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Bacterial Dye obtained from pseudomonas Fluorescens for Dyeing of Cotton

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Abstract

The world is shifting from synthetic to nature based products in today's era. Bacterial pigment production is now one of the emerging fields of research to demonstrate its potential for various industrial applications. Therefore, present research work was carried out to optimize the growth conditions of *Pseudomonas fluorescens* (Pf-6), dyeing variables, to see the effect of natural mordants with dye using different mordanting methods on cotton fabric, testing of color fastness and antimicrobial activity of the dye. Maximum pigment was extracted from *Pseudomonas fluorescens* when it was inoculated on King's B agar at 7pH and incubated for two to seven days at 25^0C in BOD incubator. Best colour was obtained on fabric when it was dyed with *the* dye keeping the pH 5 of the dye solution, 2 percent concentration of the dye, 1:30 material to liquor ratio and dyeing was done at 80^0C for 90 minutes. Overall fastness of the dyed fabric increased with mordanting.

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Introduction

Natural dyes are mainly obtained from plant sources. They have many advantages but also have certain limitations. These limitations includes low or non availability throughout the year, requirement of large amount of cultivated land for the production of plant sources, problem in transportation, lack of the reproducibility of the same shades and unstable to light and pH etc. Therefore, great concern is necessary for searching of those natural sources of textile dyes which can overcome these limitations of natural dyes. Recently, there has been increasing interest in using microorganism as a renewable source of natural dyes for textiles. Microorganisms take less time to multiply whereas plants take many days to years to grow. Moreover most of plant give dye during specific season. Thus, microorganisms can be one of the best alternate for the production of natural dyes.

Therefore, efforts have been made in the present research work to identify new bacterial source of natural dye with

objectives to optimize the growth conditions of *Pseudomonas fluorescens* (Pf-6), to optimize the dyeing variables, to assess the color fastness of dyed fabric and to test the antimicrobial activity of dye source.

Materials and methods

Materials

Plain woven cotton fabric having thread count of 82 warps and 62 wefts per inch was selected for the study. Different shades were developed on cotton fabric using five different natural mordants viz. amla, walnut bark, bahera, pomegranate rind, harad and synthetic mordants viz. alum, copper sulphate, ferrous sulphate, tannic acid and tartaric acid along with the dye obtained from *Pseudomonas fluorescens* (Pf-6).

Methods

Optimization of growth conditions

Growth conditions of the bacteria were optimized on the basis of growth medium, pH of culture media, incubation time and incubation temperature in order to obtain maximum pigments.

Optimization of dyeing variables

A series of experiments were conducted to optimize various dyeing variables. Optimum value of specific parameter was selected on the basis of percent absorption of dye and percent marks obtained by the dyed cotton fabric samples after visual evaluation.

Selection of mordants

Both natural and synthetic mordants were applied with the dye solution using simultaneous mordanting and dyeing technique on cotton samples. These samples were visually evaluated by the selected panel of judges to select the best two mordants from each natural and synthetic mordants.

Optimization of concentration of mordants

The concentration of natural and synthetic mordants for the dye obtained from *Pseudomonas fluorescens* (Pf-6) was optimized on the basis of visual evaluation by the panel of judges. The samples obtained highest percentage of marks was selected and the selected concentration of mordant was considered as optimum for the further experiments.

Optimization of methods of mordanting

Various shades were produced on cotton fabric by different mordanting methods with each selected natural mordants along with the dye obtained from *Pseudomonas fluorescens* (Pf-6). The dyed samples were evaluated visually to select the best method of mordanting.

Testing colour fastness of dyed samples

Dyed samples were evaluated for their colour fastness against washing, sun light, crocking and perspiration using the appropriate standardized test procedures.

Assessment of antimicrobial activity

AATCC Test Method 90-1974: Antibacterial Activity of Fabric, Detection of Agar Plate Method [1] was used for the assessment of antimrobial activity of the dye.

Results

Optimization of growth conditions

It was found that *Pseudomonas fluorescens* (Pf-6) produced excessive pigment on the King's B agar medium having 7 pH after 2 days of incubation at 25^0C. Therefore, King's B agar medium was selected for the

cultivation of *Pseudomonas fluorescens* (Pf-6).

Optimization of dyeing variables

λ-max of dye obtained from *Pseudomonas fluorescens*(Pf-6)was determined through scanning in UV-VIS region in order to record optical density of solutions before and after the dyeing. λ-max was found to be 570 nm.

pH

Cotton samples showed decrease in percent absorption from 54.22 percent (pH 5) to 23.06 percent (pH 9) with increase in pH of dye liquor. Dyed cotton samples obtained highest marks (76.0 percent) when dyed with dye liquor maintaining pH 5. Thereafter, percent marks decreased till 38.0 percent when dyeing of cotton was carried out at pH 9. Therefore, pH 5 was selected as optimum pH for dyeing of cotton with *Pseudomonas fluorescens* (Pf-6) dye.

