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COLOUR MEASUREMENTS AND FASTNESS PROPERTIES OF NATURAL FABRICS DYED WITH PIGMENT OBTAINED FROM *PSEUDOMONAS FLUORESCENS*

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ABSTRACT
The pigments produced by bacteria have received the attention of many investigators. In recent years, emphasis has been placed especially on the chemical nature of the pigments and their probable roles in the textile application. Efforts have been made in the present study to identify new sources of natural dye and to see its dyeability and colourfastness properties on protein fibers. A non-pathogenic pigment producing strains of *Pseudomonas fluorescens* were tried on silk and wool fabrics, to see whether these extracted pigments can be used as dye or able to develop colour on textile. Microbial dye from *Pseudomonas fluorescens* has been evaluated for their potential as a source for natural dyeing of silk and wool. The dyed samples have been evaluated for colour measurement and standard wash, light, rub and perspiration fastness tests. The dyed samples showed acceptable fastness properties as of other natural dye sources and are found to posses good colour strength and dyeability. The results show that dye from *Pseudomonas fluorescens* are promising as a natural colourant which would, in turn, pave the way for the discovery of a new range of environment- friendly dyes for textile materials.

KEYWORDS: *Pseudomonas fluorescens*, natural dye, colour, natural fabrics, microbial dye.

INTRODUCTION
Modern consumers are concerned about environment conservation, consumer safety, health and hygiene. Therefore, interest is created towards eco-friendly textiles. The market for the natural dyestuff is very small. Natural dyed material is not widely available to the average consumer. Emphasis has been given now a days to identify new natural sources of raw materials to be used as dye and finishing agent and to standardize the procedures for their use. Use of synthetic dyes has cut down significantly due to toxic effluent resulting from the dyeing process. Therefore, natural dyed product represents the good opportunity for value added manufacturing. The most important part of the production of natural dyes is the sourcing of the raw material. The raw material selected for the extraction of the colouring matter should be easily available at reasonable price and in large quantities throughout the year (Mukherjee *et al*., 2005) Natural colours have proved their versatility by way of application in foods, pharmaceuticals, cosmetics and in textiles. The concept of total eco-friendly apparels can be thus be introduced giving technological inputs to the textile small scale processors in rural areas which will have potential of providing employment to the rural youths strengthening their economy. On the other hand natural dyestuff are required to be carried out on the lines of ease of application, repetitive colour yield, shorter dyeing cycles, more so exactly on the pattern of synthetic dyes application. This may precisely lead to commercialization of natural colourants (Mishra, 1999).

The aim of this study has been to identify newer sources of natural dyes. Although, the hills of Kumaon region of Uttarakhand state are reservoir of dye yielding plants but in today's world there is scarcity of productive land for cultivation of dye yielding plants which is necessary to fulfill the industrial demand. Secondly, the content or amount of dye present in the plants varies greatly depending on the season as well as age of the plants (Anon, 2003). Therefore, there is upsurge need to explore some dye sources other than plant such as bacterial, lichen, fungal and algal dyes *etc*. Microbial pigments are of great structural diversity. They may be derivatives of the material classes of carotenoids, phenazine dyes, pyrrole dyes, azaquinones *etc*. (Meyer and Montforts, 2005). Biotechnology can also produce novel natural colorants and assist effluent treatment systems to remove colour. Microbial production of anthraquinone dyestuff intermediates eliminates the use of strong acids, heavy metals and high temperature dyeing. Further efforts are needed to optimize an economically viable process. Particular attention is currently being paid to microbes capable of degrading azo dyes. Attempts have been made to synthesize bacterial forms of indigo as well as fungal pigments for use in the textile industry. Certain microbes like fungi are capable of yielding up to 30% of their biomass as pigment (Ramachandran et al., 2004).

MATERIAL & METHODS

Dye source

Pigment producing, non-pathogenic strain of *Pseudomonas fluorescens*, (Strain, Pf-24) isolated from soil, were obtained from Bio-control Laboratory, Department of Plant Pathology, College of Agriculture, G.B.P.U.A.&T., Pantnagar, Uttarakhand. This strain was used for production of natural dye, which was also tested for mammalian toxicity and was found completely safe (Mishra, 2007).

Textile materials

Dyed samples of silk and wool were selected as natural textile substrate to see effect of dyes in the present study. Tasar silk and Merino wool fabric were procured from Gandhi Ashram, Pantnagar, Uttarakhand, India. The constructional details of all the experimental fabrics are given in Table- 1.

