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Surface Modification of Silk Fiber with Chitosan and studies of dyeing enhance ability with antimicrobial properties

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ABSTRACT: Biopolymers are one of the suitable alternate materials for different chemical processes because of its environmental friendly behavior. This research work shows a comparative analysis of silk dye with acid dye before and after Chitosan treatment. In this analysis silk yarns are treated with two different Chitosan 0.5% & 1.0% at temperature (40 & 60)° C respectively and dyed with acid dye at pH 6.00. The study focus on the dye uptake %, color strength value (K/S), different types of fastness properties, antimicrobial properties and the strength of dyed sample. It was found that sample treated with 1% Chitosan showed better dye uptake at 40°C and 60°C are 86.82% and 88.28% respectively, color strength value at 40°C and 60°C are 6.64 and 7.10 respectively and color fastness also increases with increase of temperature. The effects of Chitosan on the antibacterial properties of silk yarns against both of gram positive and gram negative bacteria such as *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Shigella dysenteriae* were investigated and show the antibacterial potential due to the antimicrobial property of Chitosan.

KEYWORD: Silk fiber, acid dye, chitosan, antimicrobial activity, Scanning electron microscopy and silk dyeing.

I. INTRODUCTION

Silk is a natural protein fiber, like wool fiber, due to this mechanism of dyeing silk is dependent not only on free amino and carboxyl groups but also on phenolic with accessible –OH group [1]. Because of slightly cationic character of silk with isoelectric point at above pH 5.0, it can be dyed with anionic dye such as acid, metal complexes, reactive and selected direct dyes [2]. Acid dyes become very popular due to their brilliancy, variety of hues, high wet fastness, convenient usage and high applicability despite having some problems, such as low dye ability, requirements of large amount of auxiliary agents mainly acid and high volume of discharged wastewater [3-5]. Effluent of textile plants is the most polluting amongst all industrial sectors [6, 7]. This huge amount of toxic and hazardous wastewater is finally discharge into the rivers, canals and water streams resulting in adverse effects on flora and agricultural land and thus presumed as one of the major sources for environmental pollution [8]. Moreover, some properties of silk fiber such as crease recovery wash and wear properties, photo-yellowing, water and oil staining resistance, dye-ability, and color fastness are weak and they should be improved. The specialists around the world are attempting to build up a cleaner innovation and ecologically supporting methods of silk coloring for conforming to continuously requesting ecological regulation and to save water, energy, coast and time. For this purpose, surface modification of silk by some physical and chemical techniques has been developed. Some techniques such as γ -ray radiation grafting, plasma grafting, and ultraviolet ray initiated grafting are being explored as a physical method for improving silk fiber properties [9]. These methods are some important alternative to wet treatments because they are considered as clean, dry, and environmental friendly physical techniques [10]. In graft copolymerization as a chemical technique-many monomers such as vinyl monomers (vinyl acetate, acrylonitrile, and phenylethylene), methacrylate monomers (methyl methacrylate, 2-hydroxyethyl methacrylate, and ethyl methacrylate), acrylamide monomers (acrylamide, methacrylamide, hydroxy methacrylamide) and fluoroacrylate have been used [11]. In spite of these advantages, it is believed that chemical processes do not comply with environmental regulations, due to their generating of some toxic agents during processes

[12-14]. Surface modification of textile fibers by using some natural polymers such as chitin or chitosan in the textile finishing processes is considered to be the best route to overcome these problems [15, 16]. Chitosan is the derivative of chitin, a major component in marine invertebrates such as crustacean shells (shrimps, crabs and lobsters), exoskeleton of insects and some fungi, and is prepared through the deacetylation of chitin [17, 18]. The unique properties of chitosan including availability, biodegradability, biocompatibility, bioactivity, cost-effectiveness, and non-toxicity, as well as bio-adhesion, sorption, and antimicrobial properties are the major reasons for its multiple applications [19, 20].

Chitosan has prospective applications in many fields, such as medicine, pharmaceuticals, textile, wastewater treatment, agriculture, food, the paper industry, cosmetics, and biotechnology [21-26]. The application of chitosan in textiles can be categorized into two main topics: the production of man-made fibers and textile wet processing, which include dyeing (improving the dye-ability), finishing (antimicrobial properties), and printing [27-29]. Up to now, work was conducted to study chitosan applications in textile the industry as an antimicrobial agent, but there has been little work done on the application of chitosan to improve the dye-ability of textiles because of increasing the cationic site in silk fiber [30]. The application of chitosan to silk could reduce the use of dyes and acid due to increased dye exhaustion, which has positive effect on textile wastewater. The majority of the chemicals used for the cationization of silk are not safe environmental use of chitosan, where a polycationic biopolymer is more eco-friendly.

