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# Artificial Cultivation of *Stereum hirsutum* and Colorimetric Analysis of Dyed Fabrics Using the Extracted Dye

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**Abstract:** Due to pollution and toxicity problems associated with synthetic dyes and pigments industry, the whole world is shifting back towards the manufacturing of natural dyes and pigments. Natural dyes have been obtained from biological origins like plants and animals; however a very little effort have so far been to use fungi as source of natural dyes despite the fact that certain species can yield considerable amount of good quality colouring substance. Present work describe the development of protocol for artificial cultivation of *Stereum hirsutum* and extraction of dye from artificially cultured fungal species, dyeing and mordanting trials with the extracted dyes on different textile materials and determination of CIEL*a*b* hue, chroma and K/S values of each dyed fabrics. Using different mordants various shades like yellow, brown and purple were produced on silk, wool and cotton fabrics. Colour coordinates like b* for silk, wool and cotton were found to be 8.41 to 24.71, 20.03 to 26.67 and 0.38 to 12.60 respectively, where as L* for silk, wool and cotton were 59.35 to 85.19, 35.02 to 71.83 and 77.34 to 90.77 respectively.

**Key words:** Natural dye, Fungi, *Stereum hirsutum*, silk, wool and cotton.

## Introduction

The use of natural colorants derived from different sources such as plant, animal, microbial including fungal biodiversity for coloring textile fabrics has now becoming the commercial reality [11]. Several synthetic colorants have been banned because they cause environmental pollution, allergy-like symptoms or are carcinogens. Among the all natural dyes, plant-based pigments have wide range of medicinal values. Natural dyes are derived from naturally occurring sources like plants (e.g., indigo and saffron); insects (e.g., cochineal beetles and lac insects); animals (e.g., some species of mollusks or shellfish); and minerals (e.g., ferrous sulfate, ochre, and clay) without any chemical treatment [2]. Because of the abundance, quicker growth and eco-friendly biological origin, the use of certain non-toxigenic filamentous fungi to produce pigments that can be used as colorants in textile applications are gaining importance to explore the possibility of developing range of shades on textile by developing dyes from fungi, to fulfill need of consumers and industries for overall development of natural dye sector. Natural dyeing using the colorants of fungi is quite common in Finland. However, published reports of scientific research on the subject are very rare [8,12]. The chromophores of mushroom dyes contain a variety of fascinating organic compounds. Their pigmentation may vary with the age and some undergo distinctive colour changes on bruising; therefore, the colours of mushrooms are

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one of the essential features used in their identification. Furthermore, the pigments of mushrooms may protect the organism from UV damage and bacterial attack or play a role as insect attractants [5]. Use of fungi for production of natural dye has certain advantages over plant dye that includes availability of material in desired quantity, intense and consistent colors etc. [7]. *Monascus* species are widely used as a microbial source for natural red pigment production [7]. Monascus red pigments are of polyketide origin and are used commercially as non-toxic colorants for colouring rice wine, "Koji", soybean, cheese and red meat [4]. The red pigments have attracted worldwide commercial interest [6,10], but little information is available on the production of this pigment by other microbial sources. It has been reported that *Penicillium* may be the potential candidate to produce polyketide structure compounds [6]. Although the natural dyeing has old age traditions, however using scientific research and develops the efficiency of dye, different kinds of hues, colour fastness properties etc can be improved. The basis for the improvements in extracting the dyes, dyeing and mordanting techniques, are research results, which, in addition, lead to better quality of the dyed products. Thus the present study was focused on to develop dye using a fungus *Stereum hirsutum* for its application on silk, wool and cotton fabrics.

## Materials and methods

### *Fungus and fabrics*

*Stereum hirsutum* obtained from NTCC (National Type Culture Collection), Forest Pathology Division, FRI, Dehradun. Pure silk, wool and cotton fabrics were purchased from Gandhi Ashram, Khadi Bhandar of Dehradun.