TABLE 1: Constructional details of fabrics

Textile Material	Constructional Details		
	Fabric count		Weave
	Warp	Weft	
Silk	104	100	Plain
Wool	76	65	Plain

Wool sample was scoured with solution containing 0.5g/liter sodium carbonate and 2g/liter non-ionic detergent solution at 40-45ºC for 30 minutes, keeping the material to liquor ratio at 1:50. The scoured material was thoroughly washed with tap water and dried at room temperature. The scoured material was soaked in clean water for 30 minutes prior to dyeing to remove the air and soften it to facilitate even dye penetration (Shankar and Vankar, 2007).

Silk was degummed as recommended by Sreenivasa and Sengupta (2007). A detergent solution containing 10ml of mild detergent (genteel) per 100ml of water was prepared and heated at 50ºC. Silk skeins were dipped into this solution maintaining the material to liquor ratio 1:40 and stirred gently for 60 minutes. It was then washed and dried in shade. Now the pre-treated experimental fabrics can be subjected to dying.

Cultivation of the bacteria in favouring pigment production

Prior to attempt the cultivation of bacteria for dye preparation, the growth conditions, which favoured pigment production, like type of medium (broth or agar), pH of medium, incubation temperature, shake or stationary culture, incubation time were determined (Mishra, 2007). Strain pf-24 of Pseudomonas fluorescens was grown on Modified King's B agar medium (pH7.0), plates were incubated in stationary condition in a B.O.D. incubator at 25^0C for 48 hrs.

Extraction of dye

After 48 hrs of incubation the slimy/mucous growth of bacterial cells that had become dark red was scraped out with the help of flat spatula from the plate, placed in a glass plate and dried in air. This dried dark red powder soluble in water was directly used as dye for textile material.

Dyeing

All the experimental fabrics has their own extent to absorb the dye, therefore, all the fabrics were dyed with optimized dyeing conditions, which produced good colours on fabrics that were colourfast. Aqueous dyeing was carried out in open water bath system. Dyeing of silk and wool fabric with extract was carried out at 10% of (on weight of fabric) at 1:30 (material to liquor ratio) for 60 minutes at acidic medium (pH-5) at 70ºC. Dyed samples were rinsed in cold water and dried under shade (Gulrajani and Gupta, 1992).

Evaluation of colour strength and colour coordinates

Silk and wool dyed samples were evaluated for their colour depth (K/S) as well as for colour coordinates (L*, a*, b*, C*, hº) to analyse or quantify its dyeability. Dyed samples were evaluated by using CIELAB colour cordiantes with illuminant D65/10º observer on Macbeth Colour-Eye 3100 3100 of Kollmorgen Instruments (USA) spectrophotometer, at the maximum absorbance wavelength (λ_{max}) of the corresponding dyed sample. Five measurements were made for each sample and average results were recorded. The colour strength (K/S) measurements were based on the ratio of total light absorbed and scattered S by the substrate as developed by th Kbelka Munk equation.

Values of colour coordinates were computer calculated from reflectance data which reflects:

L^*= lightness/darkness axis (L^*=0 for black and L^*=100 for white)

a^* = red-green axis (+ a^* for red and -a^* for green)

b^* = yellow-blue axis (+ b^* for yellow and -b∗ for blue)

C^*= chroma $(a^2 + b^2)^{1/2}$

hº = hue ($\tan^{-1}$ b/a)

Colourfastness tests

Silk and wool samples dyed with Pf-24 dye were further used for testing colourfastness against light, washing, crocking and perspiration.

Wash Fastness Test was carried out as per the recommendations of IS: 3361-1979 (test-2) in a "launder-O-meter." AATCC (1975) rating scale was used for assigning wash fastness scores for change in colour and degree of staining.

Light fastness test was done as per the recommendations of IS: 686-1957. Treatment for improving colorfastness to light the red colour obtained from Pf-24 without mordanting faded very rapidly in the presence of sunlight and such dyeing is therefore of limited practical use. For improving the resistance to light of textiles dyed with the plum red pigment extracted from Pf-24 was subjected to post-treatment with a thiourea solution as per the procedure given

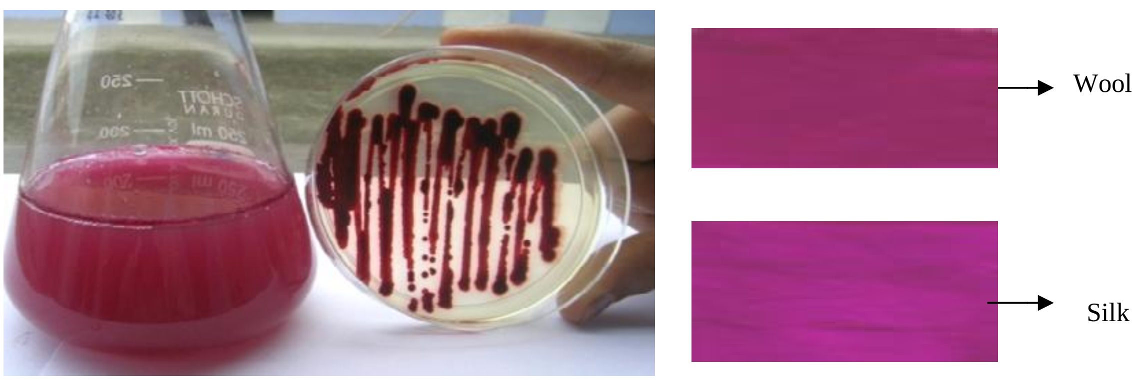
by Shirata *et al.* (2000). The dyed material was immersed in 5% thiourea solution keeping material to liquor ratio 1:30 for 15minutes at room temperature. It was then drained and air-dried. The colorfastness of this dyed and treated material against light was determined and the results were compared with the results of light fastness of untreated dyed samples.
Fastness to rubbing (dry and wet rubbing) and perspiration fastness was carried out as described, IS: 766-1956 and IS: 971-956, respectively.