II. MATERIALS AND METHODOLOGY

A. Materials and Chemicals

100% scoured and bleached silk yarn was used and its count was 5 Tex. Chitosan was used as cationizing agent and prepared in the laboratory in the standard method. White Chitosan chips were used 1.0% distilled acetic acid was used to dissolve the Chitosan. Bestalin Red SF was used for acid dyeing. Leveling agent was used for level dyeing. All chemicals were purchased from Dysin and solvent was distilled previous use. Chitosan was used for cationization of silk with 0.5% and 1.0% and acetic acid solution was used to dissolve chitosan for making two different concentration of chitosan solution for 0.5% & 1.0%.

B. Weight of sample

The sample was conditioned at 20° C temperatures for 30 minutes. Then the sample was weighted in gm by an electronic balance.

C. Determination of polymer loading

Silk yarn was treated by Chitosan using exhaust method at 40 ° C and 60 ° C for 30 minutes. The samples were washed two times (hot and cold wash) and dried in dryer machine. Then the dried sample was conditioned 20 ° C temperature for 30 minutes. After that weight was determined and finally polymer loading was calculated by difference between conditioned weight of sample after and before application of Chitosan at 20° C for 30 minutes.

$$\text{Polymer loading, \%} = \frac{W_2 - W_1}{W_1} \times 100$$

Here, W₂=conditioned weight of the sample after treatment, W₁=conditioned weight of the sample before treatment.

D. Determination of strength

We use the Titan Universal Strength Tester which is packed with features which ensure its success in the textile industry. Standard: ISO 2062 (250mm-250mm/min).

E. Dyeing process of silk yarn

The scoured and bleached yarn was dyed with Bestalin Red SF (Dysin group) for 0.5% shade. Five samples were dyed with total weight 1.25 gm having ratio 1: 10 of M : L. The dye bath was run for 30 minutes at temperature 90° C at pH 6.0. The bath was drained at 40°C. Then the sample was washed at normal temperature until neutralization (pH =7.0). Then the silk yarn treated with 0.5% and 1.0% chitosan was dyed in similar way.

F. Measurement 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 by spectrophotometer at wave length 500 nm. The K/S of untreated and pretreated silk fabrics with Moreinga Olefera was evaluated. The reflectance value of a sample for the wavelength of 400nm - 700nm with 10 nm intervals was found using data color 600 nm Spectrophotometer. By using this reflectance value into the Kubelka Munk’s equation we can get the color strength (K/S).

Color strength, $\frac{k}{s} = \frac{(1-R)^2}{2}$ Where R is the reflectance, K is absorbance and S is the scattering.

G. Determination of wash fastness

The sample was tightening with SDC multifibre (DW). The sample was washed by 5 g/L detergent in ratio of 1: 20 of material with respect to solvent for 30 minutes at Temperature 60 ° C. Then the sample was removed and rinsed twice in cold water. Then dried and assessed for color loss and the adjacent yarn were assessed for staining.

III. RESULTS AND DISCUSSION

A. Evidence of cross-linking of chitosan with silk

The evidence of cross-linking of chitosan with silk is given by FT-IR (Fourier Transform Infrared Spectrometer, a SHIMADZU FT-IR spectrophotometer, Japan in the spectra range 600 -4500 cm-1 using the KBr disc technique). The infrared spectrum of untreated silk, 0.5% and 1.0% chitosan treated silk dyed silk are studied here. From the spectrum of untreated and chitosan treated silk are approximately same except the peak absorb at 1635 cm⁻¹ which indicate the N-H bond absorption. From the peak of FTIR spectrophotometer, chitosan treated silk spectrum is