### Chemical & analytical

All the chemicals *viz*., acetic acid, alum, copper sulphate, ferrous sulphate, potassium dichromate and stannous chloride used as mordants were of L.R. grade of chemicals and procured from local dealers. Different media like Potato Dextrose Agar (PDA), Czapex Dox Agar (CDA), Sabouraud Dextrose Agar (SDA), Oat Meal Agar (OMA), Mannitol Salt Agar (MSA) and Potato Dextrose Broath (PDB) were purchased from local market. Pure Khadi silk, wool and cotton were used in dyeing experiments. The U.V. spectra were recorded on a UV spectrophotometer (Chemito make; Model 2700. The CIEL*a*b*, hue, chroma and K/S values were determined on a Reference Bench-top Spectrophotometer (Make- Gretag Mac Beth; Colour-Eye 7000A).

### Growth of fungus on different media

After visual observations of fungal species growing in NTCC, *Stereum hirsutum* was selected for pigment production in different culture media. In order to study the growth and high pigment production by the fungus, five different solid media were used. The culture media screened for high pigment production were Potato Dextrose Agar (PDA), Czapex Dox Agar (CDA), Sabouraud Dextrose Agar (SDA), Oat Meal Agar (OMA), Mannitol Salt Agar (MSA) under aseptic condition. The plates were incubated at 24°C ± 1°C for a week. The different media were observed for pigment production and the best culture media was selected for further experiments. The culture was maintained on PDA slants. Accordingly for growing *S. hirsutum* in liquid culture medium, PDB (HiMedia) was employed. The medium was then transferred to conical flask. It was then plugged with non absorbent cotton plugs and sterilized in autoclave at 121°C ± 1°C at 15 lbs pressure for 15 minutes. Further the seven days old culture was used to prepared culture discs by cork borer (5 mm) and placed into the conical flasks containing 50 ml PDB and incubated at 24°C ± 1°C for 14 days in different conditions viz. shaking and stagnant position. The temperature and incubation period provided for the growth of fungi was same in both conditions whereas 150 rpm was given in case of shaker culturing.

### Measuring fungal biomass in shaking and static culture:

At room temperature (RT) the fungal mycelium in PDB was filtered using pre autoclaved and pre weighted whattman filter paper ($W_1$). Filtered mycelium was oven dried at 60°C for 6 h and reweighed the filter paper with mycelium ($W_2$) and samples were again dried in oven till their weights became constant.

The fungal dry weight (W) was calculated by formula:

$W = W_2 - W_1$

**Extraction of dye from liquid broth**

The liquid broth 400 ml (including mycelium) was boiled for 60 minutes, filtered as above and residual mycelium was transferred in beaker having 400 ml distilled water. The mycelium with water was again boiled for 60 minutes and then filtered in order to extract maximum dye in the broth and mycelium. Both extracts were pooled and solid content in the extract was determined to get the percentage yield.

**Determination of percentage absorption of dye absorbed by various fabrics**

Dye solutions were prepared and the optical density of each solution was measured after diluting 1 ml of solution to 100 ml with water and optical density at 207 nm (λmax) was measured. Then after dyeing the fabrics the remaining dye solution was makeup to 100 ml and OD was measured again. Calculation of absorption percentage (%) was carried by using the following formula:

$$\% \text{ Absorption} = \frac{\text{OD before dye} - \text{OD after dye}}{\text{OD before dye}} \times 100$$

**Dyeing and mordanting of different fabrics**

The filtered extract was used directly for dyeing the fabrics. The experiments were carried out in acidic, alkaline and neutral medium and it was observed that acidic medium gives the darkest shade on the fabrics. Thus all the dyeing experiment was carried out in acidic medium. Dyeing on silk, wool and cotton samples was carried out by post mordanting method, which produced improved shades in terms of hue and darkness. The dye bath was heated to 80-85$^o$C and dyeing of silk and wool fabrics was continued for 45 min. by maintaining the temperature and the same level of the solution. The dyeing of cotton was carried out at 95-98$^o$C for 60 minutes. The dye bath was cooled to room temperature. The dyed samples were directly immersed in the mordant bath by keeping the fabrics to liquor ratio (MLR) of 1:40. Mordant concentration of 0.5 g per 100 ml was found to be optimum by visual observations of dyed samples prepared under different conditions. The criteria for evaluation were the depth of color, evenness of dye and brightness of shade. The bath was heated to a temperature of 80-85$^o$C for 30 minutes for fixing the dye on silk and wool samples where as for cotton samples the mordanting was continued for 45 minute at a temperature of 95-98$^o$C. The samples were taken out from the bath, washed with soap solution and water and then dried in shade. The mordanted samples (7 nos.) of silk, wool and cotton were named as SH1: without mordant; SH2: Alum; SH3: Copper sulphate; SH4: Ferrous Sulphate; SH5 Potassium Dichromate; SH6: Stannous chloride and *Terminalia chebula*: SH7.