RESULTS AND DISCUSSION

The study was carried out for dyeing silk and wool samples with dye extracted from Pf-24 strain of *Pseudomonas fluorescens.* The possible reasons related to the results are discussed herewith. The present study deals with dyeing of protein fibers followed by assessing dyeability (in terms of K/S value and colour coordinates) and colourfastness properties. This is first study of using Pseudomonas fluorescens, a bacterium, as dye source hence; much information is not available to compare the finding of the present study.

Cultivation, pigment extraction and dyeing

The dye from strain Pf-24 has dyeing property which is accomplished from our studies that can have a great potential in textile coloration. Colonies on MKB (Modified King's B) agar plate of strain Pf-24 produced excessive plum red pigment. They were raised, whole, circular, convex and smooth and ranged from 1 to 3mm in diameter after 48 h of incubation. The colonies have a mucoid appearance and tacky consistency (Fig.1a). Strain Pf-24 incubated on King's B agar medium at pH-7.0 was optimized which incubated at 25ºC for 2 days for getting maximum pigment of desired colour. Intracellular red pigment was scraped, dried and was directly used for dyeing purpose.
Dyeing of silk and wool fabric with extract was carried out at 10% of (on weight of fabric) at 1:30 (material to liquor ratio) for 60 minutes at acidic medium (pH-5) at 70ºC. Dyed samples (Fig. 1b) were rinsed in cold water and dried under shade.



(a) **(b)**

FIGURE 1 (a) Pigmented growth of Pseudomonas fluorescens (Pf-24) in broth and agar (b) dyed fabric samples

Colour Characteristics

Colours of all the dyed samples was assessed through the computer colour matching by calculating K/S and colour values (L*, a*, b*, C* and hº). The colour expression is a mixture of such attributes as hue (red, yellow, blue, green) brightness, darkness, lightness and dullness. Colour coordinates and depth of colour of various dyed samples were evaluated (Table-2).

TABLE 2: The colorimetric data of samples dyed with Pseudomonas fluorescens

Dyed sample	K/S	Colour values				
		L*	a*	b*	C*	hº
Silk	8.09	53.94	51.02	-12.82	52.61	345.90
Wool	13.68	49.99	23.74	-13.79	24.07	321.02

Colour depth or K/S value of silk samples dyed with dye extracted from Pf-24 was 8.09. Table- 2 depicts that wool sample showed 13.68 K/S value and gave deep shade with blue-red colour tone. Higher dye uptake on silk could be attributed to dyeing in acidic medium in which electrostatic forces between the positively charged side chain of the protein fiber and the dye molecules would be expected to play a major role in dye adsorption (Dayal and Dobhal, 2000).
L* represents the lightness and darkness of the sample. The low L* values of the wool dyed samples as compared to the L* values of silk dyed sample in the Table 3 showed that the dye is substantially absorbed by wool samples and gave deeper shade. Regarding hue (red, green, yellow and blue) it was found that the lower a* and b*values of the dyed wool samples indicate the decrease in redness and yellowness, respectively. The C* values or chroma indicate that silk dyed sample was more bright as compare to wool sample.

Colour coordinates (a* and b* values) in the Table 4.12(a) clearly indicate that dyed wool samples exhibited blue-red tone. Very little difference in hº value was found in between wool and silk samples.

Among protein yarns the colours are more pronounced in case of wool than silk, the reason being the presence of arginine, cysteine and glutamic acid on wool, which provides additional carboxyls and amino groups for their interaction with the dye and mordants molecules while alanine, glycine and serine are the major constituents of silk with no additional carboxyl and amino groups as in case of wool. But the relative change (percentage increase/decrease) in L*, a*, b* values is almost the same in case of wool and silk due to the basic nature(amino acids) of both the fibers (Dayal and Dobhal, 2000).

