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Exploring Textile Dye from Microorganisms, an Eco-friendly Alternative

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Authors' contributions

This work was carried out in collaboration between all authors. Author SA conceptualized and designed the research methodology. Author PS contributed to sample collection and processing. Author SLS contributed to carry out the whole research work. Authors SLS, NS and SA contributed equally to data interpretation and manuscript preparation. All authors read and approved the final manuscript.

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ABSTRACT

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 regions 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

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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 the potentiality 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 hazard associated with processing and synthesis of synthetic dye, a global interest has been 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 through chemical processing [2]. To limit the environmental impact, construction of highly efficient effluent treatment plants could be an option. Another effective way is to make use of environment friendly dyes and chemicals [3]. Excessive use of colorants in dyeing industries and unproductive dyeing capabilities of those dyestuffs result in mixing up with the waste water, which ultimately finds its way to release into the environment. Rai et al [4] reported that during 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 and product, especially for application as food colorants, child textile and leather industries, is at the zenith of mind of conscious personalities [6, 7].

This study was conducted to find out some natural 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 and are amenable for bulk production in a short time. Bacteria like *Chromobacterium violaceum, Serratia marcescens* and *Chryseobacterium* sp. have the potentiality to produce violet, red and yellow-orange pigments [8]. But the source of pigment producing bacteria is not easily tractable in the environment. They are found mainly in harsh condition of industrial effluent at tropical and subtropical regions. Similarly, a range of fungus like *Monascus purpurus*, *Emmerciella* sp., *Penicillum* sp., *Fusarium* sp., *Thermomyces* sp., *Aspergillus* sp. etc. have been reported to be effective pigment producers [6, 9, 10].

In several studies, it has already been reported that natural dyes are restarted to be used in coloring fabrics like cotton, silk, wool, nylon etc. [11]. Moreover,the growing interest on natural colorants also keeps the researchers busy to innovate convenient techniques, to improve the product and to use them in an authentic way. However, as being natural, the product has some drawbacks on high yield production. The principle hurdles are low predictability and extractability; very minute amount of dye might be extracted from kilograms of raw materials. To cope up with this hindrance, it is usually being 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 [12]. Microorganisms, for instance, bacteria as well as fungi are reported to be potent pigment producer [10, 13]. So, study with these creatures from environmental samples will unveil their hidden capability and application to mankind.

2. MATERIALS AND METHODS

2.1 Sample Collection

In this study, a total of 113 samples were collected from soil, air and water. From all those samples, both bacteria and fungus were isolated and investigated for pigment production. Air samples were collected using settle plate techniques whereas soil and water samples were collected using proper methods described by APHA [14]. During sample collection, materials used for collection were sterilized properly and transferred immediately to 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. Water samples were enriched in nutrient broth media and then transferred to nutrient agar plates. After incubation on nutrient agar media at 37℃ for 24 hours, colony characteristics of the isolates were observed. Pure culture of the bacterial isolates was subjected to Gram staining and biochemical characterization such as oxidase, catalase, coagulase, citrate utilization, sugar utilization, starch hydrolysis, gelatin hydrolysis, motility, indole production, urease production, methyl red and voges-proskauer tests to identify the isolates.

2.2.2 Isolation and Identification of Fungi

As a wide range of fungus produces colorful spores, they were the main target for pigment production and extraction. All the samples were transferred to potato dextrose agar and incubated at 25℃ for 5-7 days and developments of color shades were observed. Fungal colonies with noticeable colors were further transferred into the Malt Extract agar (MEA) media and Molasses media (MM) for bulk production on individual plates. Fungal strains were confirmed by staining with lactophenol cotton blue under microscope.

2.3 Cultivation of Microorganisms

Due to the 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; $(NH_4)_2SO_4$ 1.0 g; $MgSO_4.7H_2O$ 0.5 g; K_2HPO_4 1.4 g; KH_2PO_4 0.6 g; $NaMoO_4.2H_2O$ 0.8 mg; $ZnSO_4.H_2O$ 0.8 mg; $FeCl_3.6H_2O$ 0.8 mg; $MnSO_4.2H_2O$ 0.4 mg; $CuSO_4.5H_2O$ 0.08 mg and pH was adjusted at 5.6 [15]. For cultivation, individual flasks were poured with 150 ml liquid media. Using sterile cork borer (12 mm in diameter) fungal isolates were inoculated into the chemically defined media and incubated at 25℃ in a dark place for 4-6 weeks. After proper incubation, fungal biomasses 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). After filtration, the fungal mat on the filter paper was collected and dried at 60℃ for one to 12 hours until it turns desiccated and then grounded into powdered form. The powdered materials were then stored and treated with methanol and ethanol for dye extraction.

