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PIGMENT PRODUCTION FROM *TRICHODERMA SPP.* FOR DYEING OF SILK AND WOOL

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ABSTRACT
There are many areas of application of biotechnology in the textile industry, one of which is production of novel colorants using microorganisms that have certain distinct advantage over those produced from plants and animals. The present study is an attempt to isolate and optimize the fermentation condition of *Trichoderma spp.* to explore the possibility of extracting colors and its application in terms of dyeing silk and wool fabrics. It was found that the optimum culture conditions for *Trichoderma spp* .was potato dextrose broth at a temperature of 28ºC in 25 days in static conditions. Dyed samples were also evaluated in terms of percentage absorption and color value which was found to be greater for wool than silk. The fastness test of the final dyed samples revealed to have good to excellent fastness for washing and rubbing. *Trichoderma spp.* has demonstrated antifungal property.

KEYWORDS: Natural dye, Microbial pigment, Fungus, *Trichoderma spp*, Dyeing, Anti-fungal property.

INTRODUCTION

Colors are considered natural when derived from biological sources--plants or microorganisms. The increasing awareness of health and pollution hazards of chemical dyestuffs has led to a resurgence of interest in natural and mineral colors. Microbial pigments have meaningful advantages over artificial and inorganic colors. Microbes can be used as an alternative source of natural dyes to develop environment friendly dye production technologies for textile industries. These multiply very fast and are capable of growing on large scale on a variety of raw materials requiring limited space. Moreover, standardization and optimization of growth conditions and pigment production is possible. Attempts have been made to synthesize bacterial and fungal pigments to be used in the textile and leather industry (Velmurugan et al 2009, Chba et al 2006, Mapari et al 2005, Shin et al 1998, Hamlyn 1995). Microbes can produce a large amount of stable pigments such as anthraquinones, carotenoids, flavonoids, quinines and rubramines. Fungi contain several anthraquinone compounds which have been identified as their secondary metabolites (Nagia and El-Mohamedy 2007). The production and evaluation of such textile colorants are currently being investigated at the British Textile Technology Group (BTTG). Raisanen and coworkers (2002) dyed wool with anthraquinone carboxylic acid isolated from fungus *Dermocybe sanguine.* The fungi such as *Fusarium oxosporum, Trichoderma viride* and *Alternaria sp*. were studied for color production and tested on various cellulose fibers. Earlier studies confirmed the non toxicity and biodegradability of the fungal pigments (Youssef and Ibrahim 2008, Daniel et al 2007, Ferreira- Leitao et al 2007). Results from different studies showed that the strains of *Trichoderma spp.* inhibit pathogens through production of antifungal antibiotics and or/hydrolytic enzymes, these restrict the growth of other fungus that can be detected by formation of a zone of inhibition around the pathogens. *Trichoderma* species are free living fungi that occur in nearly all the soils and other natural habitats. They grow rapidly in artificial culture media and produce large number of green or white conidia from conodiogeneous cells. Several species of the genus *Trichoderma* received attention mainly due to their importance in biological control of soil borne plant pathogens. In the present work, efforts have been made to screen and isolate *Trichoderma spp.* and optimize its fermentation conditions for maximum pigmentation. Thereafter, the substantivity of the pigment is checked on different textile substrates, and their color measurement and color fastness was ascertained. Finally the anti microbial property of *trichoderma spp.* was tested.

MATERIALS AND METHODS

Textile material

Dyeing was done on silk and wool fabric. A mulberry silk of thread count 119 x 116 and pure 2 x 2 twill woven woolen fabric of thread count 59 x 58 was used respectively.

Isolation of *Trichoderma spp.*

Tricoderma sp was isolated from soil near college premises; 1 gm of soil was dissolved in 10 ml of distilled water in a test tube. 0.1ml of the soil solution was then spread onto the Potato dextrose agar (PDA) plates with the help of sterile spreader. All onto the Potato dextrose agar (PDA) plates were then kept in B.O.D incubator at 28^0 C for 3-4 days. Different fungal colonies appeared on Potato dextrose agar (PDA) plates from which pure cultures of the fungi were obtained by transferring them onto a fresh Potato dextrose agar (PDA) plates and then kept again in an incubator at 28^0 C for 3-4 days. Potato dextrose agar

(PDA) is a solid medium containing extract of 300g peeled potato, 2.5g glucose, 15g agar in 1000 ml distilled water to grow fungi.

