Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.4, No.7, 2014

Extraction of Natural Dyes from Fungus – An Alternate for Textile Dyeing

Aishwarya Anchana devi*
PG and Research Department of Biotechnology, Women's Christian College,Chennai 600 006
*Email of the Corresponding Author :dr.anchanababu@gmail.com

Abstract
A dye is a coloured substance that has an affinity to the substrate to which it is being applied. The majority of natural dyes are from plant sources – roots, berries, bark, leaves, and wood, also from animals and microbes. Natural dyes are non-toxic, non-polluting and less health hazardous. Moreover, their antioxidant and antimicrobial nature further adds to their positive effects. The main idea of extracting dyes from natural sources is to avoid the environmental pollution and also to avoid toxic and allergic reactions associated with synthetic dyes. These natural dyes have emerged as an important alternative to synthetic dyes. In the present study, The pure cultures of fungal strains, *Trichoderma*spand *Aspergillus*sp were isolated from soil samples using PDA plates. The fungal cultures were grown under static condition in PDB for the production of pigment. These pigments were tested for their colour production properties by taking absorbance at different time intervals during the incubation period. A set of 3 pieces of cloth (cotton, silk & silk cotton) were used for dyeing with the fungal pigments in both pre mordanted and unmordanted conditions. The fungal filtrates were bio autographed using chromatographic techniques (TLC & CC) to purify and identify the compounds present in it. The purified samples was analyzed further by GC – MS and the compounds present in the filtrates were identified.
Keywords : Natural dyes, *Aspergillus*sp. *Trichoderma*sp. ,GC-MS

1.Introduction

There is a growing interest in the revival of natural dyes in textile coloration. The prominence of natural dyes slacked down because synthetic dyes had some advantages over natural dyes like colour fastness, good reproducibility of shades, brilliance of colours and easy to use and also for ready availability of pure synthetic dyes of different types/ classes and its cost advantages, most of textile dyers/ manufacturers shifted toward use of synthetic colourant. But, almost all synthetic colourants being synthesized from petrochemical sources through hazardous chemical processes pose threat towards its eco-friendliness. Natural dyes produce very uncommon, lustrous, soothing and soft shades as compared to synthetic dyes. Application of natural dyes has potential to earn carbon credit by reducing consumption of fossil fuel (petroleum) based synthetic dyes.
Microbial dyes have some advantages over plant and animal based dyes as microbes are fast growing and have the potential of being standardized commercially. Microbial dyestuffs produce rare colour ideas and are automatically harmonizing. Unlike, non-renewable basic raw materials for synthetic dyes, these natural dyes are usually renewable and biodegradable and generally have a higher compatibility with the environment than synthetic dye therefore, no disposal problem of this natural waste.

1.1 Fungal pigments as Eco-dyes

Microbes can produce a large amount of stable pigments such as anthraquinones, carotenoids, flavonoids, quinines and rubramines. Fungi are more ecological and interesting source of pigments, as they produce stable colourants. Fungi contain several anthraquinone compounds and pigments such as delphinidin, melanin and volatile organic compounds (VOC's) which have been identified as their secondary metabolites.

For example, the following scheme may be representative of natural anthraquinone compounds:

Primary Metabolite (Lipid) ⟹ **Low MW Intermediate (Acetyl Coenzyme)** ⟹ **Anthraquinone**

Many fungi cells are involved in the production of different shades of dye and pigments as their intermediate metabolites such as – *Alternariaalternata, Aspergillusniger, Curvularialunata, Hapilopiusnidulans, Omphalotusolivascens, Boletopsisgrisea, Phaeolusschweinitzii, Hypomyceslactiflourum, Pisolithustinctorius, Sarcodonfuscoindicus, Trichodermavirens, Monascuspurpureus, Isariafarinosa, Emericellanidulans, Dermocybesanguinea, Fusariumverticillioides and Penicilliumpurpurogenum.* Pigment production by fungiowing to their genetic background of the species is taken into consideration as a taxonomic feature**.**The production and evaluation of microbial pigments as textile colorants is currently being investigatedby the British Textile Technology Group (BTTG).
An effective biotechnological solution to manufacture of these dyes and other dyestuff intermediates will impart the following benefits:

- The medium in which these fungal cells grow contain no expensive or toxic chemicals.
- The process is carried out at a low temperature (around 30° C) compared to the fuel – consuming very high temperatures in the synthetic process.

- The process is typically run at neutral pH as opposed to very high acidic or alkaline conditions in the synthetic process.

