure of Bixin. Of features of natural dyes, especially its herbal type is ability of complex formation with metal ions through cordinance link. The metal ions available in rack structures have the ability of connecting with protein chains of silk yarns. Also chitosan is de-acetylation derivative of kitten which is a non-toxic, natural and biodegradable polymer. Figure 2 shows chemical structure of chitosan.

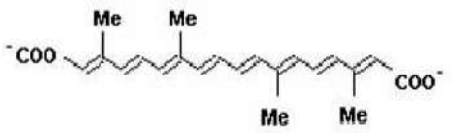


Fig (1) Bixin chemical structure

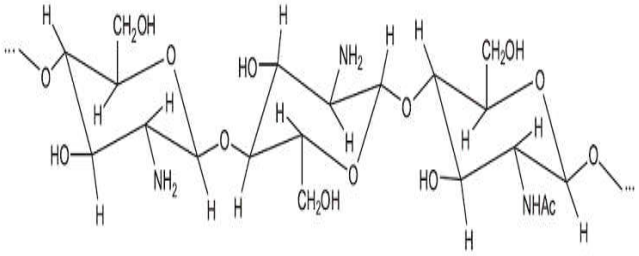


Fig (2) chitosan chemical structure

The mechanism of anti-bacterial feature is due to positive load on it. The wall of bacteria has negative load; [3] thus, chitosan adheres to this wall and removes its uniformity, then it causes disorder in permeability of wall leading to cytoplasm deposit and finally removal of bacteria. This improves stabilized property of fabrics completed with racked chitosan. For facilitation, all fabrics of experiment were codified as shown in table 1.

Table (1) samples coding

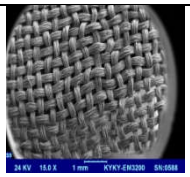
Samples coding	Sample names
A^1	First racked samples , completed with chitosan 0.1%, racked with nick and then dyed and then dyed
A^2	First samples completed with chitosan 0.1%
A^3	First samples racked with ick, completed with chitosan 0.6% and then dyed
A^4	First samples completed with 0.6% chitosan, racked with nick and then dyed

MATERIALS AND METHODS

Introduction of materials

In this study, the 100% silk fabric of Tus Co. was used which its features are shown in table below:

Table (2) features of consumed fabric

SEM image	Warp-Woof Density	Manufacturer	Producer	Fiber Type	Components	Name
	warp: 67, woof: 71	Tus silk Co.	Iran	wreathy	100% silk	silk

Annatto grains (*Bixaorellena* L)was selected as natural dye for silk fabric dying; the detergent used and standard method used were LNB(Ludwigshafen, BASF, Germany) and AATCC, respectively. Chitosan was provided from KimiyaGostarCo; also racks of iron sulfate, potassium de chromate, aluminum sulfate and acetic acid were bought from Merck Co, Germany. The negative hot bacteria (*Escherichia coli*) and positive hot bacteria (*Staphylococcus aureus*) were also used in this experiment. This study was dedicated by microbiology laboratory of medical faculty of Shahid Beheshti University in which the reflective spectrophotometer machine (X.rite model), USA was utilized to determine reflection factor percent and color power of samples. The rubbing stability test machine (Sherli Co.) was used to determine rubbing stability. Also the atomic absorption device (PG-990, Iran) was used in this study.