Fungal Cultures for Pigment Production

Cultures of *Tricoderma sp.* was grown on Potato dextrose broth (PD) and incubated at 3 different temperatures i.e. 15°C, 28°C and 37 °C as stationary cultures for 3 weeks for pigment production.

Screening of Dye

Fungal culture broths showing color were filtered out after 3 weeks of incubation period. The fungal mycelia and the culture filtrates were tested for color production and dyeability. The mycelium and the filtrate for each of the selected fungi were divided into 4 parts. In one flask only filtered solution was taken, in the second flask mycelium was crushed using homogenizer and then autoclaved, in the third flask along with the crushed mycelium alcohol was added and autoclaved, and in the fourth flask filtered solution and solid mycelium together were autoclaved. This was done to extract any intracellular pigment in the mycelia. However, even after homogenizing and autoclaving no more pigment got extracted and thus homogenization as a step for pigment extraction was eliminated. The colored filtrate was used to dye the textile samples.

Preparation of fabric

Silk: Silk was degummed with a detergent solution containing 10ml of mild detergent (ezee) per 100ml of water and heated at 50°C. Material liquid ratio (MLR) was kept 1:40 and silk was stirred for 60 minutes. After degumming, bleaching was done at 85°C for 1 hour by keeping the material liquid ratio (MLR) 1:30 in a solution containing 0.9% hydrogen peroxide and 10% sodium carbonate. It was rinsed and dried in shade.

Wool: Wool was washed with the solution containing 0.5gm/litre sodium carbonate and 2gm/litre non-ionic detergent (Lissapol) at 40-45°C for 30 minutes, by keeping the material liquid ratio (MLR) 1:50. Scoured material is thoroughly washed with water and dried.

Dyeing

The colored filtrates from all the broths were then used for dyeing wool and silk. Before dyeing the pH of the dye was checked. The samples were pre mordanted with 5% of ferrous sulphate and copper sulphate on the weight of the fabric. Finally the unmordanted and mordanted wool and silk each weighing 1 gm were dyed in 50 ml of colored filtrate i.e. Material liquid ratio (MLR) =1:50 for 45 minutes at 70-80^0 C. After dyeing washing of the samples were carried out at boil for 5 minutes in lissapol followed by rinsing with cold water.

Percentage Absorption of Dyed Samples

Percentage absorption of the dyed fabrics was also calculated on Spectrophotometer 107 (systronics) to find out the uptake of dye stuff by each fibre.

The percentage absorption of the dyed sample was calculated using the following equation:

$$\text{Percentage absorption} = \frac{\text{O.D before dyeing - O.D after dyeing} \times 100}{\text{O.D before dyeing}}$$

Color Measurement Analysis and Fastness Properties

Color values like K/S. L*, a* and b* readings were calculated on computer color matching system of Macbeth-color eye 3100.

Dyed samples were tested for wash fastness and rub fastness according to ISO standards.

S.No	Type of test	Equipment	Standard
1	Wash fastness	*digi* WASH-INX ^{TM} (ISO) Launderometer	IS : 687,3361,764,765 & 3417 ISO 105 : CO1/CO2/CO3 CO4/CO5
2	Crocking: dry and wet	*crock* METER-I ^{TM}	IS : 76 ISO 105 -X12 AATCC-8