The application of natural dyes in textile industry for various purposes, viz. dyeing of yarns, which are then woven into cloth, carpet or any other usable form; dyeing of cloths woven earlier; block printing, where the textile materials are printed with the help of printing blocks; kalamkari where the 'kalam' or pen is used to draw beautiful designs on the cloth.

Apart from being used in textile industry, natural dyes are now days used in cosmetics, leather, food and pharmaceutical industry. In food industry natural anthraquinones have been used as a colouring agent for beverages, sweets and other foods. Also, natural colourants, especially hydroxyanthraquinones, those occurring in fungus *Dermocybesanguinea,* like emodin, dermocybin and dermorubin, were mentioned as potential hair dye that is used to colour human hair. Fungus is also being used in treating colouredwaste water from the textile industry. Fungal systems appear to be most appropriate biological agent in the treatment of colored and metallic effluents and are able to degrade a wide variety of recalcitrant organo pollutants, including various types of dyes and decreases toxicity and have aroused interest in using them in bioremediation.

Toxicity is the ability of a substance to cause damage to living tissue, impairment of nervous system or severe illness when ingested, inhaled or being absorbed by skin. Earlier studies have confirmed the non-toxicity and biodegradability of the fungal pigments. Fungal dyes when tested on human skin for toxicity showed no sign of rashes, swelling, or any other form of allergy, indicating the non-toxic nature of the dye to human skin.

2. Materials and Methods

2.1 Isolationand Characterization of Fungi

The fungal organisms were isolated from soil samples randomly collected from the college premises , Serially diluted and plated on to Potato Dextrose Agar and incubated at 37° C for 4-5 days and observed for fungal colonies. After colony identification, to study the microscopic features of the fungi Lacto Phenol Cotton Blue (LPCB) staining technique was performed.

2.2 Culturing and Extraction of Pigment

Fungal Cultures which was characterized earlier were grown on Potato Dextrose Broth (PDB).Two clean conical flasks were taken and 200mL of distilled water was added along with 4.8g of PDB, a pinch of copper sulphate was added to increase the pigmentation and autoclaved at 121°C for 15minutes and allowed to cool. All the flasks were inoculated by 2-3 mycelia disk of 5-12mm in diameter with the help of a sterile cork borer obtained from the PDA culture plates.The flasks were incubated at 28°C in a static condition for 3-4 weeks for pigment production.

2.3 Screening of Dye

Fungal culture broths showing various colours were filtered out after 3 weeks of incubation period using Whatmann filter paper no: 4 in a conical flask. The culture filtrates were tested for colour production and dyeability by taking absorbance at different time intervals using colorimeter . The coloured filtrate was used to dye different textile samples.

2.4 Mordanting

For each organism dye, a set of cotton, silk and silk cotton clothes weighing 1g and measuring 3cm x 3cm was used. 1-3% of ferrous sulphate and potassium dichromate were used as mordants.The clothes were washed with warm water before mordanting.The clothes were treated with mordants using a conical flask at 60°C for 30 minutes or 180-200°F for 1 hour with MLR 1:20 and the temperature was maintained with the help of a thermometer.After the treatment they are cooled with water overnight.

2.5 Purification of Dye by Alumina Column Chromatography

A 20mL syringe was taken and used as the column, it was packed with activated alumina. A cotton ball was plugged at the bottom to seal and control the flow of the sample.10mL of ethanol and 10g of alumina was mixed in a beaker to obtain a slurry, this was filled in the column and left undisturbed until it settled down to 4-5cm height.Some amount of ethanol was kept at the top of the alumina (1mm) so as to not to allow it to dry.1mL of the extract was poured on top of the column.10mL of ethanol was added was carefully added to the column and was allowed to dip through.The uncolored eluent was collected as waste; but as soon as the coloured compound began to emerge it was collected in a beaker and stored in eppendorf's.

2.6 Identification of Dye Components

After purification of the dye by column chromatography, the purified fractions were analyzed using Gas chromatography- mass spectrophotometry.

2.7 Dyeing of the Fabric with Fungal Pigment

For dyeing with the fungal extract, two sets of clothes was used for each organism in both mordanted and unmordanted state and compared for higher dye reproducibility.The MLR is 1:50; 50mL of extract was taken in a beaker and clothes weighing 1g was immersed into it.

The dyeing was carried out for 45 minutes at 70-80°C and the temperature was maintained with the help of a thermometer. The sample was washed by boiling for 5 minutes in tween 80 and rinse with cold water and dry it in sunlight.