Methods

The 1gr silk fabric was washed using anionic detergent 20% with ratio 1:50. The 5% grains were poured (0.25 cc) in a basin with ratio 50:1. Also chitosan 0.1% and 0.6% was poured in temperature of 30C with color solution (25 cc) in a basin with ratio 1:40. Then, dyed sample were washed and dried with cold water for non-absorbed dyes. Colorful components of dyed silk were measured with annatto in presence of racks and chitosan using reflective spectrophotometry device. Completed fabric was washed 5 times using AATCC124-1996 standard test at temperature of 46C. Then, samples of anti-bacterial fabrics were used applying AATCC-100 standard method and two bacteria-*Staphylococcus aureus* and *Escherichia coli*-as indicators of hot positive hot negative microorganisms, respectively. In this method, duration of

bacteria growth is 24 hours. Afterwards, the number of bacteria is measured using images of plate; number of colonies are calculated and put in the relation below to compute percent of anti-bacterial effect:

Percent of decrease in number of bacteria colonies = $\frac{A-B}{A} \times 100$

Where, A and B are control sample and complete sample, respectively. The FTIR device (Tensor 27 Bruker model, Germany) was used to measure infrared spectrometry in wavelengths of 1000-35000 nm at room temperature.

RESULTS AND DISCUSSION

As it is observed in table 3, a*, b*, and K/S for all silk fabrics completed with chitosan are more than that of without completion with chitosan. [4]Increase of chitosan density causes increase in a*, b*, and K/S; but value of L* is decreased. [5] Value of L* for a non-complete fabric is 65.19 and $C_{0/1\%}$.D و $C_{0/6\%}$.D are equal to 62.043 and 61.039, respectively. An increase in density of chitosan causes more annatto dye absorption in the silk fabric. Also, sample M_{Fe} .$C_{0/6\%}$. D has the least L*. Color power of dyed fabric samples is calculated from Cubel-Mank relation below:

$K/S = (1-R)^2/2R$ (1)

Where, R and K/S are reflection and color power of samples, respectively. When fabrics become darker, K/S also increases. [6] The color power of samples (K/S) for non-completed fabrics (D) is equal to 6.061 and its amount for chitosan with density of 0.1 and 0.6 increases to 8.235 and 8.224. This is because increasing density of chitosan to 0.6% will enhance absorption percentage of anion dye; this could be because of increase in aniongroups' absorption of annatto dye on the silk fabric completed with chitosan. [7] Table 3 shows calorimetric parameters (L*, a*, and b*).

Table (3) brightness, a*, b* and K/S

Sample name	L*	a*	b*	K/S
A^3_{Fe}	53/241	30/404	50/85	8/93
A^4_{Fe}	54/02	30/335	50/8	8/875
A^3_{cr}	56/087	30/282	50/78	8/847
A^4_{Cr}	56/098	30/25	50/768	8/8
A^3_{cu}	56/653	30/19	50/743	8/623
A^4_{cu}	56/921	30/147	50/696	8/525
A^3_{Al}	57/064	30/143	50/627	8/52
A^4_{Al}	57/087	30/143	50/578	8/5
A^1_{Fe}	58/029	30/12	50/578	8/465
A^2_{Fe}	58/87	30/056	50/53	8/426
A^1_{Cr}	58/946	30/054	50/435	8/423
A^2_{Cr}	59/073	29/99	50/332	8/35
A^1_{Cu}	59/095	29/987	50/3	8/332
A^2_{Cu}	59/145	29/986	50/243	8/332
A^1_{Al}	59/96	29/87	50/243	8/323
A^2_{Al}	61/039	29/76	50/24	8/3
$C_{0/1\%}$.D	61/078	29/72	50/148	8/235
$C_{0/6\%}$.D	62/043	29/678	50/123	8/224
D	65/19	29/53	50/024	6/061

Figures 3 and 4 show the percent of decrease in bacteria *Staphylococcus aureus* and *Escherichia coli* for all samples. Results indicate that sample $M_{(Fe,Cu,Al,Cr)}$.$C_{0/6\%}$.D has a high anti-bacterial effect against Escherichia coli compared to *Staphylococcus aureus*. Increase in chitosan density will absorb more annatto dye to the silk fabric; [8] since it increases chitosan density and as a result, the antibacterial influence increases. Also, repeated washing decreases anti-bacterial features particularly in sample M_{Cu} .$C_{0/6\%}$. D. The percent of bacteria reduction also receives to 100% and antibacterial percent will receive to 99.9 % after repeated washing. The bacteria colonies are calculated and substituted in equation 1.