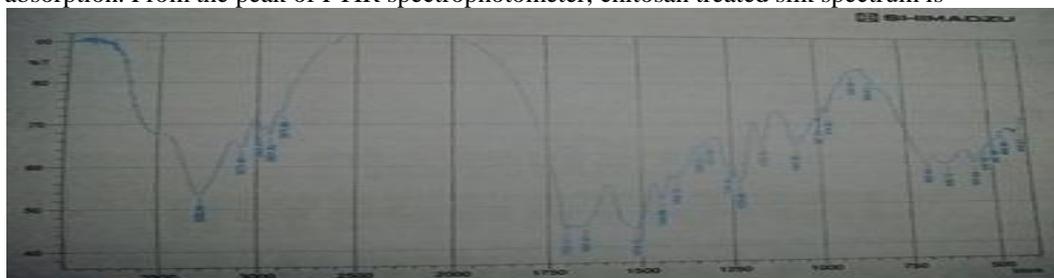


Figure 1: FTIR Peak of 1.0 % chitosan treated silk yarn

compared with untreated silk yarn. All peaks of chitosan treated are sharper then untreated silk as the intensity decreases. This data proves that cross-linking of chitosan with silk yarn polymer increase the percentage of hydroxyl group peak in (3200-3350) cm⁻¹ and 2959 cm⁻¹ and 1690 cm⁻¹ are the peak of C-H bond for asymmetry and symmetry stretching. By observing the spectrum of 1% chitosan treated dyed silk, it is seen that the absence of 1635 cm⁻¹ absorption which proves the dye molecule react with the cationic site (NH₃⁺) and bonded with the silk polymer and colored it.

B. Analysis of polymer loading

Polymer loading % of treated yarn with different chitosan concentrated is given below:

Table01: Polymer loading of different Chitosan conc. (%) Treated silk yarn

Chitosan conc. (%)	Temperature (° C)	Polymer Loading (%)
0.5%	40	1.47
	60	1.516
1.0%	40	2.624
	60	2.804

Table02: Polymer loading of different chitosan conc. Treated yarn at 40⁰ C Temp.

Yarn type	Chitosan conc.(%)	Weight before treatment (W ₁) gm	Weight after treatment(W ₂)gm	Polymer loading(%)	Average polymer loading
1	0.5%	0.249	0.253	1.60	1.47%
		0.236	0.239	1.27	
		0.256	0.262	1.34	
		0.253	0.257	1.58	
		0.256	0.260	1.56	
2	1%	0.238	0.241	1.26	2.624%
		0.245	0.249	2.63	
		0.272	0.276	3.47	
		0.261	0.265	2.53	
		0.243	0.246	3.23	

It is observed that treated yarn with 0.5% chitosan concentration at 40⁰ C and 60⁰ C temperatures has a average polymer loading of 1.47% and 1.516% respectively. Similarly yarn treated with 1.0% chitosan concentration at 40⁰ C and 60⁰ C temperature have polymer loading of 2.624% and 2.804%. We found from the table 02 that polymer loading increases significantly with the increase of chitosan concentration % and temperature.

C. Analysis of tensile strength and extension (%)

Tensile strength and extension of silk untreated and treated with chitosan conc. is given below:

Table03: Untreated and treated Silk yarn tensile Strength and extension (%)

Chitosan (%) conc.	Treated Temperature (°C)	Average Tensile / Load Centi-Newton (CN)	Average Extension (%)
0%	N/A	161.96	13.38
0.5%	40	158.97	12.59
	60	160.56	10.96
1%	40	160.09	10.78
	60	150.23	10.69

From the above observation, the average load of the untreated sample is 161.96 CN and the average extension percentage is 13.38%. The average load of samples is 158.97 CN and 160.56 CN where the average extension is 12.59% and 10.96% treated with 0.5% chitosan at 40°C and 60°C temperatures respectively. The average load of samples is 160.09 CN and 150.23 CN where the average extension is 10.78% and 10.69% treated with 1% chitosan at 40°C and 60°C temperatures respectively. So it is found that with the increase of Chitosan conc. (%) and temperatures, the silk yarn falls its strength slightly.

D. Analysis of color strength (k/s) value

The effect of pre-treatment with different concentration of cationic agent (chitosan) on K/S value of silk dyed with Bestalin Red SF is shown in the bellow table.

Table04: K/S values of treated and untreated dyed silk with Bestalin Red SF

Chitosan (%) conc.	Treated Temperature(°C)	Average K/S value
0%	N/A	4.5
0.5%	40	5.12
	60	5.56
1%	40	6.64
	60	7.10

From the above observations of the table04, It is found that the average k/s value of untreated sample is 4.5. The average k/s value of sample treated with 0.5% chitosan is 5.12 and 5.56 at 40°C and 60°C temperatures respectively. The average k/s value of sample treated with 1.0% chitosan is 6.64 and 7.10 at 40°C and 60°C temperature respectively. So we can say that K/S value increases with the increase of chitosan concentration and temperature and K/S values of treated silk yarn are higher than the untreated silk yarn.

