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Reddish brown pigments from *Alternaria alternata* for textile dyeing and printing

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Reddish brown pigment has been extracted from dry mycelium of *Alternaria alternata* in methanol and then evaluated for dyeing efficacy on cotton fabrics. The pigment producing fungus *A. alternata* is grown in maize grain broth maintained at *p*H 6. Shade of Sienna is obtained on cotton. Dyed cotton fabric has recorded a grey scale rating of 2-3 and 4-5 for colour fastness and multi fibre staining respectively. Maximum dye absorption of 63% has been observed on cotton fabrics. The antimicrobial property of dyed fabric is also tested. Potent antimicrobial activity is observed against *Staphylococcus epidermis* (42 mm) and *Streptococcus pyogenes* (39 mm).

Keywords: *Alternaria alternata*, Fungal pigment, Natural dyeing, *Staphylococcus epidermis*, *Streptococcus pyogenes*

1 Introduction

Recently the use of natural dyes on textile materials has been attracting more and more scientists due to the wide viability of natural dyes and their huge potential[1-5], in spite of the better performance of synthetic dyes. However, the limitation of natural colourants is their poor fastness, limited shades and low brilliancy on the dyed textile. It has been well known that apart from a variety of plants and animals, microorganisms also produce pigments[6-10]. Recently, there has been increasing interest in using micro organisms as a colour source since the cost efficiency, labour, extensive land requirement and use of expensive solvent for extraction are higher in plant materials. In nature, colour/pigment production occurs in certain algae, fungi, bacteria and small crustaceans. Microorganisms produce various pigments like carotenoids, melanins, flavins, quinones, prodigiosins and more specifically monascins, violacin or indigo[11]. Secondary metabolites from microbes can be utilized for industrial applications and leather dyeing[12]. Microbes have advantages of versatility and productivity over higher forms of life in the industrial-scale production of natural pigments and dyes. Also most of these pigments are reported to have antimicrobial properties which makes their use very lucrative. Similar to plants, there is a long history of the utilization of fungi by mankind as remedies in everyday life. The Mayans used fungi to treat intestinal ailments nearly 3000 years ago[13]. The bio- transformation by fungi has been used for food production since Neolithic times. The earliest types of fermented food were beer, wine and leavened bread, followed by the early Chinese who produced fermented soy foods. Various studies confirm that the pigments produced from *Monascus purpureus*, *Emericella* spp. *and Penicillium* spp. pose no toxic effects[14-15] and the pigments are biodegradable[16] and contain negligible amount of phenolic component[17-18]. The application of fungal pigments in dyeing of cotton, silk and wool have been reported in several studies[19-21]. Fungi are rarely used in natural dyeing, even though some species are known to possess stable colourants. The biodegradability and non-toxicity of the fungal dyes which are diverse in structure and perform functions that are not always known is also confirmed from several previous studies[22]. However, dyes from fungus or the utilization of fungal pigments for textile applications is fairly investigated. The genus *Alternaria* contains 300 accepted species that widely occur in soil and organic matter. There are several reports on the diverse bioactivity of the wide assortment of secondary metabolites produced from the cosmopolitan, saprophytic and endophytic fungi *A.alternata*. The potential applications of *Alternaria* metabolites as antitumor agents, herbicides, and antimicrobials as well as other promising bioactivities have led to considerable interest for diversified industrial applications[23]. However, very limited information is available on the utilization of pigments

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from *A.alternata* in textile applications. In the present work, the reddish brown pigment of *Alternaria alternata* has been extracted for its substantivity in dyeing and painting of selected textile fabrics. The fabric properties such as colour fastness and antimicrobial activity are also ascertained.

2 Materials and Methods

2.1 Alternaria Culture Conditions

A. alternata (MTCC 2724) strain was obtained from microbial type culture collection and gene bank (MTCC), IMTECH, Chandigarh. The culture was revived on sterile potato dextrose agar (HIMEDIA, India) medium and incubated at 28°C. The 21st day mycelial culture served as inoculum for further studies. Erlenmeyer flasks (250 mL) of 200 numbers, containing 50 mL of maize grain extract (MGE) medium maintained at *p*H 6 were taken individually as substrate. The flasks containing medium were autoclaved at 121°C for 15 min. The flasks were inoculated with 6mm mycelial discs of 21 day old *A. alternata* (MTCC 2724) and incubated at 28°C ± 2°C as stationary cultures for 9 days. The flasks containing *A. alternata* were withdrawn on 9th day and the mycelial mat was collected by filtration using Whatman no.1 filter paper. The harvested biomass was air dried at 40°C for 24 h and the dry mycelium was used for pigment extraction.

2.2 Extraction of Pigments from *A.alternata*

Dry mycelium (35 g) was pre weighed, soaked in 30mL of methanol and well ground using a mortar and pestle. The mixture was allowed to stand overnight and then taken for refluxing in Soxhlet apparatus (Borosil, India) with 3L methanol. The extraction was continued up to 48 h to extract the pigment. The pigment was then condensed using a rotary evaporator (Lark, India) and subsequently centrifuged at 1000 rpm for 20 min. The reddish brown supernatant was collected and condensed to 20 mL.