2.5 Extraction of Color

The powdered pellets were subjected to dissolve in absolute methanol and ethanol 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 with dissolved materials was again filtrated with vacuum filtration and clear filtrates were allowed to dry using water bath at 45-50℃.

2.6 Coloring of fabrics

2.6.1 Preparation of mordant

For this experiment, alum (aluminum potassium sulfate) was used as a mordant. But using more alum has its own drawback to make the yarn sticky. To subside this condition, alum was generally combined with cream of tartar [16]. As mordant, 5% solution of alum was prepared in hot water.

2.6.2 Preparation of dyes

The respective dried powders of extract were dissolved in methanol. Two ml of each dyeing

components were added to 18 ml of Sulfur and Vat chemicals, warmed at 60-70ºC in a closed bath for 1 h. The liquor was filtered and used for dyeing. 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 remove sizing and desizing impurities. The cotton fabric was boiled for 20 min in a solution containing 4% liquid detergent, 8% soda ash and 2% caustic soda in water and washed off in clean water. The fabric was soaked in 3% solution of bleaching powder for 20 min and finally rinsed well with plenty of cold water. On the other hand, the silk fabric was treated only with 4% liquid detergent and then rinsed with cold water.

2.6.4 Mordant and dyeing

The freshly prepared mordant was mixed with dyeing solutions on constant stirring. The proportion of mordant-dye mixture depends on the fabric and the desired color intensities. In this study, 16 sq-inch of fabric to be dyed was put in 80 ml of warm mordant solution and kept for 30 min. After squeezing, the fabric was put into 20 ml of dyeing liquor for 1 h at different temperatures for better efficiency. As control, a piece of same fabric was treated as it was done for test fabrics, except using autoclaved distilled water instead of dyeing liquor. The dyed fabrics as well as control were washed thoroughly in liquid detergent and plenty of cold water till the water ran clear [17, 18].

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.

2.7 Hypersensitivity of Dye on Human Skin

It has been reported by many scientists that fungal spores have a direct role in hypersensitivity reaction on human. As the isolated dyes were of fungal origins, those were subjected to allergic or hypersensitivity testing using a standard method called “Patch test” [19]. 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 with standard disk diffusion technique described by Kirby & Bauer [20]. Measured amount of pure bacterial cultures were swabbed over Muller Hinton agar (OXOID, UK). Imipenem (10 µg) (OXOID, UK) was used as standard antibiotic disk and commercially available blank disks (6 mm) (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. After transferring the tested disks, plates were incubated at 37℃ for 18 hours.

3. RESULTS AND DISCUSSION

In this study, searching for a better alternative of synthetic dye was the prime concern. On that perspective, optimization of bacterial and fungal dye from the environment and their application in different types of fabrics was inspected as better alternative natural dye. Pigments could be produced as active biocatalyst or as secondary metabolites of microorganisms, for example, β-carotene, canthaxanthin, lycopene, monoscoflavin, zeaxanthinetc [21]. Beside

pigment production, these metabolites might have the potential to be active against different pathogenic microorganisms. Among 113 samples, a wide range of bacteria and fungus were isolated. Bacterial isolates included *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	*Aspergillus* sp.
	Fusarium sp.
Soil	*Aspergillus* sp.
	Fusarium sp.
	Trichoderma sp.
	Penicillius sp.
Water	*Aspergillus* sp.
	Trichoderma sp.

A number of fungal isolates were found to produce visible pigments of different shades. *Aspergillus* sp., which produced black spores both in the culture plate and cultivation broth, was responsible for the green shade after extraction. Meanwhile, the *Penicillum* sp. produced red color in the exhaustion broth. It was really surprising that most of the isolated fungal species were able to produce different colors of spores but after extraction those colors were not recoverable. The main reason behind this miracle might be the lack of solubility of colors or those pigments were not extracellular. Dried powdered biomasses were weighted and solvent extracted with methanol and ethanol. Among seven types of pigments finally isolated, 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 dye fabrics. The absorbance spectra of extracted dyes were also measured by spectrophotometer under a range of different wavelength (Fig. 1).