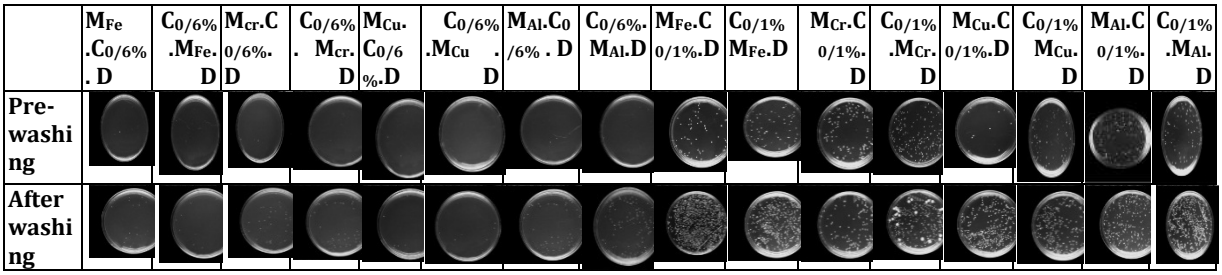


Figure (3) antibacterial effect of samples $M_{(Fe,Cu,Al,Cr)}.C_{0/6\%}.D$, $C_{0/6\%}M_{(Fe,Cu,Al,Cr)}.D$,$M_{(Fe,Cu,Al,Cr)}.C_{0/1\%}.D$,. ، $C_{0/1\%}M_{(Fe,Cu,Al,Cr)}.D$ against bacteria *Escherichia coli*

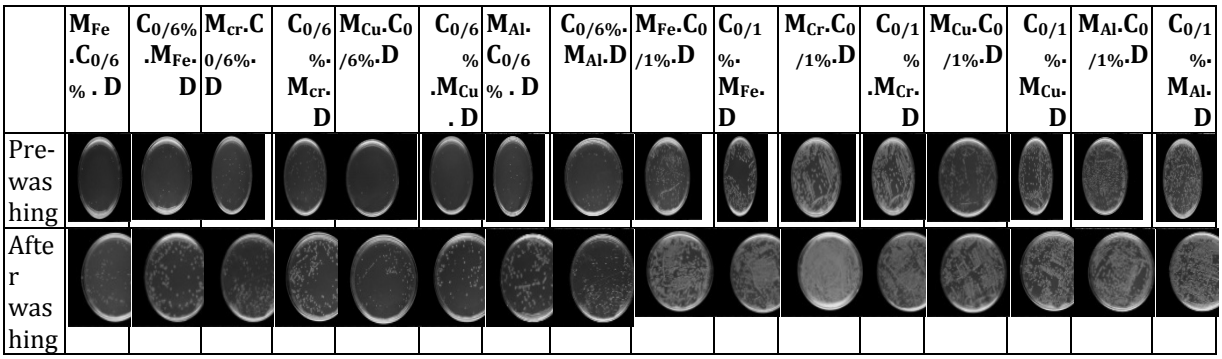


Figure (4) decrease of colonies in samples $M_{(Fe,Cu,Al,Cr)}.C_{0/6\%}.D$, $C_{0/6\%}M_{(Fe,Cu,Al,Cr)}.D$,$M_{(Fe,Cu,Al,Cr)}.C_{0/1\%}.D$ against bacteria *Staphylococcus aureus*

Table (4) decrease of bacteria in samples of $M_{Fe,Cu,Al,Cr)}.C_{0/6\%}.D$, $C_{0/6\%}M_{(Fe,Cu,Al,Cr)}.D$ against bacteria *Escherichia coli*