**Determination of colour coordinates**

All the dyed and mordanted silk, wool and cotton samples were tested for their L*, a*, b*, C*, $h^0$ and K/S values using Reference Bench Top Spectrophotometer. CIE stands for Commission Internationale de l'Eclairage system of colour analysis, an international clearinghouse for colour research at universities and research laboratories who developed CIE L* a* b* or CIELAB colour scale to be used by everyone for comparison of colour values. L*, a*, and b* signify respectively the luminosity or lightness, redness or greenness and yellowness and blueness of a sample. Positive a* is red and negative a* is green where as positive b* is yellow and negative b* is blue.

Other aspects, like hue, chroma and K/S ratio of the dyed fabrics were also studied so as to get a more precise result of the colour coordinates.

**Hue**

Hue is the attribute by which a sample differs from achromatic (i.e. hueless) colour of same lightness.

**Chroma**

It is the colourfullness at a given luminance level. Chroma is the colourfulness of an area judged in proportion to the brightness of a similarly illuminated area that appears to be white or highly transmitting.

**K/S Values**

Kubelka-Monk equation defines a relationship between spectral reflectance (R in %) of the sample and its absorption (K) and scattering (S) characteristics, represented as follows:

$$K/S = \frac{[1 - 0.01 R^2]}{2[0.01 R]}$$

K/S grows to infinity as reflectance decreases to zero

## Results and discussion

### *Growth of fungus on different media*

Liquid media used for this experiment was selected on the basis of the pigment production by the fungal colonies in solid media. As per the observation by experiment, best growth was observed in PDA therefore, PDB was used for further experiments. As per the visual observations pigment production was clearly more in the flask kept in shaker (150 RPM) where as very little pigment was produced in static position. During the course of experiments the change in colour density from 7 days to 14 days was noticeable.

**Fungal biomass in shaking and static cultures**

The experiment was carried out in triplicate and the dry weight of the fungal mycelium was calculated using the formula ($W_2$-$W_1$) and mean was calculated as per the observation in Table 1, it is evident from data the dry weight of fungal mycelium was more in the static position when compared with shaker.

**Extraction of dye from liquid broth**

The liquid broth 400 ml was boiled for 60 minutes and filtered at room temperature. The residual mycelium was again extracted for 60 minutes with 400 ml distilled water and the filtrate was pooled. Solid content of extract was determined by evaporation of 25 ml of extract (thrice). The average solid content was 2.3 g per 100 ml of extract. The remaining 710 ml extract was used for dyeing

**Calculation of percentage of dye absorption on different fabrics**

The experimental data presented in Table 2 indicate wool absorbed the maximum of dye con-

**Table 1. Dry weight of fungal mycelium under shaker and stagnant position**

| Name of fungi | Dry weight of fungal mycelium under shake culture | | | |
|---|---|---|---|---|
| | Dry weight of filter paper($W_1$) | Dry weight of filter paper with fungal mycelium ($W_2$) | Dry weight of fungal mycelium $W= W_2 - W_1$ | Average weight |
| *Stereum hirsutum* | 1.08 | 2.44 | 1.36 | 1.33 |
| | 1.06 | 2.41 | 1.35 | |
| | 1.12 | 2.40 | 1.28 | |
| | **Dry weight of fungal mycelium under static culture** | | | |
| | 1.04 | 2.58 | 1.54 | 1.58 |
| | 1.06 | 2.68 | 1.62 | |
| | 1.08 | 2.66 | 1.58 | |