2.3 Dyeing and Painting of Selected Fabrics

2.3.1 Pre-treatment of Cotton Fabric

Cotton fabric (25 g) was pre-soaked in distilled water and then cooked in 5L of distilled water with 5 g of soap nut for 1h at 70°C to remove dirt, grease and oil. The cloth was then rinsed thoroughly with running water. The scoured fabric was treated with alum (1%) for 45 min at 70°C, rinsed in running water and finally allowed to dry.

2.3.2 Dyeing of Cotton Fabric

The condensed pigment (20 mL) was redissolved in 1L distilled water. Dyeing experiments were carried out on pretreated cotton fabric using the reddish brown pigment of *A.alternata*. Pretreated cotton was dyed at material liquor ratio (MLR) of 1:50 at 70°C for 45 min. After dyeing, the fabric was treated with 1% acetic acid and washed thrice in running water. The dyed fabric was then rinsed with cold water and shade dried before testing colour fastness.

2.3.3 Painting of Silk Fabric with A. alternata Pigments

Painting was done on commercially available tussar silk fabrics. The silk fabric was pinned on four sides to a drawing board. *A.alternata* pigment (1 g) was mixed with natural binder (10 mg crushed tamarind seeds, 10 mg chalk, and cooked in 50 mL water) and then used for painting. The cloth was then allowed to shade dry.

2.4 Determination of Fabric Properties

2.4.1 Percentage Absorption of Dye

Percentage absorption of the fungal pigment by dyed fabrics was calculated using UV-Visible spectrophotometer (VARIAN, US) as per the following equation:

$$\text{Absorption}\,(\%) = \frac{\text{OD before dyeing} - \text{OD after dyeing}}{\text{OD before dyeing}} \times 100$$

2.4.2 Colour Fastness

All the dyed fabrics were tested for colour fastness to washing, perspiration, rubbing and light according to standard methods in The Regional Laboratory, Textile Committee (Ministry of Textiles, Government of India) Chennai. The colour fastness to commercial laundering at 40°C was determined according to ISO: 105: C06 - 1997; and colour fastness to perspiration according to ISO: 105- E04- 1996; rubbing according to ISO: 105: X 12 - 2002 and light according to ISO: 105: B02 - 1999. Colour change of the fabrics was assessed against grey scale rating.

2.4.3 Antimicrobial Property

The cotton fabric dyed with the reddish brown pigment of *A.alternata* was tested for its antimicrobial properties using standard disc diffusion method with slight modifications[24]. The test organisms used for the study were obtained from MTCC, Chandigarh, India. The selected test pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Streptococcus pyogenes*, *Trichoderma viridae* and

Curvularia lunata were screened against the fabric dyed with reddish brown pigment of *A.alternata*. The sterile nutrient agar and potato dextrose agar plates were inoculated with the test bacterial and fungal strains using lawn culture method. The dyed cloth (2 mm) was autoclaved and placed at the centre. Cotton fabric without dyeing was used as control for the above experiments. The petriplates were incubated at 28°C ± 2°C for 24 h for bacterial cultures and 48 h for fungal cultures and observed for clear zone of inhibition.

3 Results and Discussion

A reddish brown colour pigment (Sienna shade) extracted from the dry mycelium of 9th day culture of *A. alternata* using methanol has been applied on cotton [Fig. 1(a) & (b)]. Absorption of 63.33% reddish brown pigment on cotton is obtained, using the following equation:

$$\text{Absorption}\,(\%) = \frac{2.013\text{-}0.738}{2.013} \times 100 = 63.338$$

The dyed and painted fabrics have been processed for the determination of colour fastness to washing at commercial laundering (40°C) as per grey scale rating. The fastness to washing is found to be fair. The results show good to excellent colour fastness to perspiration and rubbing. Analysis of wet and dry rub fastness shows that the dry rub fastness ranges from 4 to 5, indicating less staining and good fastness (Table 1).

Previous reports on brown pigments from *Alternaria alternata* expressed good to very good fastness properties to washing, perspiration and light when dyed on wool[25]. The investigations on olive colour pigments from *Alternaria alternata* dyed on wool show 4-5 (grey scale) rating, indicating least staining and excellent wash fastness. These dyes have also been tested for toxicity to human skin and found to be safe[26]. The percentage absorption of *Trichoderma* is found to be 44.58% for silk and 48.37% for wool respectively. Analysis of rub fastness shows that the dry rub fastness of *Trichoderma* is 5, i.e. no staining, hence have excellent dry rub fastness[27]. The pigment, obtained from *P. sanguineus*, when subjected to dyeing on cotton and silk yarns shows promising dyeing ability on cotton yarns[28]. The cotton and silk yarns treated with dye extract of *Ganoderma lucidum* by enzymatic treatment (lipase) with tannic acid and dye fix solution result in reddish brown shades of colour with excellent wash fastness (4,4-5) to commercial laundering and acid (4-5, 4-5) as well as alkaline (4, 4-5) perspiration fastness in grey scale rating[29]. Earlier research reported the production of magenta pigment by *Phoma herbarum* in the presence of nylon-6 fibers and suggested that fabrics can be dyed using microorganisms[30].