Sample names	Pre-washing antibacterial percent	After washing antibacterial percent
A^3_{cu}	100	99/9
A^3_{Fe}	100	99
A^3_{Al}	100	99/3
A^3_{cr}	99/8	98/4
A^4_{cu}	99/9	96/5
A^4_{Fe}	99/99	95/1
A^4_{Al}	98/7	94/5
A^4_{Cr}	98	89
A^1_{cr}	97/7	81/8
A^1_{Fe}	93/9	70
A^1_{Al}	92/3	69/8
A^1_{cr}	91/2	69/6
A^2_{cu}	91/9	68
A^2_{Fe}	90/2	58/4
A^2_{Al}	86/1	56
A^2_{cr}	84	54
D	0	0

Table (5) decrease of bacteria in samples $M_{(Fe,Cu,Al,Cr)}.C_{0/6\%}.D$, $C_{0/6\%}M_{(Fe,Cu,Al,Cr)}.D$,$M_{(Fe,Cu,Al,Cr)}.C_{0/1\%}.D$,.$C_{0/1\%}M_{(Fe,Cu,Al,Cr)}.D$ against bacteria *Staphylococcus aureus*

Sample names	Pre-washing antibacterial percent	After washing antibacterial percent
$M_{cu}.C_{0/6\%}.D$	99/9	98/8
$M_{fe}.C_{0/6\%}.D$	99/9	89/9
$M_{Al}.C_{0/6\%}.D$	99	86
$M_{cr}.C_{0/6\%}.D$	98/9	80/9

$C_{0/6\%}$. M_{cu}.D	98/8	71/8
$C_{0/6\%}$. M_{Fe}.D	96/4	71/4
$C_{0/6\%}$. M_{Al}.D	94/9	70/1
$C_{0/6\%}$. M_{cr}.D	89/4	69/2
M_{cu}.$C_{0/1\%}$.D	61/6	0
M_{Fe}.$C_{0/1\%}$.D	59/2	0
M_{Al}.$C_{0/1\%}$.D	56/8	0
M_{cr}.$C_{0/1\%}$.D	55/3	0
$C_{0/1\%}$. M_{cu}.D	50/2	0
$C_{0/1\%}$. M_{Fe}.D	45/3	0
$C_{0/1\%}$. M_{Al}.D	40/2	0
$C_{0/1\%}$. M_{cr}.D	0	0
U	0	0

Results of infrared spectroscopy in spectrophotometry (ATR/FTIR) of chemical bonds on the surface of yarns are shown in figures 5-7. Figure 8 shows that FTIR of raw sample is related to hydroxyl and amine groups. [9] First type amides (NH_2) and hydroxyl (OH) groups show absorption bond of 3276.76 and 687.64, respectively. There is also an absorption bond in 1229.64 related to tensional CN. Asymmetric bridges are observed in 1164; also when raw ample is compared with $M_{(Fe,Cu,Al,Cr)}$. $C_{0.6\%}$ .D and **$C_{0/6\%}$.** $M_{(Fe,Cu,Al,Cr)}$.D, it is observed that peaks 3276.33,1165,1619 and 1229.64 related to amine group (NH_2),(C-O-C), tensional NH and flexural CN are decreased; [10] but these peaks are equal for $M_{(Fe,Cu,Al,Cr)}$.$C_{0/1\%}$.D $C_{0/1\%}$. $M_{(Fe,Cu,Al,Cr)}$.D and raw sample. Also peak OH is equal for samples of OH$M_{(Fe,Cu,Al,Cr)}$.$C_{0/1\%}$.D و $C_{0/1\%}$. $M_{(Fe,Cu,Al,Cr)}$.D, $M_{(Fe,Cu,Al,Cr)}$.$C_{0/6\%}$.D ،$C_{0/6\%}$. $M_{(Fe,Cu,Al,Cr)}$.D .