**Table 2. Percentage (%) absorption by different fabrics**

| No. | Fabrics | Calculation of Absorption % | | |
|---|---|---|---|---|
| | | Before dyeing OD | After dyeing OD | % Absorption |
| 1. | Silk | 2.191 | 1.430 | 34.7 |
| 2. | Wool | 2.191 | 1.169 | 46.6 |
| 3. | Cotton | 2.191 | 1.881 | 14.1 |

tent (46.6 %) followed by silk (34.7 %) and least amount of dye was absorbed by cotton (14.1 %.). It was further supported by visual observations which show that wool has darkest shade followed by silk and cotton.

**Dyeing and mordanting of silk, wool, and cotton fabrics using the extracted dye**

The filtered extract 710 ml was used directly for dyeing the fabrics. Silk, wool and cotton fabrics (44 g) were immersed in the extract and 1050 ml distilled water was added for maintaining the material to liquor ratio (MLR) 1:40. On the basis of experiments in acidic, alkaline and neutral medium and it was observed that acidic medium gives the darkest shades to the fabrics. Thus all the dyeing experiment was carried out in acidic medium (pH-4.0) by adding glacial acetic acid. The dyeing of silk and wool fabrics were carried out for 45 minutes by maintaining the temperature and the same level of the solution while dyeing of cotton was carried out at 95-98°C for 60 minutes. The dye bath was cooled to room temperature. The dyed samples were directly immersed in the mordant bath by keeping the fabrics to liquor ratio of 1:40. Yellow, brown, purplish and grey shades were produced on silk, wool and cotton fabrics using various natural and synthetic mordants.

Sharma *et al.*, [9] investigated three fungal species; *Trichoderma virens*, *Alternaria alternata* and *Curvularia lunata* to get pigments for the purpose of textile dyeing. Highest optical density was achieved in potato dextrose broth at 28°C in 25 days under static conditions, where as in present study with *Stereum hirsutum* highest optical density was obtained under shaking condition. Hamlyn, [3] investigated the production and evaluation of microbial pigments as textile colorants. Atalla, [1], studied eleven fungal species *Acrostalagmus* sp., *Alternaria alternata*, *Alternaria* sp., *Aspergillus niger*, *Bisporomyces* sp., *Cunninghamella*, *Penicillium chrysogenum*, *Penicillium italicum*, *Penicillium oxalicum*, *Penicillium regulosum*, *Phymatotrichum* sp for preparation of dye.

**Determination of colour coordinates of dyed fabrics**

The data (Table 3) indicate that dye obtained from *Stereum hirsutum* produced big variation in color coordinates. As the greater the 'L' values lighter the shade. Maximum dark color was obtained with *Terminalia chebula* fruit pericarp (TC) in silk (L=59.35) and cotton (L=77.34) but in wool (L=35.02) with potassium dichromate, whereas the lightest shade on silk (L=87.15) and wool (L=73.75) was obtained when mordanted with stannous chloride, but in cotton lightest shade was obtained when mordanted with alum (L=90.77). Maximum redness (a*) was found using T.C as a mordant in silk (a*= 6.38) and wool (a*= 8.77) but in cotton (a*= 3.99) with ferrous sulphate. Minimum redness was found in silk (a*= - 0.90), wool (a*= - 0.84) and cotton (a*= - 0.74) when these all were treated with copper sulphate. Negative values (-) of a* indicate the greenness characteristics of the dyed silk, wool and cotton fabrics.