The fabric dyed with reddish brown pigment of *A.alternata* has been tested for its antimicrobial potential against 4 bacterial and 2 fungal strains. The maximum zone of inhibition (42 mm) is observed in *Staphylococcus epidermis* followed by *Streptococcus pyogenes* (39 mm). No activity is observed against *Escherichia coli*, *Staphylococcus aureus*, *Trichoderma viridae* and *Curvularia lunata*. Study on six isolates of *P. sanguineus* shows inhibition of *S. aureus*[31].

The anti-bacterial activities of brownish red dye extract of *Ganoderma lucidum* (MCRC 001) have been tested on seven pathogenic and non-pathogenic bacteria. The alkaline extract (0.1% NaOH) of *Ganoderma lucidum* (MCRC 001) shows maximum

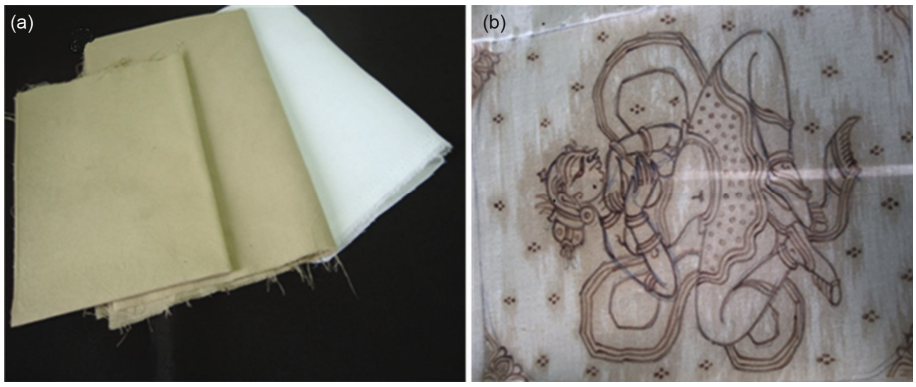

Fig. 1—(a) Dyeing and (b) painting of fabrics with reddish brown *A.alternata* pigments

Table 1—Colourfastness report of dyed and painted textiles

Fabric	Wash fastness			Perspiration fastness						Rub fastness		Light fastness
				Acidic			Alkaline					
	CC	SC	SW	CC	SC	SW	CC	SC	SW	Dry	Wet	(CC)
Dyed	2-3	4-5	4-5	4	4-5	4-5	4	4-5	4-5	5	4-5	2
Painted	2	4-5	4-5	3-4	4-5	4-5	3-4	4-5	4-5	4	3-4	3-4

Grey scale rating: 5- excellent; 4- good; 3- fair; 2-poor; 1- very poor, CC - change in colour, SC-staining on cotton, SW-staining on wool.

zone of inhibition against *Bacillus cereus, Bacillus subtilis, Bacillus megaterium* and *Janthinobacterium lividum.* The alkaline, aqueous and methanol extract of *Ganoderma lucidum* (MCRC 001) are found to be inactive against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*[29]. Actinomycetes have been investigated by various researchers[32] including those who have reported that the crude extract of antifungal compounds is active against *R. stolonifer*, *A. flavus*, *F.oxysporum* and *Alternaria*. Previous research has proved the antibacterial activities of orange pigment extracts of *P. sanguineus* (MCRCPS02 and MCRCPS04) against *S. aureus* and *K. pneumoniae*[28].

Alternaria alternata produces about 60 different metabolites. One of the most important groups of metabolites produced by *Alternaria* is tenuazonic acid and derivates of this compound. The compound has been characterized as an anti-tumour agent[33-34], but it also has antiviral[35], antibiotic[35-37], and insecticidal[38]. The -dibenzopyrones Altenuene , Alternariol and Alternariol-5-methylether are other typical *Alternaria* secondary metabolites. Studies on biosynthesis and chemistry of these metabolites can unfurl the ways to develop promising pharmaceutically or agro-chemically important products.

4 Conclusion

It is inferred from the study that pigments from microbes can serve as safer alternatives for textile dyeing industries. The pigments from fungus *A.alternata* can be utilized as safe textile dyes on cotton. The culture conditions can be optimized to get maximum pigment production. The fermentation conditions can be standardized so that the dyes can be produced at commercial scale in ecofriendly manner. These dyes can be applied on cotton with good to excellent colour fastness to perspiration and rubbing. This study serves as first report on the dyeing efficacy of reddish brown pigments of *A.alternata* on cotton fabrics and thus provides a scope for further studies in the above to obtain and elucidate a wide range of secondary metabolites for application in other industries.

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