E. Analysis of dye exhaustion percentage (%)

By analysis dye exhaustion (%) of treated untreated silk dyed with 0.5% and 1% shade, dye exhaustion (%) of treated and untreated dyed silk with Bestalin Red SF is also listed in the table.

Table05: Dye exhaustion (%) of different chitosan conc. (%) treated and untreated silk

Chitosan (%) conc.	Treated Temperature (°C)	Average Exhaustion (%)
0%	N/A	81.05
0.5%	40	84.25
	60	85.81
1%	40	86.82
	60	88.28

From the above observation, it is found that the average value of untreated sample is 81.05%. The average exhaustion (%) of samples treated with 0.5% chitosan is 84.25% and 85.8% at 40°C and 60°C temperature respectively. The average exhaustion (%) of samples treated with 1.0% chitosan is 6.64 and 7.10 at 40°C and 60°C temperatures respectively. So we can say that with the increase of chitosan conc. (%) the exhaustion percentage also increases.

F. Analysis of wash fastness

We analyzed color fastness to wash of treated untreated silk dyed with 0.5% and 1.0% shade. Wash fastness had assessed for color change and color staining with respect to ISO 105 C02. Fastness rating of different test samples is represented in table.

Table06: Wash fastness rating of different chitosan conc. (%) treated and untreated silk yarn

Chitosan Conc. (%)	Yarn type	Color Change	Color Staining					
			Acetate	Cotton	Nylon	Polyester	Acrylic	Wool
0.5%	Untreated Silk with Acid Dye	3-4	4	4	3	3	2	3-4
	Treated Silk with Acid Dye	4	4-5	4	3	3-4	2-3	3-4
1%	Untreated Silk with Acid Dye	3-4	4-5	4	1-2	3	4	3
	Treated Silk with Acid Dye	4	4	4	2-3	4	4-5	4

The table 06 gives the evidence that there is a little bit difference between treated and untreated dyed silk yarns. All samples dyed with Bestalin Red SF show good wash fastness. The average fastness rating for color change and color staining is 3-4 but the treated sample shows better value than the untreated sample for color change.

IV. Antimicrobial studies of silk yarn

The encouraging results of preliminary toxicological studies on silk yarn with different chitosan 0%, 0.5 % and 1.0% at temperature 40° C and 60 ° C provide good opportunities for the development of their safe use in industries. The

antimicrobial activity confirms their safe uses in any fields and establishes them as environment friendly materials [31]. In 60 ° C temperature, the yarn shows the high the color strength (k/s) value, polymer loading, exhaustion percentage (%) and wash fastness. So that to carry on the antimicrobial activity, the silk yarn at 60° C temperature was used. The antimicrobial activity confirms their safe uses in any fields and establishes them as environment friendly materials [32]. The antimicrobial activity test is the further measurement to require some views of environmental aspects as standard scale in well diffusion method [33-34]. To carry out the study, some tests are very close for experiment such as screening of anti-bacterial activity and antifungal activity.

A. Antibacterial Working Procedure

Raw materials are used as test chemical for antibacterial work and different concentrations were prepared using serial dilution technique taking one initial concentration as higher 20% gm/mL. Chemicals were dissolved in dimethylsulfer oxide (MDSO), and they act as positive control. Nutrient agar was prepared according to the instruction leveled in the container and kept in freezer for longer use. Dilute alcohol were used to ensure for septic environment during the culture preparation and bacterial inoculation. Media was spread in preti-plate at first and keep 40 minutes for solidification of media. Then bacterial suspension was uniformly spread using sterile hockey stick on a sterile petri dish on agar media. Then sample of 20 µml with solution were taken in each well in marking on petri-plate (5 mm diameter holes cut in the agar gel, 40 mm apart from one another) with control as DMSO in each plate. The systems were incubated for 24h at 36±1°C, under aerobic conditions. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in mm and recorded. Tests were performed in triplicate. Incubator for bacterial growth was maintained at temperature 37±1°C [34].

