

# ECO-FRIENDLY TEXTILE DYEING: HARNESSING FUNGAL PIGMENTS FOR SUSTAINABLE FABRIC COLORING

## INTRODUCTION

Fungi are eukaryotic microorganisms. Fungi can occur as yeasts, molds, or as a combination of both forms. Some fungi are capable of causing superficial, cutaneous, subcutaneous, systemic or allergic diseases. Yeasts are microscopic fungi consisting of solitary cells that reproduce by budding. Molds, in contrast, occur in long filaments known as hyphae, which grow by apical extension. Hyphae can be sparsely septate to regularly septate and possess a variable number of nuclei (Michael R. McGinnis, Stephen K. Tyring, 2003).

*Trichophyton rubrum* is a human pathogenic fungus. As a dermatophyte it causes athlete's foot, fungal infection of nails, jock itch and ringworm. To find an appropriate treatment for the disease, an accurate diagnosis is mandatory. Therefore, the disease of fungal infections involve in many cases microscopic examination and fungal cultivation. Under alkaline conditions, *T.rubrum* has a red pigmentation, whereas at acid conditions, *T.rubrum* has a yellow pigmentation (Oliver Blechert *et al.*, 2019).

Domain	Eukaryota
Kingdom	Fungi
Division	Ascomycota
Class	Eurotiomycetes
Order	Onygenales
Family	Arthrodermataceae
Genus	<i>Trichophyton</i>
Species	<i>T.rubrum</i>

*Aspergillus* is a genus consisting of several hundred mold species found in various climates worldwide. It is an asexual spore-forming structure common to all *Aspergillus* species;

around one-third of species are also known to have a sexual stage (Geiser DM, 2009). While some species of *Aspergillus* are known to cause fungal infections, others are of commercial importance.

Domain	Eukaryota
Kingdom	Fungi
Division	Ascomycota
Class	Eurotiomycetes
Order	Eurotiales
Family	Aspergillaceae
Genus	<i>Aspergillus</i>

Secondary metabolites are structurally heterogeneous low molecular mass molecules that are not essentially required to ensure growth of the particular organism that produces them. A lot of microorganisms produce a variety of secondary metabolites including Carotenoids like soil dwelling bacteria and fungi (Berdy, 2005). It is obvious that evolution of these secondary metabolites over hundreds of millions of years occurred because microorganisms used them as chemical signals for communication, to defend their habitat or to inhibit the growth of competitors (Brakhage, 2011). Fungi produce a large number of secondary metabolites, which have roles in range of cellular processes such as transcription, development and intercellular communication.

Many microbial secondary metabolites have been found to have biological activities such as anticancer, anti-inflammatory, antioxidant and antibiotic properties. Interestingly, some of biological activities can also be associated with pigments produced by fungi (Celestino JDR *et al.*, 2014). Fungal pigments are grouped into carotenoids, polyketides, melanin, azaphilones (polyketide derivatives) etc (Nystrom M *et al.*, 2008). Fungal pigments have demonstrated potential significance to human health boosting interest in understanding and developing fungal pigments for therapeutic applications. Pigment derived from marine fungi attracts plenty of

attention due to its complex and unusual chemical structures, which have tremendous antioxidants and antimicrobial activities (Lin L, Xu J, 2020).

For a long time, filamentous fungi have been used for the industrial production of commercially relevant products, including enzymes, antibiotics, feed products, and many others. The bio-refinery concept, i.e., converting substrates to value-added products, is widely accepted within the research community. Therefore, research towards the diversification of established and future facilities for the production of numerous novel and valuable products as well as by-products through fermentation is presently a hot topic. First generation ethanol plants are good example where side stream products (e.g., ethanol, animal feed and CO<sub>2</sub>) by producing substances such as organic acids, enzymes, ethanol, biomass for food and/or feed applications and pigments (Ferreira JA *et al.*, 2016). In particular, the interest for fermentation-derived pigments in the food and feed industry has increased in recent years (Duffose L *et al.*, 2014). The interest in food-grade pigments is because of the pigment's ability to enhance the product's natural color in order to indicate freshness, appearance, safety and sometimes even to add a novel sensory aspect to attract consumers (Malik K *et al* & Caro Y *et al.*, 2012).