Antifungal Property

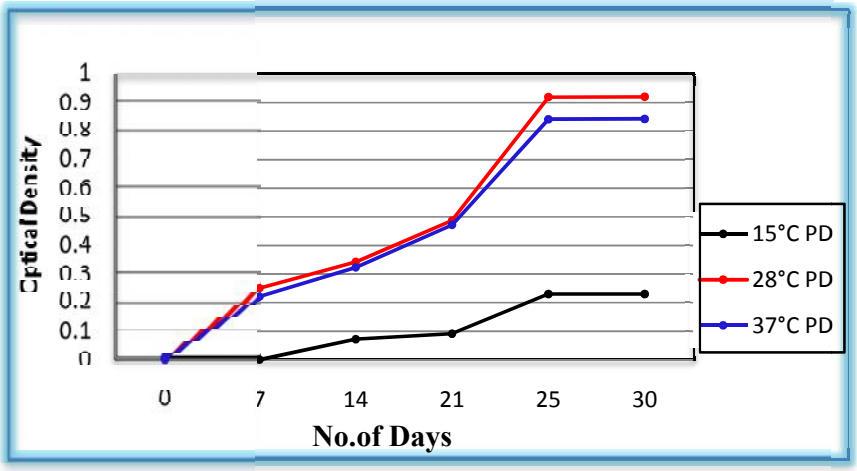
Strains of *Trichoderma* have been known to inhibit growth of other microbes through production of antimicrobial agents and/or hydrolytic enzymes. This restriction of the growth of other fungi is evident through formation of a zone of inhibition around the growth of microbes .For testing antimicrobial property of the selected *Trichoderma* strain in present study, Potato dextrose agar (PDA) plate was point inoculated on one side with *Trichoderma* and on the other side with other fungal strains like *Alternaria* and *Fusarium*. The plates were kept in an incubator at 28 ºC ± 2 ºC for 3-4 days and visually analyzed for formation of any zone of inhibition.

Toxicity to Human Skin

The dyed silk and wool samples were tested for red marks, swelling, rashes etc, if any, on ten subjects for three successive days by taping a 1x1 inch swatch on the upper arm.

RESULTS & DISCUSSIONS

The fungi *Tricoderma* chosen in present study produced pigmentation in submerged culture. The optimum culture medium, temperature and time for *Trichoderma sp* was (PD) potato dextrose broth at 28°C for 30 days. (Fig.1) The pH for potato dextrose medium before inoculation was 6, however, pH before dyeing (after culturing) for *Trichoderma* became 5.6. Hence the dye liquor became more acidic in nature. *Trichoderma* produced yellow color on wool and silk respectively.



Trichoderma sp.

FIGURE 1. Optical density of fungal culture filtrates recorded for over a period of 30 day

Percentage Absorption of Dyed Sample

The spectrophotometer works on the principle of spectrophotometry based on Beer-Lambart law. It gives the value of absorbance in terms of optical density in visible range. The spectrophotometer measure the amount of light of a specified wave length which passes through a medium. According to Beer's law, the amount of light absorbed by a medium is proportional to the concentration of the absorbing material or solute present. Thus the concentration of a colored solute in a solution may be determined in lab by measuring the absorbency of light in a given wavelength. The optical density is a synonym for absorbance.

Table 1 gives the optical density of the fungal filtrate and their corresponding percentage absorption for wool and silk. As can be seen, the percentage absorption of Trichoderma is 44.58% for silk and 48.37% or wool respectively. As expected wool has higher percentage absorption than silk (Table 1).The wool fiber contains equal amount of amino and carboxyl groups which ionize and form zwitter ion. At low pH the hydrogen ions are absorbed by carboxyl groups present in wool. At high pH, the protein looses hydrogen ions leaving behind ionized groups. Thus wool absorbs maximum dye in acidic medium. (Mathur and bhandari, 2001).

TABLE 1- Percentage absorption of silk and wool dyed with *Tricoderma*

		Trichoderma PD broth (28°C)		
S.No	Textile fabric	O.D before dyeing	O.D after dyeing	Percentage absorption (%)
1	SILK	0.767	0.425	44.58
2	WOOL	0.767	0.396	48.37

Color Measurement Analysis

CIE *L*a*b** (CIELAB) is the most complete color space specified by the International Commission on Illumination (*Commission Internationale d'Eclairage*, hence its *CIE* initialism). It describes all the colors visible to the human eye.The three coordinates of CIELAB represent the lightness of the color (***L****=higher value indicates lighter shades and lower value indicates deeper shades), its position between red/magenta and green (***a****, negative values indicate green while positive values indicate magenta) and its position between yellow and blue (***b****, negative values indicate blue and positive values indicate yellow).

Wool and silk samples were compared through computer color matching system and arrived at K/S, L*, a*, b* readings. The readings are recorded in the Table 2. It can be seen that wool has higher **K/S** value than silk.

TABLE 2. K/S, L*, a*, b*values of final dyed samples of silk and wool with *Trichoderma sp*

S.No	Textile fabric	*Trichoderma* PD broth (28°C)			
		K/S	L*	a*	b*
1	Silk	0.90	85.27	-2.23	21.81
2	Wool	2.00	82.91	-4.07	35.18

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