Silk and cotton fabrics were treated with the extracted color and incubated about 120 min. After proper incubation and wash, the shades of red and green colors in the fabrics were observed. Visually the color retention quality of the silk was relatively better than cotton (Fig. 2). It was shown that the absorbance range of red and green colors peaked at 450-650 nm which was related to color absorbance spectrum.

After pouring the fabric into dyeing liquor, the time was recorded for best dye uptake moment. 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 75-85% in range comparing to cotton whose best uptake about 75-90% was occurred after 120 minutes (Fig. 3). Some natural dyes also reported to have best exhaustion time of about 60 minutes [8].

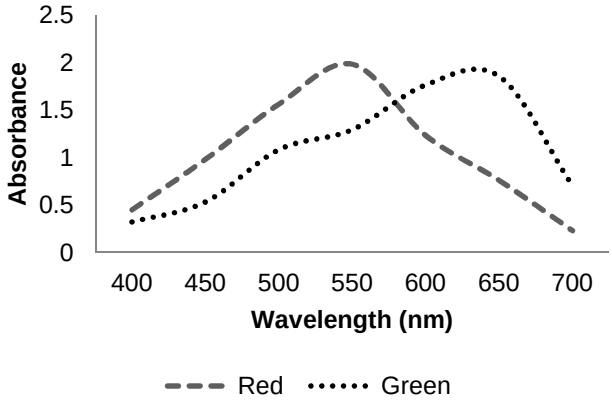

Fig. 1. Absorbance spectra of red and green color dye in different wavelengths

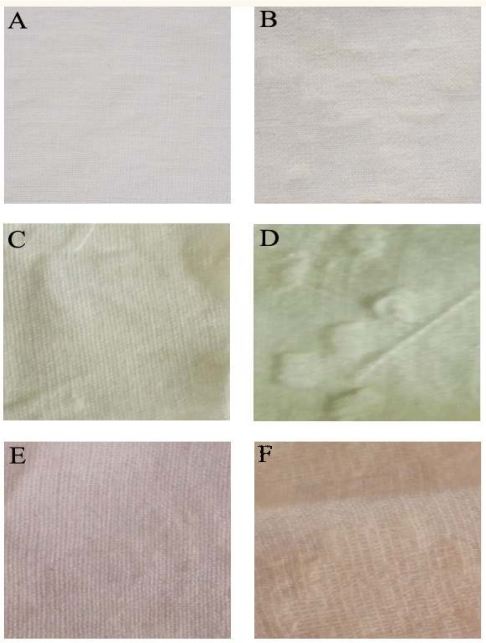

Fig. 2. Color of cotton and silk fabric before and after dyeing with two extracted colors: A. Untreated cotton, B. Untreated Silk, C. Cotton dyed with green color, D. Silk dyed with green color, E. Cotton dyed with red color, F. Silk dyed with red color

Temperature played a crucial role on dyeing process. The experiment was continued within a different temperature ranges. Dye uptake was started at 30℃ for both silk and cotton (15-25%) but best outcome (80-95%) was recorded at 65-70℃ (Fig. 4). Above the optimum uptake temperature, the dye uptake rate became subsided (45-60%) at 80℃, which could be the reason of instability of natural biomolecules at higher temperature.

Eight types of pathogenic bacteria were tested with extracted dyes for their antimicrobial potentiality. Colors extracted from *Aspergillus* sp. and *Penicillium* sp. showed antimicrobial properties against a range of bacteria. Though not much effective, they have shown potentiality to inhibit microorganisms, for example, *Klebsiella pneumoniae*, *Shigella sonnei* and *E. coli* (Fig. 5) to a certain extent. Hence it can be assumed that the extracted dye can be used as a coloring agent of baby diaper, human mask or hospital clothing etc [7, 22].

Hypersensitivity testing was done on human skin with both allergic and non allergic individuals. Among 21 individuals no one showed to be hypersensitive to the extracted dye. Hypersensitivity 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 fabrics used for human wear.

