ANTIMICROBIAL EFFECT OF CI BASIC GREEN 4 (MALACHITE GREEN) AGAINST SOME PATHOGENIC BACTERIA

CI BAZİK YEŞİL 4 (MALAHİT YEŞİLİ) BOYASININ BAZI PATOJENİK BAKTERİLERE KARŞI ANTİBAKTERİYEL ETKİSİ

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ABSTRACT

In this study, antimicrobial and dyeing properties of CI Basic Green 4 dye and dyed acrylic fabrics have been studied against the common pathogens *Escherichia coli, Bacillus subtilis, Staphylococcus aureus,* and *Pseudomonas aeruginosa*. CI Basic green 4 dye was effective against selected test bacteria *E. coli*, *B. subtilis*, *S. aureus, and P. aeruginosa* respectively. The dyed acrylic fabric with malachite green cationic dye displayed various antimicrobial activity (reduction rate: 0-90.00 %) against selected microbes.

Key Words: Malachite green, Antimicrobial, CI Basic Green 4, Acrylic, Cationic dye.

ÖZET

Bu çalışmada, CI Bazik Yeşil 4 boyası ve boyalı akrilik kumaşa ait boyanma özelliği ve yaygın patojen *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* ve *Pseudomonas aeruginosa* bakterilerine karşı antibakteriyel özellik çalışılmıştır. CI Bazik Yeşil 4 boyası seçilen test bakteri olan *E. coli*, *B. subtilis*, *S. aureus ve P. aeruginosa* karşı etkilidir. Malahit yeşili katyonik boyası ile boyanan akrilik kumaş seçilen bakterilere karşı farkı antibakteriyel aktivite (düşüş oranı: % 0-90) göstermiştir.

Anahtar Kelimeler: Malahit yeşili, Antimikrobiyal, CI Bazik Yeşil 4, Akrilik, Katyonik boya

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1. INTRODUCTION

Cationic dye act as bases and when made soluble in water, they form a colored cationic salt; this can connect with the anionic sites on the surface of the substrate. These dyes are used in the coloration of acrylic fiber that widely used synthetic fabrics due to a combination of desirable properties, such as soft wool-like handle, good elasticity and mechanical properties (1). Textile materials are known to be susceptible to microbial attack, as the fibers provide a large surface area and absorb moisture, thus generating a suitable environment for microbial growth and multiplication. Damage to textiles by micro-organisms can occur at nearly all stages of manufacture. This often leads to objectionable odor, product deterioration, dermal infection, allergic responses and risks associated with transfer of microbes (2-3). Bacteria are living organisms that have only one cell. Under a microscope, they look like balls, rods and spirals. If bacteria form a parasitic association with other organisms, they are classed as pathogens. Pathogenic bacteria are a major cause of disease and infection. The properties of textile materials against harmful bacteria are important for user (4). In recent decades, there has been an increasing tendency towards the prevention of microbial attack on textile materials by providing the necessary requirements such as oxygen, moisture, nutrients and suitable temperature to counteract bacterial growth and multiplication (5-6). Many cationic dyes possess antimicrobial activity and the most commonly used antimicrobial colorants are triphenylmethane dyes (7-9). Various finishing chemicals, especially silver related products, have been applied to textile materials for their antibacterial properties (10-13). Sawa *et al.* investigated six basic dyes antimicrobial activity against selected two bacteria (14). Their study demonstrated that used cationic dyes have antibacterial activity depending on the chemical structure of dyes. Demir *et al*. reported that chitosan posses antimicrobial effect (15). The aim of this study was to determine what effect the application of Malachite Green cationic dye to acrylic fabrics had on antimicrobial activity. For this purpose, this dye was tested against selected bacteria both in solution *in vitro* and dyed fabrics *in situ*.

2. MATERIAL AND METHOD

2.1. Dye

The commercial triphenylmethane dye, Synacryl M Green 4 (CI Basic Green 4) was used as obtained from the dye manufacturer (Alfa Chem, Turkey) without further purification. The dyestuff structure is shown in Figure 1.

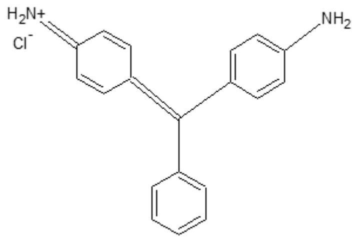

Figure 1. The chemical structure of the CI Basic Green 4 (Malachite Green) cationic dye

2.2 Fabric and Dyeing

The scoured acrylic fabrics used in this study were supplied by Aksa Acrylic Company (Turkey). The characteristics of the fabric were plain, knitted and a weight of 150 g/m2. The dyeing was carried out at two different depth, 1%, and 2% owf (on weight of fabric), at 1:20 liquor ratio, and at pH 5-5.5. The dyeing procedure started at 25 ^{o} C and after remained in the dyeing bath 10 min the temperature was increased to 95 ^{o} C (1 ^{o} C/min). The acrylic fabrics were dyed for 45 min (16). The dyed fabric samples were further treated with 1g/l of the non-ionic detergent Setalan BNH (Setas Chem, Turkey) at boil for 15 min, and rinsed in first hot and then with cold water. In order to examine the dyeing performance of the dyed acrylic fabric, the color strength and color fastness to light, washing and rubbing were carried out according to, ISO 105-B02, ISO 105-CO6:1997 C2S, and ISO 105-X12 respectively. The dyeing diagram is given in Figure 2.

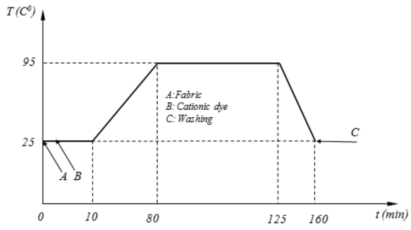

Figure 2. Acrylic fabric dyeing diagram

2.3. Color Strength

The surface reflectance measurements of the dyed acrylic fabrics with MG dye was determined using Konica Minolta 3600d spectrophotometer and color measurements software (RealColor v1.3). Color intensity was expressed as K/S values of the dyed samples using the Kubelka-Munk equation:

$$K/S = \frac{(1 - R)^2}{2R} \quad (1)$$

Where R is the decimal fraction of the reflectance of the dyed sample, K is the absorption and S is the scattering coefficient (17).

2.4. Test Bacteria

Cultures of the following five different bacteria were used in the study: Before the antimicrobial tests, all bacteria from the fresh culture were grown in nutrient broth at 37 ^{o} C for 18 h. Each bacterium culture had 10^7 cfu (colony forming unit) /ml cells.

2.5 Antimicrobial Tests

In Vitro the antimicrobial effect of the MG cationic dye varied when they were present in the solution and held intimately by the acrylic fabric. In the first set of experiments diffusion agar test method was used for the aqueous sterilized MG dye solutions of three different (1%, 2% and 5%) concentration levels. A nutrient agar medium (g/l: peptone 5.0; beef extract 1.5; yeast extract 1.5; NaCl 5.0; agar 20; pH 7.5) was prepared and autoclaved at 121 ^{o} C for 15 min. Test organisms were grown overnight at 37 ^{o} C, in 10 ml nutrient broth. 1 ml bacterial cultures were added to the cooled 100 ml nutrient agar (1 %). Sterilized Petri plates with three wells opened (4 mm in diameter) were prepared with an equal thickness of nutrient agar. 60 microliters of MG dye solution was poured into wells on nutrient agar. *E. coli, B. subtilis, S. aureus,and P. aeruginosa* test Petri plates were incubated at 37 ^{o} C for 24 h. At the end of this period, zone of inhibition (ZOI) formed on the medium were measured in millimeters (mm) (18-19).

In situ set of experiments, AATCC 100 test method was used for the bacteriostatic activity (inhibition of multiplication) as well as bactericidal activity (killing of bacteria) of dyed acrylic fabrics against selected bacterial species. The 48 × 48 mm of sterile fabric sample (undyed and dyed) was introduced into the 10 ml nutrient broth inoculated with the desired microbe and incubated at 37 ^{o} C overnight (18 h). At 0 and 24 h, the number of colonies in the tubes was counted using the serial dilution method on nutrient agar. The reduction percent caused by each concentration of the dyed and undyed samples on test bacteria was expressed as follows:

$$R = \frac{C_0 - C}{C} \times 100\ (\%) \quad (2)$$

Where C_0 (cfu) is the number of microbial colonies on the control fabric (undyed fabric) and C (cfu) is the number of microbial colonies on the dyed fabric. R (%) is the reduction in bacterial population (20).

Table 1. The properties of the used bacteries

Bacteria codes	Bacteria names	ATCC number	gram +/-
B1	*Escherichia coli*	ATCC 11229	gram -
B2	*Bacillus subtilis*	ATCC 6633	gram +
B3	*Staphylococcus aureus*	ATCC 25923	gram +
B4	*Pseudomonas aeruginosa*	ATCC 27853	gram -

3. RESULTS AND DISCUSSION

3.1. Color Strength and Fastness Properties

Acrylic fabrics were dyed with MG cationic dye at two concentrations (1 and 2 % owf). In Table 2, the color strength and fastness results are presented according to the dyeing depths of dye. The K/S values of the dyed fabrics increase depending on the dyeing concentration; light fastness of the dyed fabrics is moderate, and the washing and rubbing fastness levels of the dyed fabrics are good.

3.2. Antimicrobial Activity of Cationic Dyes in Solution

The three different concentrations of MG dye were screened for their antimicrobial activity against selected microbes (*E. coli, B. subtilis, S. aureus, and P. aeruginosa*). Examples of a clear ZOI by MG against selected microbes can be seen in Figures 3.

The effect of MG dye concentrations on antimicrobial activity was studied further and the results are summarized in Table 3. A zone of inhibition (diameter, mm) was determined in each case. It was observed that an increase in dye concentration lead to an increased ZOI as can be seen by the enhancement in the zone diameter. The preliminary screening showed that MG dye was effective against all the microbes in all concentrations.

Table 2. Dyeing performance of acrylic fabrics with MG cationic dye

Dyeing depth (%)	Color stren. (K/S)	Light Fast.	Washing fast.		Rubbing fast.	
			Chan.	Stain.	Dry	Wet
1	39.08	3	5	4/5	5	4/5
2	43.76	3/4	5	4/5	5	4/5

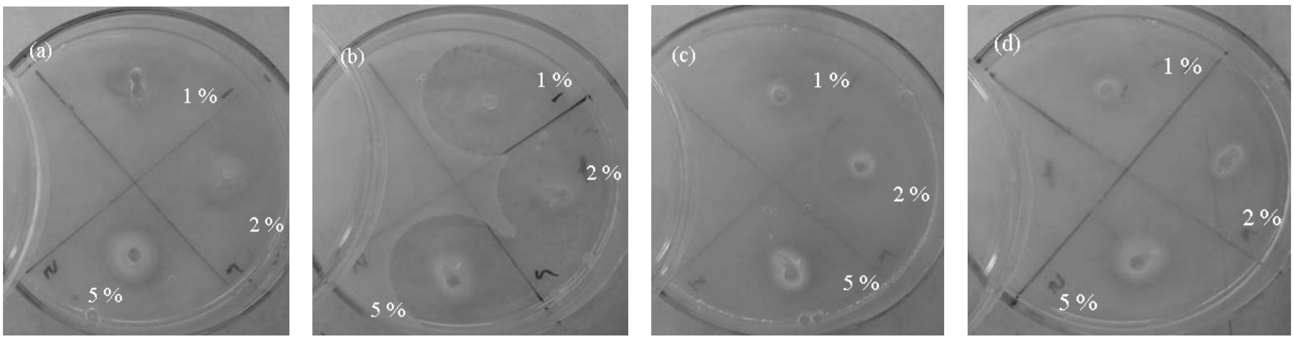

Figure 3. Antimicrobial activity of the MG dye against selected microbes: *E. coli* (a), *B. subtilis* (b), *S. aureus (c), and P. aeruginosa* (d) (the dye is effective at all concentrations, 1%, 2%, and 5% respectively)

Table 3. Zone of inhibition for cationic dyes against selected microbes

Dye conc. (%)	Zone of inhibition (diameter, mm)			
	E. coli	*B. subtilis*	*S. aureus*	*P. aeruginosa*
1	17.00	38.00	24.00	11.46
2	21.33	38.00	25.33	14.00
5	21.66	41.66	26.33	15.33

-: ineffective

3.3. Antimicrobial Activity of MG Dye on the Dyed Acrylic Fabrics

The percentage of microbial reduction of acrylic fabrics dyed with MG dye at two different dyeing concentration levels (1%, and 2%) are given in Figure 4. Acrylic fabric dyed with MG dye exhibited excellent microbial reduction against *S. aureus* (88.50-90.00 %), *B. subtilis* (55.40-59.90 %) and *P. aeruginosa* (84.60-88.90 %). However acrylic fabric dyed with MG dye was ineffective against *E. coli.* This was probably due to the adherent of *E.coli* bacteria in special to the dye would be decreased by formation of a complex structure comprising both acrylic fibre and MG dye. The maximum antimicrobial activity on dyed acrylic fabric was achieved with 2% dyeing concentration against *S. aureus* (90.00 %); the minimum antimicrobial activity on dyed acrylic fabric was achieved with 1% dyeing concentration against *B. subtilis* (55.40 %).

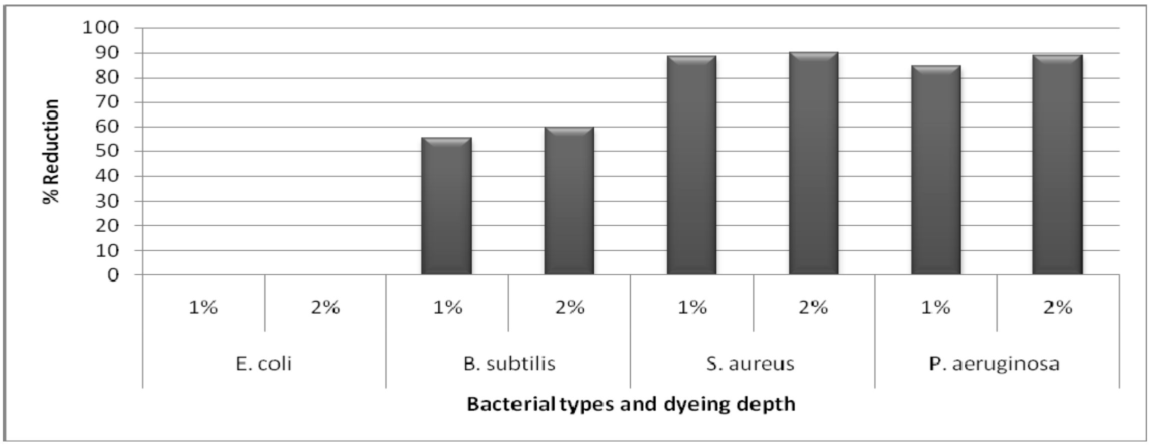

Figure 4. Antimicrobial activity of dyed acrylic fabrics

4. CONCLUSIONS

Malachite Green cationic dye and dyed acrylic fabric was tested against pathogenic bacteria: *E. coli, B. subtilis, S. aureus, and P. aeruginosa*. In the first part of the study aqueous solutions of the MG dye was prepared and tested against selected microbes. In the second part of the experiment acrylic fabric dyed with the dye was tested against the same microbes. While MG dye solution was effective various levels against selective all bacteria in diffusion agar test method, dyed acrylic fabric was not active against *E. coli.*

In Conclusion, it can be said that MG dye has excellent antibacterial property in liquid form, however, on dyed fabrics, this antimicrobial effect decreases probably due to having a complex structure interaction between the dye and fabric. MG dye can be applied onto acrylic fabrics goods to protect them from the negative effects of *B. subtilis, S. aureus, and P. aeruginosa* pathogenic microbes.

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