2.8 Percentage Absorption

To analyze the fabric properties, percentage absorption of the dyed fabrics was calculated on UV spectrophotometer at 500-600 nm and the percentage was determined for both mordanted and unmordanted dyed fabrics separately using the following formula:

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

3. Results and Discussion

3.1 Isolation of Fungi

The pure cultures of *Trichoderma*sp and Aspergillussp were isolated from soil sample using Potato Dextrose Agar plates. Further confirmed by observing the microscopic features of the Fungi by staining withLactophenol cotton blue .

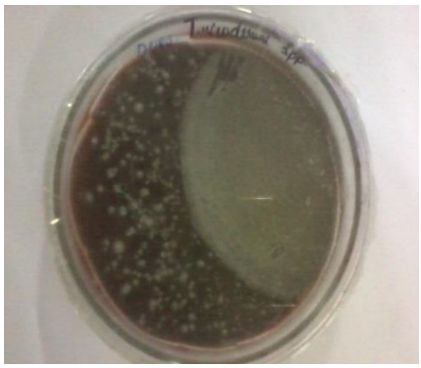


Figure 1 :*Trichoderma*sp

Figure 2 *Aspergillus*sp

3.2 Culturing and Extraction of Pigment

Mycelial disks were inoculated in Potato Dextrose broth(PDB) for the production of fungal pigment which was extracted by filtering with Whatmann filter paper number 4. The filtered extracts were used for dyeing the fabric. The PDB showed pigment production after 3 weeks of incubation (Figure 5 & 6).

(Both Culture broths after one week)

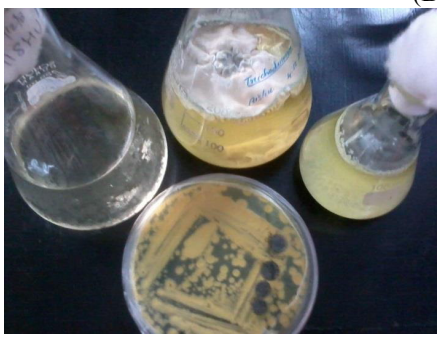


Figure 3: *Trichoderma*sp

Figure 4: *Aspergillus*sp

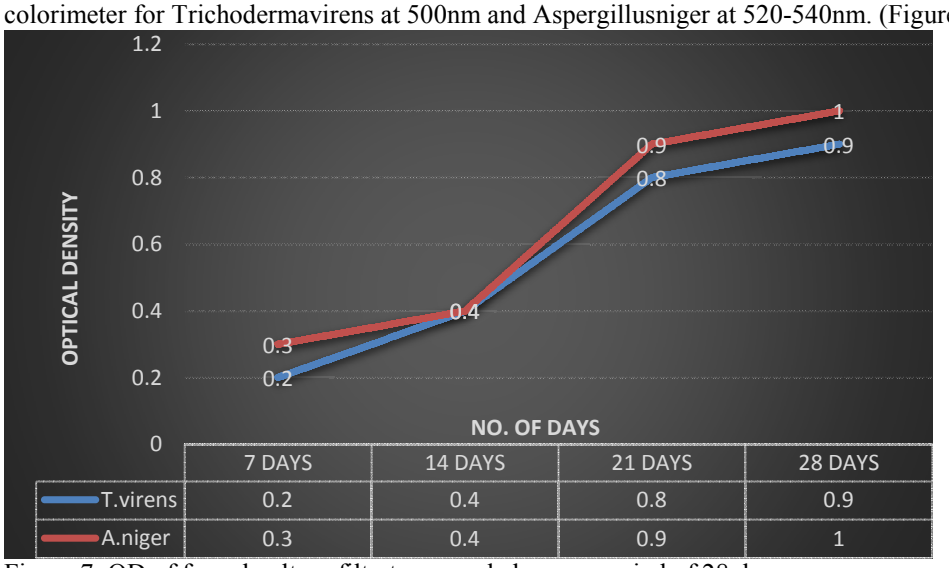
(Both Culture broths after three week)

Figure 5: *Trichoderma*sp

Figure 6:*Aspergillus*sp

3.3Screening of Dye

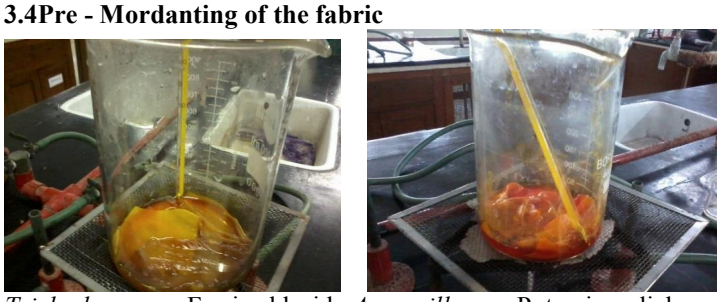
The fungal filtrates was tested for colour production by taking absorbance at different time intervals using colorimeter for Trichodermavirens at 500nm and Aspergillusniger at 520-540nm. (Figure 7)