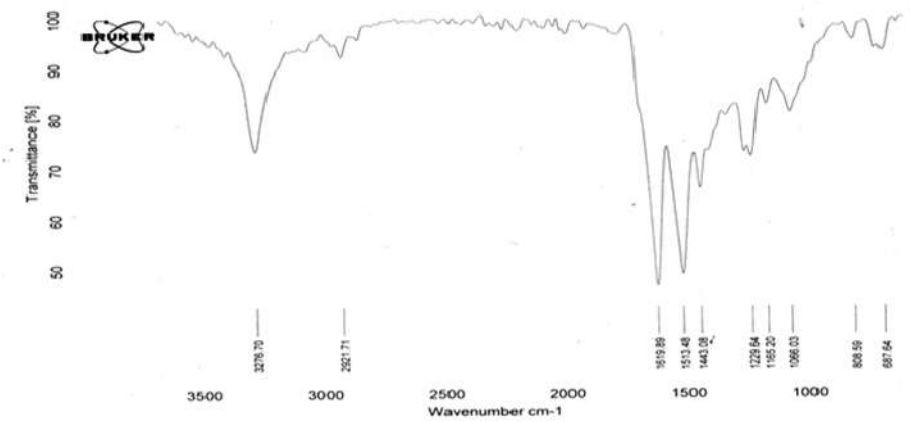


Figure (5) FTIR of raw sample

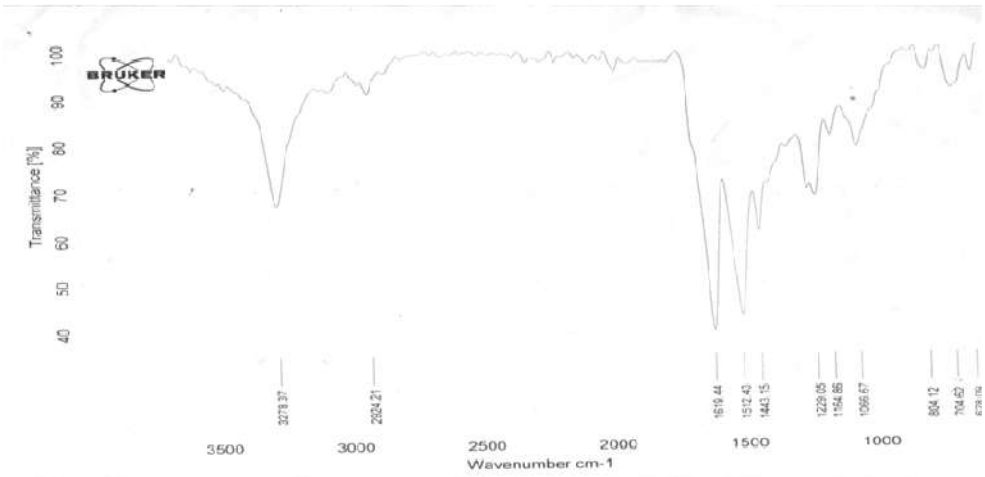


Figure (6) FTIR of samples $M_{(Fe,Cu,Al,Cr)}$.$C_{0/1\%}$.D and $C_{0/1\%}$. $M_{(Fe,Cu,Al,Cr)}$.D