There is growing scrutiny over the detrimental impact of synthetic colorants on both the environment and consumer's health, which has shifted the focus towards natural coloring alternatives. Hence, international demand for natural pigment is quickly growing in the cosmetic, pharmaceutical, food and textile industries. Natural colorants can be used for diverse industrial applications, such as dyes for fabric and non-fabric substrates such as paper, leather, coatings and paints, and as additives in food and cosmetics (Kalra *et al.*, 2020).

Among the various ever increasing applications of biotechnology in industries, dyeing of fabrics, leather and wood has found a prime importance. A worldwide interest has surfaced in development of procedures/extracting techniques for the production of various pigments from natural sources such as plants, animals, cell cultures, tissues, etc (Dziezak , J.D. , Francis, F.J. , 1987). Scientists are continuously being pressurized to turn towards natural pigment producers due to increasing safety hazards associated with many artificial synthetic colorants being widely used in foodstuff, cosmetic and pharmaceutical industry. Microorganisms are now also being

applied to produce drug compounds in presence of natural substrates such as milk and whey so as to reduce the harmful effect of chemical synthesis on environment (Shrivastava M *et al.*, 2017). Pigments to be extracted from microorganisms is advantageous as although there are a number of natural pigments maximum are being produced through plants and hence are not available in sufficient quantities (Lauro, G.J. , 1991).

Colored substances known as dyes are used to impart color to variety of material. The use of such synthetic dyes in dyeing industry results in dye containing waste water which increases the environmental pollution. Some of these dyes have potential potent carcinogen posing serious health hazard. The use of food colorants as additives in the food industry is a significant factor for both food manufacturers and consumers in determining the acceptability of processed food (Ana Abad *et al.*, 2010).

Color has always played an important role in the life of all organisms on earth. Human life has become truly “colorful” due to the use of colors in all its aspects, including clothes, food and furniture. Much archaeological evidence has shown that the use of pigments as coloring agents has been practiced since ancient times (Rao *et al.*, 2017). Pigments, especially synthetic ones, have occupied for entire market due to their wide range of applications in different industries since their discovery in the 19<sup>th</sup> century. Different attributes such as low production costs, ease of production and superior coloring properties have largely contributed to the establishment of synthetic pigments in the market. However, the use synthetic color has been found to be detrimental to human health and the environment because of their adverse impacts (Downham A, Arora, 2014). Many disadvantages of synthetic pigments, such as poor degradation, longer persistence, potential to cause cancers, organic and eco-friendly pigments in the current era.

Synthetic pigments have teratogenicity, toxicity and carcinogenicity. Compared with synthetic pigments, natural pigments have the advantages of natural, bright colors, high safety, significant nutritional value and pharmacological features (Cho *et al.*, 2002). Hence, the trend of replacing synthetics with natural pigment is growing (Y.E. Kim *et al.*, 2020). Natural pigments come from the tissues or metabolites of animals, plants and microorganisms. Among natural pigments, pigments of microbial origin have many advantages, such as low cost, resistance to

various environmental factor influences, easy to be industrialized, can overcome many defects of animal and plant origin of pigments, and have higher application potential (Indra Arulselvi *et al.*, 2014).

Pigments are produced in the fungal cytoplasm as either a result of environmental stress conditions (temperature, dehydration, salinity or pH) or the stimulus from other pathogenic microorganisms. Once produced, these pigments are either secreted or released into their respective culture media or incorporated into the cell wall of the fungi producing them. The fungal pigments play vital roles in the survival and protection of the fungi such as providing rigidity to the wall, resulting in cross-linkage among the hyphae, protection against UV-radiations, protection from oxidative free radicals, environmental extremes (due to increased resistance as a result of the accumulation of pigments in the cell wall providing rigidity), and hydrolytic enzymes produced by other microorganisms. Besides the wide utilization by fungi, these pigments are also utilized by humans for agricultural (food colorants/taste medium/fertilizers), medicinal (antimicrobials/antibiotics), and industrial (textile colorants) purpose. Therefore, there is an increased interest in fungal pigments and pigment-producing fungi worldwide owing to their multiple benefits.