	7 DAYS	14 DAYS	21 DAYS	28 DAYS
T.virens	0.2	0.4	0.8	0.9
A.niger	0.3	0.4	0.9	1

Figure 7: OD of fungal culture filtrates recorded over a period of 28 days

3.4Pre - Mordanting of the fabric



*Trichoderma*sp– Ferric chloride *Aspergillus*sp– Potassium dichromate

Figure 7: Mordanting the fabric using different mordants

Different mordants was used for each set of dye, these mordants when added to the cloth gave different shades of colours. They helped in better absorbing of dye and increased its fastness properties. (Figure 7)

3. 5 Purification of dye using Alumina Column Chromatography

The fungal extracts was purified using activated alumina slurry and ethanol and the fractions was collected to identify the compounds present in it.

3.5 Dyeing of different fabrics using Fungal pigment

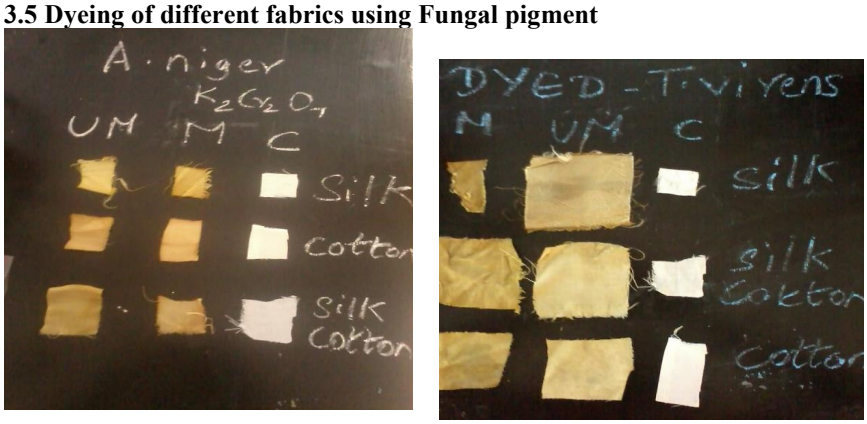


Figure 8 : Pre mordanted and unmordanted dyed fabrics with fungal pigments

The pre mordanted and unmordanted fabrics was dyed with the fungal pigments to obtain shades of colours and a comparative study was done.

3. 6 Gas Chromatography - Mass Spectrophotometry

The compounds present in the fungal dye extracts were identified by GC – MS. Methanol was used as the solvent for both the fungal filtrates. The peaks obtained was compared to the standard library and maximum similar compounds present structurally in the filtrate and library were matched and identified. (Figure 9 &10).

1. *Aspergillussp* Compound identified was 1H- Indole, 2- methyl- 3- phenyl Library searched: C:\DATABASE\NIST98 . L
2. *Trichoderma*sp: Compound identified was Ethyl tridecanoate / tetradecanoic acid Library searched: C:\DATABASE\NIST98 . L

3.7 Percentage Absorption

The fabric properties were studied by measuring the amount of colour absorbed by the mordanted and unmordanted set of cloths dyed with fungal extracts using UV spectrophotometer at 500 – 600 nm. Percentage absorption was calculated using the following formula:

Percentage absorption (%) = O.D before dyeing - O.D after dyeing × 100 / O.D before dyeing

Table 3: Percentage of AbsorptionORGANISM: *Trichoderma*sp

TYPE	CONTROL	MORDANTED	UNMORDANTED	PERCENTAGE ABSORBED	
				MORDANTED	UNMORDANTED
SILK	75.09	8.43	17.74	88.77	76.37
COTTON	94.95	9.11	16.47	90.40	82.65
SILK COTTON	56.06	7.40	15.94	86.79	71.56

Table 4: Percentage of AbsorptionORGANISM: *Aspergillus*sp

TYPE	CONTROL	MORDANTED	UNMORDANTED	PERCENTAGE ABSORBED	
				MORDANTED	UNMORDANTED
SILK	75.09	27.77	29.53	63.01	60.67
COTTON	94.95	25.93	28.30	72.69	70.19
SILK COTTON	56.06	22.25	23.38	59.77	58.29

The mordanted fabrics showed higher percentage of dye absorbance than unmordanted fabrics as the metal mordants reacts with the textiles to form a co – ordinated complex between natural dye molecules and metallic mordant for dye fixation on mordanted textiles.