Table07: Data recorded for 20% gm/mL sample

Pathogens/ Sample	Silk yarn with 1.0% chitosan(mm)	Silk yarn with 0.5 % chitosan (mm)	Silk yarn with 0% chitosan(mm)	Control
<i>Bacillus cereus</i>	9.0	6.0	4.0	0.0
<i>Stophylococcus aureus</i>	8.0	6.0	5.0	0.0
<i>Escherichia coli,</i>	6.0	4.0	4.0	0.0
<i>Salmonella typhi</i>	6.0	3.0	2.0	0.0
<i>Psedomonous aeroginosa</i>	5.0	3.0	0.0	0.0
<i>Shigella dysenteriae</i>	5.0	4.0	0.0	0.0

The table07 is the evidence of antimicrobial activity increases with the increase the percentage of chitosan explained in figure 03.

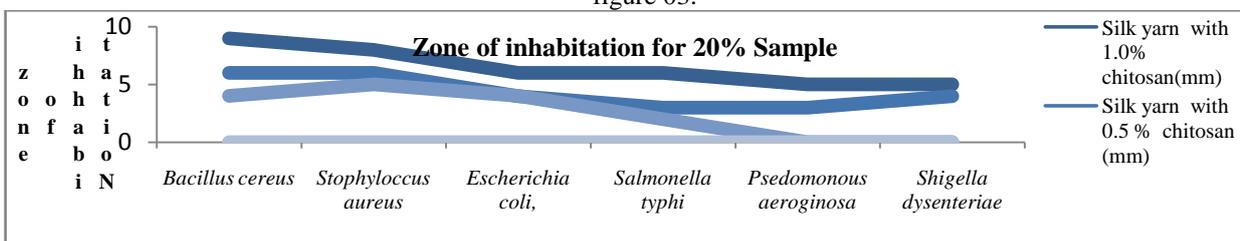


Figure 2 : Graph of 20% gm/mL sample against different bacteria

Table08: Data recorded for 10% gm/mL sample

Pathogens/ sample	Silk yarn with 1.0% chitosan(mm)	Silk yarn with 0.5 % chitosan (mm)	Silk yarn with 0% chitosan (mm)	Control
<i>Bacillus cereus</i>	5.0	2.0	0.0	0.0
<i>Stophylococcus aureus</i>	4.0	2.0	0.0	0.0
<i>Escherichia coli</i>	3.0	2.0	0.0	0.0
<i>Salmonella typhi</i>	3.0	0.0	0.0	0.0
<i>Psedomonous aeroginosa</i>	3.0	1.0	0.0	0.0
<i>Shigella dysenteriae</i>	3.0	2.0	0.0	0.0

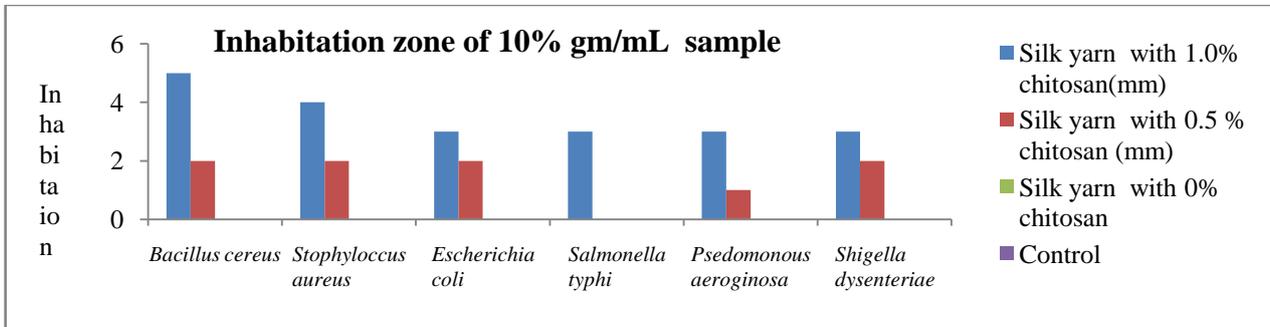


Figure 3 : Graph of 20% gm/mL sample against different bacteria

Table09: Data recorded for 1% gm/mL sample

Pathogens/ sample	Silk yarn with 0% chitosan(mm)	Silk yarn with 0.5 % chitosan (mm)	Silk yarn with 1.0% chitosan (mm)	Control
<i>Bacillus cereus</i>	0.0	0.0	0.0	0.0
<i>Staphylococcus aureus</i>	0.0	0.0	0.0	0.0
<i>Escherichia coli,</i>	0.0	0.0	0.0	0.0
<i>Salmonella typhi</i>	0.0	0.0	0.0	0.0
<i>Pseudomonas aeruginosa</i>	0.0	0.0	0.0	0.0
<i>Shigella dysenteriae</i>	0.0	0.0	0.0	0.0