The maximum yellowness was obtained by using TC in silk (b*=24.71) and wool (b*=26.67) but in cotton (b*=18.18) with ferrous sulphate. Cotton has lesser K/S values in comparison with silk and wool, indicating their lighter shades. Cotton dyed and mordanted with TC has maximum (1.67) K/S values where as cotton dyed and unmordant has minimum (0.27) K/S value. The K/S values for silk shows that sample mordanted with stannous chloride has the lowest K/S value (1.12) while T.C has the highest K/S value (10.16). In wool the highest K/S value was obtained when sample was mordanted with potassium dichromate (26.48) where as the lowest K/S value was obtained with alum (3.94). Sharma *et al.*, [9] investigated dye extraction from fungal species in which they reported L* for silk and wool fabrics dyed using *Trichoderma virens* (silk-85.27; wool 82.91), *Curvularia lunata* (silk-75.73; wool - 72.45) and *Alternaria alternata* (silk-50.15; wool 49.64). In the present study silk and wool dyed using *Stereum hirsutum* dye L* value for unmordanted silk and wool were 83.15 and 70.28 respectively. These indicate the shades are similar in darkness.

**Conclusion**

In this era of eco friendly products, *Stereum hirsutum* a fungus can be successfully cultivated and used as a source of natural dyes for textile

**Table 3. Determination of colour coordinates of dyed fabrics**

| No. | Sample | L* | a* | b* | C* | h0 | K/S |
|---|---|---|---|---|---|---|---|
| | | Dye obtained from *Stereum hirsutum* | | | | | |
| | | | | Silk | | | |
| 1 | SH 1 | 83.15 | 2.77 | 8.60 | 9.03 | 72.11 | 2.06 |
| 2 | SH 2 | 85.19 | 2.54 | 8.41 | 8.79 | 73.22 | 1.32 |
| 3 | SH 3 | 79.69 | -0.90 | 10.48 | 10.52 | 94.91 | 1.72 |
| 4 | SH 4 | 69.99 | 5.90 | 17.23 | 18.21 | 71.09 | 4.15 |
| 5 | SH 5 | 71.60 | 2.50 | 21.93 | 22.07 | 83.49 | 3.63 |
| 6 | SH 6 | 87.15 | 1.55 | 10.61 | 10.72 | 81.71 | 1.12 |
| 7 | SH 7 | 59.35 | 6.38 | 24.71 | 25.52 | 75.51 | 10.16 |
| | | | | Wool | | | |
| 1 | SH 1 | 70.28 | 4.60 | 21.11 | 21.61 | 77.71 | 4.24 |
| 2 | SH 2 | 71.83 | 4.18 | 20.74 | 21.16 | 78.62 | 3.94 |
| 3 | SH 3 | 58.62 | -0.84 | 24.03 | 24.04 | 92.00 | 8.41 |
| 4 | SH 4 | 60.31 | 5.23 | 20.03 | 20.70 | 75.37 | 7.52 |
| 5 | SH 5 | 35.02 | 7.24 | 27.10 | 28.05 | 75.05 | 26.48 |
| 6 | SH 6 | 73.75 | 3.46 | 23.29 | 23.55 | 81.55 | 4.38 |
| 7 | SH 7 | 43.89 | 8.77 | 26.67 | 28.08 | 71.99 | 18.93 |
| | | | | Cotton | | | |
| 1 | SH 1 | 90.03 | 1.07 | 1.66 | 1.98 | 57.26 | 0.27 |
| 2 | SH 2 | 90.77 | 1.26 | 0.38 | 1.32 | 16.68 | 0.31 |
| 3 | SH 3 | 88.39 | -0.74 | 3.01 | 3.10 | 103.87 | 0.41 |
| 4 | SH 4 | 82.93 | 3.99 | 18.18 | 18.61 | 77.61 | 1.37 |
| 5 | SH 5 | 86.81 | 0.39 | 1.11 | 1.18 | 70.84 | 0.77 |
| 6 | SH 6 | 90.12 | 0.27 | 5.92 | 5.93 | 87.36 | 0.73 |
| 7 | SH 7 | 77.34 | 1.55 | 12.60 | 12.69 | 83.00 | 1.67 |

application. The studies indicate that PDB is most suitable media to grow the fungus. The dyed and mordanted fabrics exhibited very attractive shades on silk, wool and cotton fabrics. The dye may find use in dyeing of silk, wool and cotton which will help to augment the export of natural dyed garments to the countries where the use of azo dyes has been banned.

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