Fabric	pH	Pf-6 dye (λ_{max}- 570 nm)	
		Percent absorption	Percent marks
Cotton	5*	54.22	76
	6	42.99	66
	7	40.10	53
	8	25.26	47
	9	23.06	38

Selected pH
O.D. – Optical density

Table 1: Percent absorption and percent marks obtained by cotton samples dyed with *Pseudomonas fluorescens* (Pf-6) dye at different pH

Concentration of dye

Fabric	Conc. of Dye (g/100 ml)	Pf-6 dye (λ_{max}- 570 nm)	
		Percent absorption	Percent marks
Cotton	0.25	30.28	48
	0.5	35.12	52
	1.0	44.52	57
	2.0*	43.86	68
	4.0	52.85	53

***** Selected concentration O.D. – Optical density

Table 2: Percent absorption and percent marks obtained by cotton samples dyed with *Pseudomonas fluorescens* (Pf-6) dye with different concentration

Percent absorption of cotton samples increased with increase of dye concentration and found to be maximum when concentration was 4.0 g/100 ml. The reason behind this may be that when there is increase in the dye particles in dye solution, more dye molecules will be absorbed by the textile material. Mondal (2007) also stated that when there is increase of dye concentration, number of dye particles in the dye liquor was increased. So, absorption of dye is increased due to the van der wall's forces which exist between the dye particles and the fiber molecules. Consumers are the ultimate user of textiles and their preference is foremost criteria for the selection of dyed

textile material. Thus, results of visual evaluation was given more weightage for selection of coloured textile material which is carried out on the basis of luster, evenness of dye, depth of shade and overall appearance. Therefore, from economic point of view as well as on the basis of visual evaluation, 2.0% concentration was selected as optimum for dyeing of cotton fabric.

M:L ratio

Fabric	M:L Ratio	Pf-6 dye (λ_{max}- 570 nm)	
		Percent absorption	Percent marks
Cotton	1:30*	47.42	56.10
	1:50	46.60	51.69
	1:70	38.79	45.87
	1:90	35.95	40.98
	1:110	30.16	37.65

***** Selected material to liquor ratio O.D. – Optical density

Table 3: Percent absorption and percent marks obtained by cotton samples dyed with *Pseudomonas fluorescens* (Pf-6) dye keeping different material to liquor ratio

Percent absorption decreased with increase in material to liquor ratio. Cotton fabric obtained highest percent absorption when dyeing was carried out with material to liquor ratio of 1:110. It may be because the concentration of dye material decreased when the material to liquor ratio was raised. This resulted in the low percent absorption by the textile material with increase in material to liquor ratio. On visual evaluation, cotton fabric samples secured highest percent of marks when the dyeing was carried out maintaining the material to liquor ratio 1:30. Therefore, material to liquor ratio 1:30 was selected as optimum for further experiments.

Dyeing temperature

Fabric	Dyeing Temp. (^{0}C)	Pf-6 dye (λ_{max}- 570 nm)	
		Percent absorption	Percent marks
Cotton	50	38.61	42.98
	60	41.55	49.62
	70	44.28	53.74
	80*	50.99	59.49
	90	48.16	56.91

***** Selected dyeing temperature O.D. – Optical density

Table 4: Percent absorption and percent marks obtained by cotton samples dyed with *Pseudomonas fluorescens* (Pf-6) dye at different dyeing temperature

It is clear from the Table that percent absorption of cotton sample was minimum i.e. 38.61 when dyeing was carried out at 50^0C. Increase in percent absorption of dyed samples was observed with increase in dyeing temp till 80^0C. Thereafter, decline in percent absorption of cotton (48.16 percent) was observed with further increase in dyeing temperature till 90^0C. Therefore, 80^0C was selected as the optimum dyeing temperature for the dyeing of cotton fabric with *Pseudomonas fluorescens* (Pf-6) dye.

Dyeing time

Minimum percent absorption (31.35 percent) was obtained when cotton fabric was dyed for 30 minutes. Percent absorption increased with increase in dyeing duration and reached

to its maximum (42.25 percent) when dyeing was carried out for 90 minutes. This cotton sample also obtained highest percent marks (58.09 percent) when visually evaluated. Therefore, duration of 90 minutes was selected as optimum dyeing duration for the dyeing of cotton fabric with *Pseudomonas fluorescens* (Pf-6).

Fabric	Dyeing time (minutes)	Pf-6 dye (λ_{max}- 570 nm)	
		Percent absorption	Percent marks
Cotton	30	31.35	32.58
	45	33.59	39.0
	60	36.41	47.35
	75	39.53	51.76
	90*	42.25	58.09

Table 5: Percent absorption and percent marks obtained by cotton samples dyed with *Pseudomonas fluorescens* (Pf-6) dye for different dyeing time

Mordants

Cotton samples were mordanted with different natural mordants and dyed with both the dyes using simultaneous mordanting and dyeing technique. Selection of natural mordants was done on the basis of highest percent marks obtained by the samples when visually evaluated.