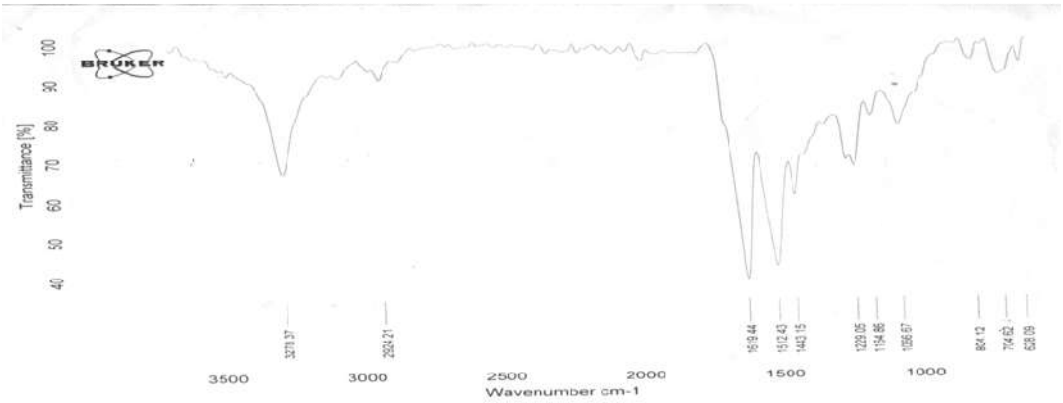


Figure (7) FTIR of $M_{(Fe,Cu,Al,Cr)}.C_{0/6\%}.D$ and $C_{0/6\%}.M_{(Fe,Cu,Al,Cr)}.D$

As seen in table 6-8, the PH effect is shown on the mentioned samples. Acid acetic 2% of samples R_f،$DC_{0/1\%}$ ،$C_{0/1\%}.M_{Cr}$ و $C_{0/1\%}.M_{Cu}$ .D، $DC_{0/1\%}.M_{Fe}$، $C_{0.1\%}.M_{Al}$.D is more obvious than acetic acid 1%. Since with adding acetic acid, functional group (NH_3) of silk becomes more positive and as a result, it leads to more negative dye and consequently more dye absorbing by leaf. [11] But in samples $DC_{0/6\%}.M_{Cr}$ و .$DC_{0/6\%}.M_{Cu}$ .D.$C_{0/6\%}.M_{Fe}$ و..$C_{0/6\%}.M_{Al}$ .D and MAl.$C0_{/6\%}$.D ،$M_{Fe}.C_{0/6\%}$.D ، M_{Cu}. $C_{0/6\%}$.D و M_{Cr}. $C_{0/6\%}$.D, adding acetic acid decreases color of sample up to 2%. Therefore, most of color lies on chitosan and less on the silk; thus, samples become more colorless. [12]

Table (6) calculation of L^* ،$M_{(Fe,Cu,Al,Cr)}.C_{0/6\%}.D$, $C_{0/6}.M_{(Fe,Cu,Al,Cr)}..D$, $M_{(Fe,Cu,Al,Cr)}.C_{0/1\%}.D$ and .$C_{0/1\%}$. $M_{(Fe,Cu,Al,Cr)}D$ in presence of acetiu acid 1 and 2%

Sample name	Acetic acid 1%	Acetic acid 2%
M_{Fe} .$C_{0/6\%}$. D	53/241	54/43
$C_{0/6\%}.M_{Fe}$. D	54/02	54/55
$M_{cr}.C_{0/6\%}$. D	56/087	56/87
$C_{0/6\%}$. M_{cr}. D	56/098	56/98
M_{Cu} .$C_{0/6\%}$. D	56/653	57/04
$C_{0/6\%}$ .M_{Cu} . D	56/921	57/03
$M_{Al}.C_{0/6\%}$. D	57/064	59/126
$C_{0/6\%}.M_{Al}$.D	57/087	59/34
$M_{Fe}.C_{0/1\%}$.D	58/029	57/04
$C_{0/1\%}$. M_{Fe}.D	58/87	57/087
$M_{Cr}.C_{0/1\%}$.D	58/946	58/046
$C_{0/1\%}$ .M_{Cr}.D	59/073	58/073
$M_{Cu}.C_{0/1\%}$.D	59/095	58/09
$C_{0/1\%}$. M_{Cu}.D	59/145	58/14
$M_{Al}.C_{0/1\%}$.D	59/96	59
$C_{0/1\%}$. M_{Al}.D	61/039	60/998
$C_{0/1\%}$.D	61/078	61/023
$C_{0/6\%}$.D	62/043	61/054
D	65/19	64/83