The O.D of the fungal filtrates recorded at different time intervals showed that *Aspergillus*sp produced a higher rate of pigment production than *Trichoderma* sp.

From the tabulation (Table 3&4), cotton fabric was found to show efficient and higher percentage of absorption than silk and silk cotton in both fungal dyes produced by the organisms.

Conclusion

The present study ,The fungal strains *Trichoderma* and *Aspergillus*produced pigments in PDB broth.The O.D of the fungal filtrates recorded at different time intervals showed that *Aspergillus*sp produced a higher rate of pigment production than *Trichoderma*sp The results of GCMS showed 1H Indole- 2 Methyl- 3 Phenyl was identified in *Aspergillus*sp and Ethyl trideconate was identified in *Trichoderma* sp. The purified dyes were dyed on the fabrics and the percentage absorption of dye onto the fabrics were measured using UV spectrophotometer and the comparative study was done and concluded that pre mordanted fabrics showed higher percentage of dye absorbance than unmordanted fabrics. Cotton fabric was found to show efficient and higher percentage of absorption than silk and silk cotton in both T.virens and A.niger dye.

References

1. Ahlstorm L, Eskilsson C.S &Bjorklund E (2005): Determination of banned Azo dyes in consumer goods, trends anal. Chem., 24(1): 49-56.
2.Bradford (1990): Methods of test for color fastness of textiles and leather, 5th edn. Society of Dyes and Colorists, UK.
3.Cappuccino, J.G. and N. Sherman (2004): Microbiology: A laboratory manual. 6th ed. Published by Pearson Educ. Singapore. Pvt. Ltd.
4. Deepshika Sharma, Charu Gupta, SinitaAggarwal&NitikaNagpal (2010): Pigment extraction from fungus for

textile dyeing, Department of fabric and apparel science, Deparment of Microbiology, Institute of Home economics, University of Delhi, India . Indian Journal of Fiber & Textile Research, Vol. 37, Pg. no 68 – 73.
5.Dufossé. L (2006): Microbial production of food grade pigments. Food technology and Biotechnology, v 44: 313–321.
6.Gill M &Steglich W (1987): Pigments of fungi (macromycetes). Progress in the chemistry of organic natural products.Volume 51, s1-317, Springer-Verlag, Wien, Newyork.
7.Gulrajani M.L & Gupta D (1992): Natural dyes and application to textiles, Department of Textile technology, Indian Institute of Technology, New Delhi, India. Indian Textile Journal: 25.
8.Johnson, L.F. and Manka, Karol (1961): A Modification of Warcup's Soil Plate Method for Isolating Soil Fungi. Soil Science 1961 92 (2) 79-84.
9.K.Karthikeyan, K. Nanthakumar, S. Suriyananarayanan and P. Lakshmanaperumalsamy, (2012): Screening of Textile Dye impacted soils for novel dye decolorizing fungi employing various nutrient media, Advances in biological Research: 6(1): 44-51.
10.Mehanta Z.A &Tiwari I.A, (2003): Natural dye – yielding plants and indigenous knowledge on dye preparation in Arunachal Pradesh Northeast India, Curr.Sci, 88(4): 1474-1480.
11.Osman A.M, Bhuyan A.M, Chattere M.X & Ahlstrom E.L, (2004): Determination of banned Azo dyes in consumer goods, Trends Anal. Chem., 24(1): 49-56.
12.Pathak H and Madamwar D (2010): Biosynthesis of indigo dye by newly isolated naphthalene-degrading strain Pseudomonas sp. HOB1 and its application in dyeing cotton fabric. ApplBiochemBiotechnol 160: 1616-1626.
13.Ranveer R. C, D.V. Jadhav, S. R. Waghmare, A. K. Sahoo and A. Chitale (2012): Production & characterization of brown coloured pigment from TrichodermaViride, electronic journal of environmental, agricultural and food chemistry ISSN: 1579 – 4377.
14.Shafiquzzaman Siddiquee, Bo Eng Cheong, KhanamTaslima, HossainKausar&MdMainulHasan (2010): Separation and Identification of Volatile Compounds from Liquid Culture of Trichodermaharzianum by GC-MS using Three Different Capillary Columns: Journal of Chromatographic Science 2012;50:358–367.
15.Youssef M.S, E.L Maghraby, O.M.O and Ibrahim Y.M, (2008): Production of Textile Reddish Brown dyes by Fungi. Int J Botany.4, 349-360.

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

Recent conferences: http://www.iiste.org/conference/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar