

Original Research Article

Exploring Textile Dye form Microorganisms, an Eco-friendly Alternative.

ABSTRACT (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

Aims: Being an ancient art, the concept of dyeing using natural resource is neither a noble issue. To develop a green and sustainable world, the natural resources are now common sought. In this study screening of pigment producing microorganisms and extraction of pigments were the prime concern.

Study design: An environmental screening was performed to isolate pigmented microorganisms. Extractability of the pigments and dying capability of textiles were also evaluated.

Place and Duration of Study: Soil, water and air were sampled and cultured from different region around Jessore, Bangladesh. Isolation and tests were performed at Jessore University of Science and Technology.

Methodology: Soil and water samples were inoculated on bacterial and fungal media. Media were exposed for 1h to air for air sampling. Pigmented isolates were cultured in bulk, dried and went through solvent extraction by ethanol and water. Soluble pigment producing isolates were identified and dying capability to cotton and silk were evaluated. Pigments were also tested for antimicrobial activity and allergic test to human skin to be used for medicated fabric.

Results: A few pigmented bacteria and several pigmented fungi were isolated. By solvent extraction, only two colors were extracted having greater solubility. The isolates were preliminarily identified as *Aspergillus* sp. and *Penicillium* sp., producing green and red color respectively. The dying capacity to cotton and silk fabrics were found satisfactory in respect to wash fastness. The extracted colors also showed antimicrobial activity against several pathogenic microorganisms. Pigments were also subjected to hypersensitivity test and found non allergic to human skin.

Conclusion: However, these dyes have potential to be used in sophisticated garment for allergic patients to chemical dyes and infants. As the biomass yielding capability of pigmented fungi were very low in the designed media, further modification of media and growth conditions are to be optimized to maximize biomass and large-scale dye production.

Keywords: Allergic, Fungi, Textile dye, Solvent extraction, Wash fastness..

1. INTRODUCTION

In the question of proliferated awareness of environmental pollution and health injury associated with processing and synthesis of synthetic dye, a global interest has raised for innovation of natural dye. The coloring stuff which is used to stain a variety of materials aesthetically is commonly known as dye [1]. Textile processing industry is remarked as one of the major environmental polluters as the disposal from these industries contains a profuse amount of chemicals. Most of the synthetic colorants are synthesized by petrochemical agents with a lot of chemical processing [2]. There are two main ways to limit the environmental impact of textile processing. One is to construct bigger and highly efficient effluent treatment plants, and the other way is to make use of dyes and chemicals that are environment friendly [3]. Excessive use of colorants in dyeing industries and unproductive dyeing capabilities of those dyestuffs result in directly mix up with the waste water released into the environment. Rai et al [4] reported that, while dyeing process an estimated about 10-35% of the colorants is wasted in the effluent. Moreover, peoples directly exposed to these dyes might fall in danger chronically as the element contains potential colon carcinogens [5]. Henceforth, the yearning demand for eco-friendly colorants especially for

application as food colorants, child textile and leather industries product is at the zenith of mind of conscious personalities [6].

This study was conducted to find out some natural resources from the environment to isolate pigment producers for dyeing from which a wide range of shades could be originated. Microorganisms like bacteria and fungi have the potentiality to produce pigments. Bacteria like *Chromobacterium violaceum*, *Serratia marcescens* and *Chryseobacterium* sp. have the potentiality to produce violet, red and yellow-orange pigments [7]. But the source of pigment producing bacteria is not easily tractable in the environment as they found in mainly harsh condition of industrial effluent, tropical and subtropical regions. Similarly a range of fungus like *Monascus purpureus*, *Emmerciella* sp., *Penicillium* sp., *Fusarium* sp., *Thermomyces* sp. etc. has been reported to be effective pigment producers [6, 8].

In several studies it has already been reported that natural dyes are started to be used in coloring fabrics like cotton, silk, wool, nylon etc. [9]. Moreover the growing interest on natural colorants also keeps the researchers busy to innovate convenient techniques, improvement of the product and uses them in authentic way. However, as being natural the product has some drawbacks on high yield production because very minute amount of dye may be extracted from per kilogram of raw materials. To cope up with this hindrance, it was suggested to apply appropriate selection techniques, mutation or genetic engineering techniques on bacteria, fungi or plant cell cultures for production of elevated amount of dyestuff [10]. Microorganisms for instance, bacteria as well as fungi are reported to be potent pigment producer [11]. So, study with these creatures from environmental sample will unveil their hidden capability and application to mankind.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

2.1. Sample collection

In this study a number of 113 samples were collected from soil, air and water. From all of those samples both bacteria and fungus were investigated and isolated. As the main aim of this study was to isolate pigment producing microorganisms, non-pigmented isolates were kept out. Air samples were collected using settle plate techniques whereas soil and water samples were collected using proper methods described by APHA [12]. During sample collection, materials used for collection were sterilized properly and transferred immediately into the laboratory after collection.

2.2. Isolation and Identification of Microorganisms

2.2.1. Isolation and Identification of Bacteria

For isolating bacteria from air and soil only nutrient agar media was used but for water samples at first it was enriched in nutrient broth media in different dilution and then they were further transferred in nutrient agar plates. After transferring they were incubated at 37°C for 24 hours. For confirming the bacterial community, isolates were forwarded to do biochemical tests.

2.2.2. Isolation and Identification of Fungi

As a wide range of fungus produce colorful spores, they were the main target for pigment production and extraction. From all of the sources the samples were transferred to the potato dextrose agar and incubated at 25°C for 5-7 days and any shades of colors were observed. If there any noticeable colors found they were further transferred into the Malt Extract agar (MEA) media and Molasses media (MM) for bulk production according to the specific color range in individual plates. Fungal strains were confirmed by staining with lacto-phenol cotton blue under microscope.

2.3. Cultivation of Microorganisms

Due to inefficiency of pigment producing bacteria the study was carried out only through fungi for pigment production. The cultivation of individual filamentous colored fungi were done by using defined mineral salt-glucose medium contained (per liter of deionized water): glucose 30 g; (NH₄)₂SO₄ 1.0 g; MgSO₄.7H₂O 0.5 g; K₂HPO₄ 1.4 g; KH₂PO₄ 0.6 g; NaMoO₄.2H₂O 0.8 mg; ZnSO₄.H₂O 0.8 mg; FeCl₃.6H₂O 0.8 mg;

MnSO₄·2H₂O 0.4 mg; CuSO₄·5H₂O 0.08 mg and pH was adjusted at 5.6 [13]. For cultivation individual flasks were poured with 150ml liquid media. Using sterile cork borer (12mm in diameter) fungal isolates were inoculated into the chemically defined media and further incubated at 25°C in a dark place for 4-6 weeks. After proper incubation fungal biomass were observed for desired color production. If any glaring color was found through exhausted broth then it was extracted directly through solvent extraction method.

2.4. Filtration of exhausted broth

The filtration of colored fungal mat over the CDM broth was done using Whattman filter No.1 with the help of Vacuum filtration apparatus (UNILAB, USA). It was observed that, the denser the fungal mat the more time was needed to filter the exhausted broth. After filtration, the fungal mat on the filter paper was collected and dried at 60°C for one to 12 hours until it turns desiccated and then converted into powdered form by using mortar. The powdered materials were then further stored and treated with methanol and ethanol for dye extraction.

2.5. Extraction of Color

When the extract turns into powdered form they were subjected to dissolve in absolute methanol and ethanol solution for solvent extraction. In case of water soluble color the colored broth was also applied to solvent extraction method. After 48 to 72 hours the solvent dissolved materials were again filtrated in vacuum filtration machine and allowed to dry using water bath at 45-50°C.

2.6. Coloring of fabrics

2.6.1. Preparation of Mordant

For the experiment we used alum (aluminum potassium sulfate) as a mordant. But using more alum has its own drawback that it causes the yarn sticky. To subside this condition, alum was generally combined with cream of tartar [14]. 5% solution of alum was prepared in hot water as mordant.

2.6.2. Preparation of Dyes

The respective dried powders were dissolved in methanol into small flakes. 2 ml of each dyeing components were added to 18 ml of Sulfur and Vat chemicals, boiled at 60-70 °C in a closed bath for 1 h. Then the liquor was filtered and stored (Figure 3). Maximum validity of that liquor was 6-8 hours.

Table 1: Weight chart of extracted dye before and after extraction

ID	Weight before extraction (mg)	Weight after extraction (mg)
Olive (2nd)	348	NC*
Black	597	80
Deep olive(1st)	362	NC*
Lemon	378	NC*
Red (S-2, 10 ⁻⁴)	586	72
Brown (1st)	463	NC*
Deep Green	1130	NC*

NC*= No soluble color produced

2.6.3. Fabric pre treatment

Two pieces of plain weave cotton and silk fabric were used for dyeing. At first, fabrics were cleansed thoroughly to keep apart starch and other impurities. The cotton fabric was boiled for 20 min in a solution containing 4% liquid soap, 8% soda ash and 2% caustic soda and was then washed off in clean water and soaked in a 3% solution of bleaching powder for 5-10 min. The fabric was given a final rinse with cold water. On the other hand the silk fabric was treated only with 4% liquid soap and then rinsed with water.

2.6.4. Mordant and Dyeing

The prepared mordant was mixed into the dyeing solutions and stirred constantly. The concentrations and volume of mordant-dye mixture will depend on the fabric (texture, thickness and area) and the color intensities desired. In this study, 2g of fabric to be dyed was put in 80 ml of warm alum solution for 30 min. After squeezing, the sample was put into 20 ml of dyeing liquor for 1 h at 60°C. For control, similar type of fabric was put into 80 ml of mordant and treated as it was done for test fabrics, and then put into 20 ml of autoclaved distilled water instead of dyeing liquor for 1 h at 60°C. The dyed fabrics as well as control fabrics were washed thoroughly in liquid soap and plenty of cold water till the water ran crystal clear [15].

2.6.5. Optimization of dye uptake quality:

Basically dye uptake property is defined by the unspent amount and exhaustion rate of dye inside the fiber. This property ensures the percent of color uptake into the fiber within given amount of time and temperature. The percent of color exhaustion was calculated by

$$\% \text{ Dye exhaustion} = [(C_g - C_t)/C_g] \times 100$$

Here, C_g is the concentration of dye used and C_t is the concentration of dye spent. The dye exhaustion properties of the dyed fabrics were measured using spectrophotometer according to ISO standard recipe.

2.7. Hypersensitivity of dye on human skin

It has been reported by many scientists that fungal spores has direct role on hypersensitivity reaction on human. As the isolated dyes were fungal origins, so those dyes were subjected to allergic or hypersensitivity testing using standard method called "Patch test" [16]. In this test the methanol soluble dyes were subjected to scratched on human skin with the help of sterile cotton bud and kept it 30 minutes for observing any redness or itching present on tested area.

2.8. Study of Antimicrobial effectiveness of extracted dye

The test was performed in a standard disk diffusion technique described by Kirby & Bauer [17]. For doing this test, at first pure bacterial cultures were swabbed over Muller Hinton agar (OXOID, UK). Imipenem (10µg) (OXOID, UK) was used as standard antibiotic disk and commercially available blank disks (6mm) (OXOID, UK) were used for absorbing dyeing materials in it and placed on plates inoculated with different bacteria. Only methanol soaked disks were used as control measure. After transferring all of the tested disks, plates were incubated at 37°C for 18 hours.

3. RESULTS AND DISCUSSION

In this study, searching for better alternative of synthetic dye was the prime concern. For that perspective, optimization of bacterial and fungal dye from the environment and their application in different types of fabrics was inspired as better alternative as natural dye. Pigments would be produced as active biocatalyst or as secondary metabolites of microorganisms for example, β - carotene, canthaxanthin, lycopene, monoscoflavin, zeaxanthin etc [18]. Beside pigment production these metabolites have the potentiality to be active against different pathogenic microorganisms. Among 113 samples, a wide range of bacteria and fungus were isolated. Bacterial isolates including *Streptococcus sp.*, *Staphylococcus sp.*, *Proteus sp.*, *Klebsiella sp.*, *Bacillus sp.*, *Pseudomonas sp.* were found but none of these were pigmented; hence kept unattended for further study. On the other hand fungal isolates including *Aspergillus sp.*, *Penicillius sp.*, *Fusarium sp.*, *Trichoderma sp.* were found. These isolates were confirmed by doing microscopy and conventional biochemical tests.

Table 2. List of fungal species found in different samples.

Sample source	Fungus
Air	<i>Aspergillus sp.</i>
	<i>Fusarium sp.</i>
Soil	<i>Aspergillus sp.</i>
	<i>Fusarium sp.</i>
	<i>Trichoderma sp.</i>
	<i>Penicillius sp.</i>
Water	<i>Aspergillus sp.</i>
	<i>Trichoderma sp.</i>

A number of fungal isolates were found to produce visible pigments of different shades and further studied. *Aspergillus sp.* which produced black spores both in the culture plate and cultivation broth was responsible for the green shade after extraction. Meanwhile the *Penicillium sp.* produced red color in the exhaustion broth. It was really surprising that most of the isolated fungal species were able to produce a different color of spores but after extraction those color was not recoverable. The main reason behind this miracle might be lack of solubility of colors of that isolates or those pigments were not extracellular.

Dried powdered biomasses were weighted and solvent extracted with methanol and ethanol. Among seven types of pigments only two types of pigments were soluble in solvent. From 598 mg of black pigmented spores only 80 mg purified green dye was extracted and from 586 mg dried red biomass, 72 mg purified red dye was devised (Table 1). After extraction dyes were further treated with Vat and Sulfur chemicals as well as mordant to color fabrics. The absorbance spectrums of extracted dyes were also measured by spectrophotometer under a range of different wavelength (Fig. 1).

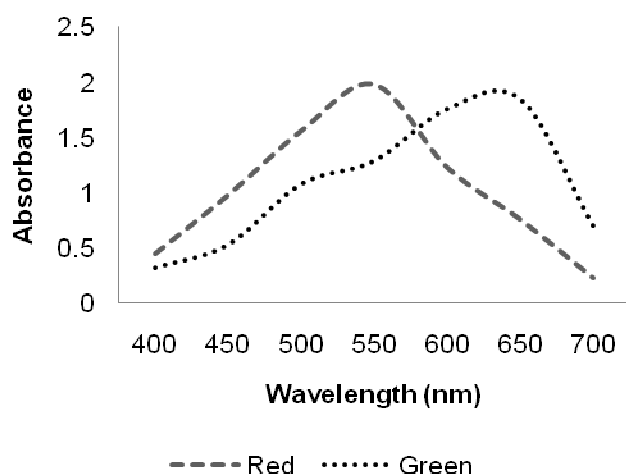
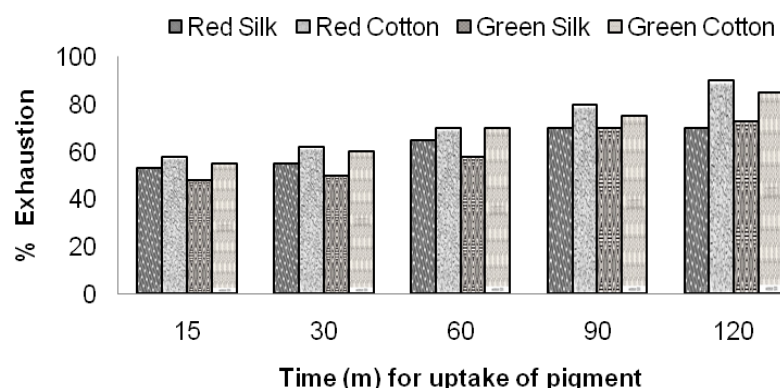


Fig. 1. Absorbance of red and green color dye in different wavelength.

Silk and cotton fabrics were treated with the extracted color and incubated about 120 min. After proper incubation and wash fastness the shade of red and green colors in the fabrics were observed. Visually the color retention quality of the silk was relatively better than cotton. It was show that the absorbance range of red and green color peaked at 450-650nm which was related to color absorbance spectrum.

After started dyeing the time was recorded for best dye uptake moment. So, it was observed that with the increase of time and maintenance of optimum temperature the dye uptake property was increased. Red and green dyes were absorbed about 45-55% after 15 min treatment for both silk and cotton fabrics. Uptake time of color in silk and cotton was varied. Silk showed best uptake after 90 minutes which was

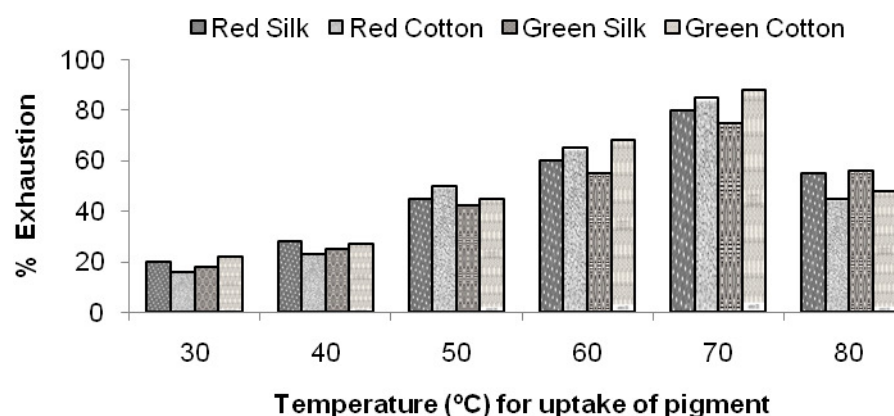
210 75-85% in range comparing to cotton whose best uptake about 75-90% was occurred after 120 minutes
 211 (Fig. 2). Some natural dyes also have best exhaustion time is about 60 minutes reported [8].