Selection of mardants

Walnut bark and pomegranate rind were selected natural mordants on the basis of highest percent marks and colour obtained.

Natural Mordants	Percent marks
Amla	53.33
Bahera	46.66
Harad	56.66
Walnut bark	73.33*
Pomegranate rind	61.66*

* Percent marks of selected mordants

Table 6: Percent marks of cotton samples dyed with *Pseudomonas fluorescens* (Pf-6) dye along with different natural mordant

Concentration of natural mordants

Cotton samples mordanted with 4.0 g walnut bark and 3.0 g pomegranate rind in combination with *Pseudomonas fluorescens* (Pf-6) dye obtained 74.35 percent and 53.45 percent marks respectively when visually evaluated. Therefore, 4.0 g walnut bark and 3.0 g pomegranate rind were selected as optimum concentration for the mordanting of cotton fabric.

Concentration g/100ml	Percent marks	
	Walnut bark	Pomegranate rind
1.0	65.33	47.33
2.0	69.68	49.76
3.0	72.0	53.45*
4.0	74.35*	51.84
5.0	73.65	48.39

*Highest percent marks

Table 7: Percent marks of cotton samples dyed with *Pseudomonas fluorescens* (Pf-6) dye along with different concentration of natural mordants

Method of mordanting for natural mordants

Best colours were produced on cotton samples when these were dyed with *Pseudomonas fluorescens* (Pf-6) and post mordanted with walnut bark and simultaneously mordanted and dyed with pomegranate rind. Therefore, post

mordanting method was selected for the mordanting of cotton samples.

Method of mordanting	Percent marks	
	Walnut bark	Pomegranate rind
I	48.0	36.33
II	48.33	43.0
III	61.33*	44.66*

I- Pre mordanting,
II- Simultaneous mordanting and dyeing,
III- Post mordanting,
*Highest percent marks

Table 8: Percent marks of cotton fabric samples with *Pseudomonas fluorescens* (Pf-6) dye along with natural mordants using different mordanting method

Colour fastness

All the cotton samples dyed with *Pseudomonas fluorescens* (Pf-6) dye and mordanted with different selected mordants and methods of mordanting were evaluated to assign the ranks on the basis of overall colour fastness properties. Sample mordanted with pomegranate rind was found best as it occupied first rank and maximum score followed by sample mordanted with walnut bark. Thus, it was observed that the colour fastness of *Pseudomonas fluorescens* (Pf-6) was improved with the use of selected natural mordants.

Mordants	MM	WF			LF	RF				PF						WMS	Rank
		CC	SC	SW		Dry		Wet		Acidic			Alkaline				
						CC	SC	CC	SC	CC	SC	SW	CC	SC	SW		
Blank	**-**	**3/4**	**4**	**4/5**	**3**	**4**	**4/5**	**¾**	**4**	**3/4**	**4**	**4**	**3/4**	**4**	**4**	**3.82**	
Walnut bark	**III**	**4***	**4/5**	**4/5**	**4/5**	**5**	**4/5**	**4/5**	**4**	**5**	**5**	**4/5**	**5**	**5**	**5**	**3.86**	**II**
Pomegranate rind	**III**	**3/4**	**4/5**	**4/5**	**4**	**4/5**	**4/5**	**4**	**4**	**4**	**4/5**	**4/5**	**4**	**4/5**	**4/5**	**4.20**	**I**

MM: Mordanting Method, **II:** Simultaneous mordanting, **III:** Post mordanting,
WF: Wash fastness,
LF: Light fastness,
PF: perspiration fastness,
RF: Rubbing fastness, **CC:** Change in colour, **SC:** Staining on cotton,
SW: Staining on wool,
WMS: Weighted mean score, * Increase in colour

Table 9: Fastness properties of mordanted cotton samples dyed with *Pseudomonas fluorescens* (Pf-6) dye

Antimicrobial properties

Qualitative assessment

Test pathogens	Inhibition zone (mm)	
	Cotton fabric	
	Control	Dyed
E. coli (gram –ve)	Nil	0.50
S. aureus (gram +ve)	Nil	0.75

Table 10: Inhibition zone exhibited by cotton fabric samples dyed with *Pseudomonas fluorescens* (Pf-6) dye

Table reveals that no inhibition zone was observed in control swatches against gram positive bacteria, *E. coli* and gram negative bacteria *S. aureus*. Cotton samples dyed with *Pseudomonas fluorescens* (Pf-6) dye exhibited the clear zone 0.50 mm and 0.75 mm respectively against *E. coli* and *S. aureus*.

Conclusion

Dye obtained from *Pseudomonas fluorescens* (Pf6) produced pink colour which is rarely obtained from plant sources.

It was found to have antimicrobial properties. Therefore, it can be used for the dyeing of children's wear or medical textiles. It can be concluded that bacterial dyes will be helpful in providing an alternative to synthetic dyes and other plant dyes.

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