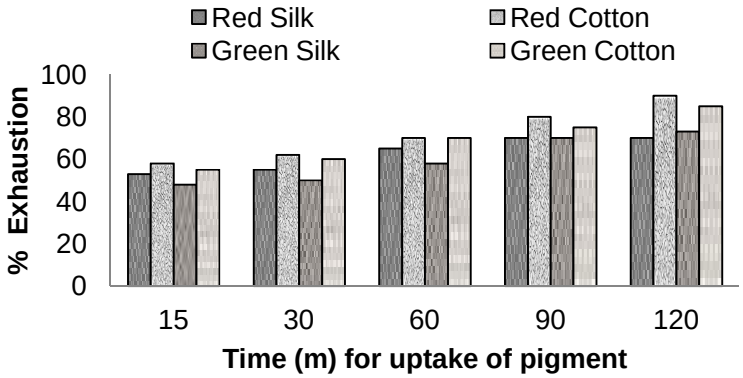

Fig. 3. Effect of time on pigment uptake into fabrics at 60^{u}C temperature

Table 3. Hypersensitivity results 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

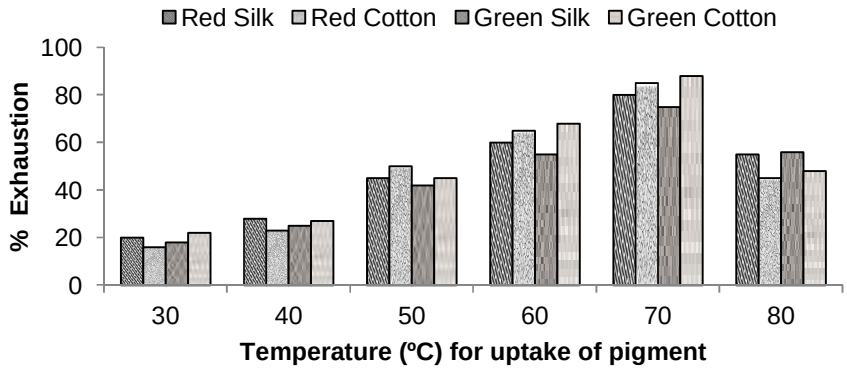

Fig. 4. Effect of temperatures on pigment uptake into fabrics

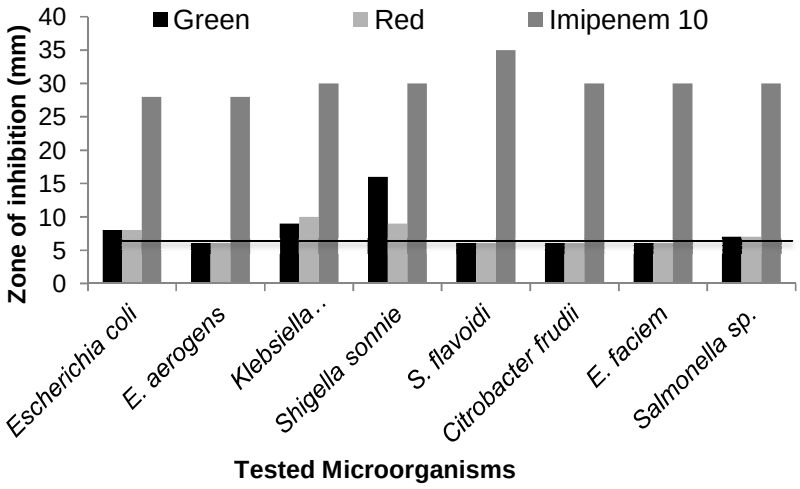

Fig. 5.Antimicrobial activity of extracted dyeing materials

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 low 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 shown no allergic symptoms. The overall dye uptake quality of two fabrics was good. Dyes have also shown moderate fastness properties for the tested fabrics. For large scale production, special media, pH and temperature will be optimized in large fermenter. Strain development should also 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 toxicity of microbial byproducts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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DECLARATION

All authors declare that the manuscript submitted to the journal is original research article and has never been submitted or published elsewhere as a full-length article. The research work was presented at the conference "Bangladesh Society of Microbiologists (BSM) 1st International Conference (28th AGM), 2015" and only the abstract was published in the conference proceedings. Available link is: "https://www.researchgate.net/profile/Shovon_Sarkar/publication/288338694_Exploring_textile_dye_from_microorganisms_an_eco-friendly_alternativeAbstract_ID_91/links/568b752708ae1e63f1fc60e9.pdf?origin=publication_detail"

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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