212

213 **Fig. 2. Effect of time on pigment uptake into at 60°C temperature.**

214 Temperature played a crucial role on dyeing fabrics. The experiment was continued within a different
 215 temperature ranges. Dye uptake was started at 30°C for both silk and cotton (15-25%) but best outcome
 216 (80-95%) was recorded at 65-70°C (Fig. 3). After optimum uptake temperature the dye uptake rate
 217 became subsided (45-60%) at 80°C which would be the reason of instability of natural biomolecules at
 218 higher temperature.



219

220 **Fig. 3. Effect of temperature on pigment uptake into fabrics.**

221
 222 Eight types of pathogenic bacteria were tested with extracted dyes for their antimicrobial potentiality.
 223 Colors beyond to *Aspergillus* sp. and *Penicillium* sp. both showed antimicrobial properties against a range
 224 of bacteria. Though not much effective, they have least potentiality to inhibit microorganisms for example,
 225 *Klebsiella pneumoniae*, *Shigella sonnei* and *E. coli* (Fig. 4). Hence it can be assumed that the extracted
 226 dye can be used as a coloring agent of baby diaper, human mask, hospital clothing and surgical gauge or
 227 cotton etc.

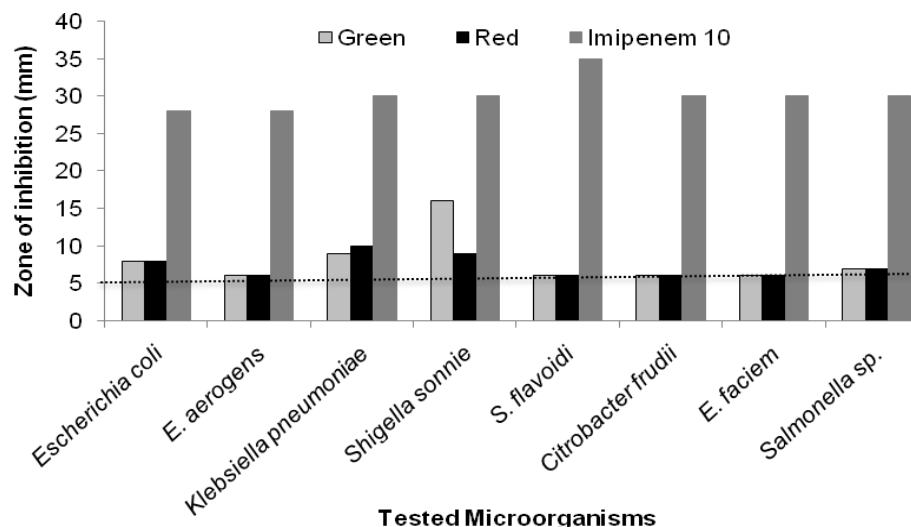


Fig. 4. Antimicrobial activity of extracted dyeing materials

Hypersensitivity testing was done on human skin with both allergic and non allergic individuals. Among 21 individuals no one showed hypersensitive to the extracted dye which was confirmed by no sign of irritation, redness or itching happen to the tested individuals (Table 3). So the natural dye can easily be allowed to the fiber and used as better alternative of chemical dye.

Table 3. Hypersensitivity result of extracted dyes on human skin

Tested Dye ID	Individual type	Redness	Itching	Irritation
Red	Allergic patient (n= 12)	N	N	N
Green	Non-allergic patient (n= 9)	N	N	N

n= Number of tested individuals; N= no reaction occurred

4. CONCLUSION

Exploration of eco-friendly textile dye and their proper use in fabrics were carried out in this study. Although the amount of biomass produced by microorganisms were very lower but they were successfully applied to different fabrics after successive extraction. Besides dyeing the extracted dyes were also effective against some pathogenic microorganisms and they had showed no allergy causing symptoms. The overall dye uptake quality of two fabrics was good. Dyes have also showed moderate fastness properties for the tested fabrics. For large scale production, special media, pH and temperature will be optimized in large fermenter. Beside that strain development should be launched for industrial level production. For the sustainability issue of the dye in the fabrics and environment further research will be needed to devise variegated shades of dye from microorganisms and reduce the toxigenicity of microbial byproducts.

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date December 2015

CONSENT (WHERE EVER APPLICABLE)

Not applicable

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

Not applicable

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