Table (7) calculation of $M_{(Fe,Cu,Al,Cr)}.C_{0/6\%}.D$, $C_{0/6}$. $a^*M_{(Fe,Cu,Al,Cr)}..D$, $M_{(Fe,Cu,Al,Cr)}.C_{0/1\%}.D$ and.$C_{0/1\%}$. $M_{(Fe,Cu,Al,Cr)}D$ in presence of acetic acid 1 and 2%

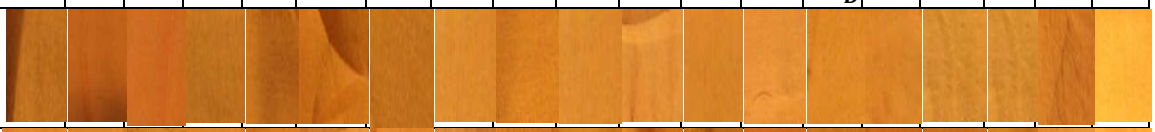
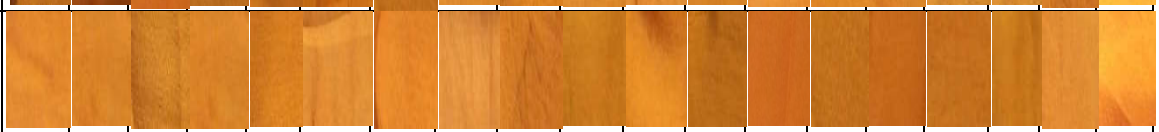
Sample name	Acetic acid 1%	Acetic acid 2%
M_{Fe} .$C_{0/6\%}$. D	30/404	30/32
$C_{0/6\%}.M_{Fe}$. D	30/335	30/302
$M_{cr}.C_{0/6\%}$. D	30/282	30/146
$C_{0/6\%}$. M_{cr}. D	30/25	30/145
M_{Cu} .$C_{0/6\%}$. D	30/19	30/11
$C_{0/6\%}$ .M_{Cu} . D	30/147	30/065

$M_{Al}.C_{0/6\%}$. D	30/143	29/98
$C_{0/6\%}.M_{Al}.D$	30/143	29/97
$M_{Fe}.C_{0/1\%}.D$	30/12	30/28
$C_{0/1\%}$. $M_{Fe}.D$	30/056	30/21
$M_{Cr}.C_{0/1\%}.D$	30/054	30/144
$C_{0/1\%}$.$M_{Cr}.D$	29/99	29/98
$M_{Cu}.C_{0/1\%}.D$	29/987	30/098
$C_{0/1\%}$. $M_{Cu}.D$	29/986	30/032
$M_{Al}.C_{0/1\%}.D$	29/87	29/96
$C_{0/1\%}$. $M_{Al}.D$	29/76	29/82
$C_{0/1\%}.D$	29/72	29/71
$C_{0/6\%}.D$	29/678	29/723
D	29/53	29/64

Table (8) calculation of b* ، $M_{(Fe,Cu,Al,Cr)}.C_{0/6\%}.D$, $C_{0/6}.M_{(Fe,Cu,Al,Cr)}..D$, $M_{(Fe,Cu,Al,Cr)}.C_{0/1\%}.D$ و$C_{0/1\%}.M_{(Fe,Cu,Al,Cr)}D$ in presence of acetic acid 1 and 2%