On considering the effect of the nature of substrates on colour depth (K/S) and L* values, wool fabric showed lowest value i.e. darker colour than silk. These results are in accordance with statement given by Micheal et al. (2003) that colour depth and their darkness, lightness, brightness and dullness depends upon the inherent properties of fibers like porosity and polarity of the experimental samples.

Colourfastness properties

All the samples were tested for colourfastness to washing, sunlight, rubbing and perspiration and results are given in Table 3. The colourfastness properties of dyed samples such as acidic and alkaline perspiration, wash fastness, light fastness and rubbing fastness were assessed with guidelines from standard AATCC tests methods.

The colourfastness properties of fabric dyed with Strain pf-24 of Pseudomonas fluorescens for wash fastness according to ISO standard tests. According to the standard grey scale for colour change and grey scale for colour staining the results are observed and presented in Table-. The colourfastness property of dyed samples to washing was around 4-5. Thus the results for colourfastness for washing were ranging from good to excellent level for both silk and wool dyed samples.

TABLE 3: Fastness properties of samples dyed with *Pseudomonas fluorescens* pigment

Dyed Sample	Wash Fastness			Light fastness	Rubbing fastness		Perspiration fastness					
							Alkaline			Acidic		
	CC	SC	SS		Dry SC	Wet SC	CC	SC	SS	CC	SC	SS
Silk	4	4-5	4-5	3	4-5	4	4	4	3-4	4	4	3-4
Wool	4	4-5	4	3-4	4-5	4	4	4	3-4	4	4	3-4

CC: Colour change, SC: Staining on cotton, SS: Staining on silk

Colourfastness to light of samples dyed with plum red pigment ranged between very poor (1) to poor (2) which has no or limited practical use. For improving the resistance to light of materials was subjected to post-treatment with a thiourea solution which exhibited tremendous change and improve the colourfastness to light. Fastness to light for treated samples ranged from fair (3) to fair to fairly good (3-4). The thiourea-treated fabric showed a slower rate of color fading, suggesting that the color fastness had improved. The color fastness of the untreated material was found between very poor to poor (1-2) to poor (2). The dyed material which had been treated with the thiourea solution did not cause any change in shade, nor did it have any adverse effect on the feel of the material. Further from Table it is evident that the colourfastness to light of the silk dyed sample post treated with thiourea was found to be fair (3) and wool sample exhibited fair to fairly good (3-4) colourfastness to light.

Colourfastness against rubbing indicates that both dyed samples i.e. silk and wool showed slight to negligible (4-5) staining on dry rubbing whereas in case of wet crocking, slight staining (4) was observed.

Dyed silk and wool samples when subjected to alkaline and acidic perspiration, showed slight (4) change in colour and slightly staining (4) on cotton and noticeably to slight staining (3-4) on silk and wool was observed. The colourfastness property of dyed samples to perspiration was around 3-4. Thus the results for colour fastness for perspiration were in moderate to good level. The fastness properties of silk and wool fabrics dyed with various natural colourants has been previously investigated by Scientists (Rajendran *et al*., 2011, Manonmani, *et al*., 2009, Kim, 2004, Susanna. 2006) and the results were in accordance with them. It has been reported that the colourfastness property to wash and light was in the range of 3 and 4 (good) respectively and fastness to dry and wet rubbing were also satisfactory (in range of 3-4).

CONCLUSION

Today when the world is shifting from synthetic to nature based products, elaborate research and development work has been started to assess the hazardous nature of synthetic dyes and natural dyes. Emphasis has been given to identify new natural sources of raw materials to be used as dye and finishing agent and to standardize the procedures for their use. Strain (Pf-24) of *Pseudomonas fluorescens* producing plum-red colour was selected for dyeing of natural silk and wool fabrics and dyeing was carried out in aqueous medium. The colourfastness properties of dyed samples were also checked against washing, light, rubbing and perspiration. Further all the dyed samples were evaluated for their colour depth (K/S) as well as for colour coordinates (L*, a*, b*, C*, hº). It was found that colours were more pronounced in case of wool as compare to silk. But the relative change (percentage increase/decrease) in L*, a*, b*, C*, hº values were almost the same for both protein fibers due to the basic constituents (amino acids) of both the fibers. On considering the effect of the nature of substrates on colour depth (K/S) and L* values, wool fabric showed lower (darker shade) than silk. The dyed samples showed acceptable fastness properties as of other natural dye sources and are found to posses good colour strength and dyeability.

Dyes extracted from *Pseudomonas fluorescens* are relatively inexpensive and can readily be produced in laboratory with certain specific equipments in short time and independent from related variables like season and age. Source of these dyes is abundantly found in soil and their tinctorial strength was like synthetic dyes. Therefore, this study will greatly help in producing large amount of natural dyes in relatively short period, which will be helpful in fulfilling industrial demand in Indian as well as world market.

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