The antimicrobial activity against the six bacteria for 20% gm/mL sample is shown that with decreasing the amount of chitosan, the antimicrobial activity decreases. That is represented by following graph

B. Antifungal Screening Test

Aspergillus niger and *Rhizopus azzahra* were used for evaluating the antifungal activity of all treated sample of yern silk with chitosan. The antifungal activity was evaluated by well diffusion method [37]. The media was altered Potato dextrose broth (abbreviated "PDB") is formulated identically to PDA, omitting the agar. Common organisms that can be cultured on PDB are molds such as *Aspergillus niger* and *Rhizopus azzahra*. all treated sample of yern silk with chitosan were dissolved in DMSO basis on their solubility for making the concentration 20%, 10% and 1% gm/mL .The 100 µL solution of ILs were taken in petri-plate. The Media of PDB was dispersed and solidified. A well of 5 mm were made in the middle of petri-plate using cork-borer and the fungal lead were place there. The plates were then kept in incubator for 96 h at 37°C. After 3 days, the fungal growth in presence of ILs, were measured and analyzed.

Table09: Data recorded for antifungal activity

Chemicals tested	Zone of growth (in mm)		Percent Growth, %	
	<i>Aspergillus niger</i>	<i>Rhizopus azzahra</i>	<i>Aspergillus niger</i>	<i>Rhizopus azzahra</i>
Control	28 mm	41mm	100.0	100.0
Silk yarn with 0% chitosan(mm)	20.5±1.0	29.2±1.0	78.57	71.34
Silk yarn with 1.0 % chitosan (mm)	19.0±1.0	23.2±1.0	67.80	81.09
Silk yarn with 0.5% chitosan(mm)	15.0±1.0	18.0±1.0	89.28	75.60

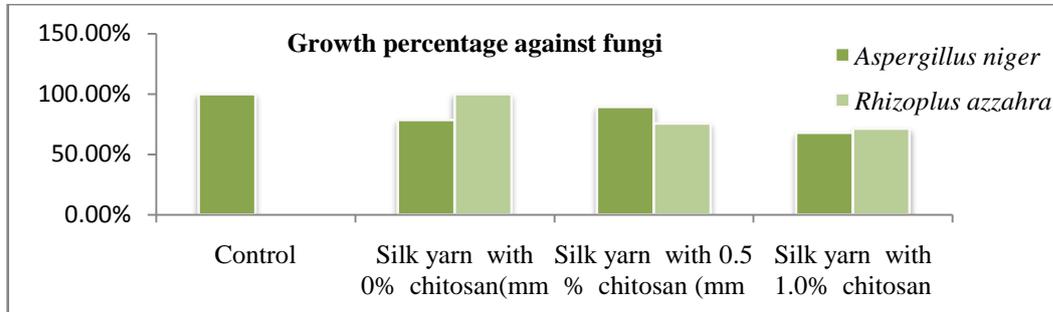


Figure 4: Graph for different silk against fungi

The antifungal test was completed and calculated the growth percentage compared with the control where the growth of control is 100% percent. The growth percentage is deduced as the following equation:

$$\text{Growth percentage, \%} = \frac{\text{Growth of fungi with ILS solution}}{\text{Growth of fungi without ILS solution as control}} \times 100$$

From the table 09, it was shown that all samples have poor antifungal activity. With increasing the percentage of chitosan, the antifungal activity increases.

V. CONCLUSION

The application of Chitosan on silk shows the satisfactory results with regard to cat-ionize silk and further in dyeing. The treated silk have significant amount of polymer loading, providing cationic site on silk determined by FTIR spectrophotometer. The modifications show an overall suitability for different acid dyes. The modified dyeing doesn't suffer either from a significant drop in color strength, wash fastness. The dyed silk yarn treated with chitosan shows higher color strength (k/s value), the higher exhaustion percentage and better wash fastness than the untreated silk yarn. But only tensile strength decreases in the increasing of chitosan percentages.

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