Sample name	Acetic acid 1%	Acetic acid 2%
M_{Fe} .$C_{0/6\%}$. D	50/85	50/786
$C_{0/6\%}.M_{Fe}$. D	50/8	50/784
$M_{cr}.C_{0/6\%}$. D	50/78	50/613
$C_{0/6\%}$. M_{cr}. D	50/768	50/606
M_{Cu} .$C_{0/6\%}$. D	50/743	50/4
$C_{0/6\%}$ .M_{Cu} . D	50/696	50/34
$M_{Al}.C_{0/6\%}$. D	50/627	50/24
$C_{0/6\%}.M_{Al}.D$	50/578	50/2
$M_{Fe}.C_{0/1\%}.D$	50/578	50/783
$C_{0/1\%}$. $M_{Fe}.D$	50/53	50/77
$M_{Cr}.C_{0/1\%}.D$	50/435	50/604
$C_{0/1\%}$.$M_{Cr}.D$	50/332	50/54
$M_{Cu}.C_{0/1\%}.D$	50/3	50/32
$C_{0/1\%}$. $M_{Cu}.D$	50/243	50/28
$M_{Al}.C_{0/1\%}.D$	50/243	50/27
$C_{0/1\%}$. $M_{Al}.D$	50/24	50/25
$C_{0/1\%}.D$	50/148	50/23
$C_{0/6\%}.D$	50/34	50/24
D	50/024	50/09

Table (9) PH effect on dyed samples in presence of acetic acid 1 and 2%

	M_{Fe} .$C_{0/6\%}$. D	$C_{0/6\%}$ .M_{Fe}. D	$M_{cr}.C_{0/6\%}$. D	$C_{0/6\%}$. M_{cr}. D	M_{Cu}. $C_{0/6\%}$.D	$C_{0/6\%}$ .M_{Cu} . D	$M_{Al}.C_{0/6\%}$. D	$C_{0/6\%}.M_{Al}.D$	$M_{Fe}.C_{0/1\%}.D$	$C_{0/1\%}.M_{Fe}.D$	$M_{Cr}.C_{0/1\%}.D$	$C_{0/1\%}$ .M_{Cr}. D	$M_{Cu}.C_{0/1\%}$. D	$C_{0/1\%}$. M_{Cu}. D	$M_{Al}.C_{0/1\%}$. D	$C_{0/1\%}.M_{Al}.D$	$C_{0/1\%}.D$	$C_{0/6\%}$ .D	D
acetic acid 1%																			
acetic acid 2%																			

CONCLUSIONS

Increase in chitosan density will increase the amount of L*,a*,b*, and K/S. among samples, $M_{(Fe,Cu,Al,Cr)}.C_{0/6\%}$.Dand $C_{0/6\%}.M_{(Fe,Cu,Al,Cr)}$.D have the least L* and the most a*,b*, and K/S. next, samples of $M_{(Fe,Cu,Al,Cr)}.C_{0/1\%}$.DabndC$_{0/1\%}$. $M_{(Fe,Cu,Al,Cr)}$.D have the most L* and the least a*,b*, and K/S. also, antibacterial

effect of $M_{(Fe,Cu,Al,Cr)}.C_{0/6\%}.D$ is more than $C_{0/6\%}.M_{(Fe,Cu,Al,Cr)}.D$, since more chitosan is absorbed on the leaf and antibacterial feature of samples increases. Thereafter, samples $M_{(Fe,Cu,Al,Cr)}.C_{0/1\%}.D$ and $C_{0/1\%}.M_{(Fe,Cu,Al,Cr)}.D$ have the most and the least antibacterial feature. Also, antibacterial durability of sample$M_{(Fe,Cu,Al,Cr)}.C_{0/6\%}.D$ is more than all. The results of FTIR confirm existence of functional groups NH2,NH,C-O- C and CN in $C_{0/6\%}.M_{(Fe,Cu,Al,Cr)}.D$ and $M_{(Fe,Cu,Al,Cr)}.C_{0/6\%}.D$. in addition, adding acetic acid 2% to samples of $M_{(Fe,Cu,Al,Cr)}.C_{0/1\%}.D$and $C_{0/1\%}.M_{(Fe,Cu,Al,Cr)}.D$ decreases brightness and increases a* and b*, respectively; but in contrast for samples of $M_{(Fe,Cu,Al,Cr)}.C_{0/6\%}.D$ and$C_{0/6\%}.M_{(Fe,Cu,Al,Cr)}D$,adding acetic acid 2% will increase brightness and decrease a* and b*.

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