The most common methods for commercial dyeing with fungal pigments are heated dye bath, high temperature dyeing and immersion dyeing. All of these methods require significant volumes of water. Both natural fibers and manufactured fibers, namely polyamide and polyester, have been tested with and without mordants. For heated dye baths (30-100°) without mordants, on both wood and silk, colorfastness to perspiration ranged from good to very good, and wash fastness ranged from good to excellent (Nagia & El-Mohamedy, 2007), with a moderate result from deep brown pigment extracted from *P.chrysogenum* (Atalla *et al.*, 2011).

Natural color alternatives in industrial environments are sourced from plant, vegetable, insect or animal substrates. Some of the color extracts obtained from plant, vegetable and related by-products are susceptible to contamination with mycotoxins and therefore limit their application (Duffose, 2018). Furthermore, the limitations of colors obtained from plant sources, are linked to light sensitivity, heat, varying pH and low solubility (Lebeau *et al.*, 2019).

According to Food Dive reports (2021), some of the imported emerging colors include blue, green, purple and bright red white, violet, green and blue are difficult to produce naturally. For these reasons, much research has been undertaken in the academic and industrial areas to study filamentous fungi as a sustainable and cheaper source of natural colorants.

Using artificial color is associated with negative health problems for consumers (Duffose L, 2006). This has generated interest in the production of alternative natural colors. The demand for natural pigments is repeatedly raised by the food industry due to the deficiencies of existing natural food colorants. Discovering new pigment producers became the aim of several researches. Pigments from microorganisms have several advantages because they are more stable and soluble than those from plant or animal sources. Microorganisms can grow rapidly, which can lead to high productivity, and can produce various pigments throughout the year without limitations (Jiang Y *et al.*, 2005, Gunasekaran S *et al.*, 2008).

Nature is replete with pigment-producing organisms: plants, animals and microorganisms. Microbial pigments are cost effective, they eliminate the need for large labor, they are easy to handle and can tolerate diverse conditions like pH, light and heat effectively, and are more stable against physiological changes (Kalra R *et al.*, 2020, Venil K *et al.*, 2020). They provide dynamic production and extraction means and they are readily available, and their availability is not dependent on seasons (Lagashetti AC *et al.*, 2019). Microbial pigments being secondary metabolites possess several properties like antibacterial, antifungal, anti-cancerous, antioxidants, anti-mutagenic, anti-proliferative, immunosuppressive and anti-diabetic and they are biodegradable, non-toxic and eco friendly (Cobanoglu SanYazici A, 2022, Numan M *et al.*, 2018). Due to these properties, they have application in various industries like food, cosmetics, textiles, leather, paper and pharmaceutical industries. Thus, using microbial pigments in different industries provide sustainable development goals for mankind.

Generally, pigment production in industrial scale has been carried out using submerged fermentation (SmF) (Babitha S *et al.*, 2006). However, solid state fermentation (SSF) has emerged as an effective way due to the high production yield (Feng Y *et al.*, 2012). In addition,

by SSF process, a relative low cost process can be achieved, especially when agro-industrial wastes are used as substrate (Subhashree *et al.*, 2011). Solid liquid extraction (SLE) is the most common technique for the removal of pigment from fermented substrate by SSF( Capozzi *et al.*, 2017, Nimnoi *et al.*, 2011). A suitable extraction technique helps in increasing the extraction yield, besides prevent the degradation of the extracted pigments (Wang Y *et al.*, 2018).

Currently in the leather industry, more than 90% of leathers are dyed with azo-dyes (Page C, 2001) and many of these synthetic dyes extensively used all over the world have negative impact on human health and the environment (Fuck W.F *et al.*, 2011). It is estimated that about 10% of unexhausted dyes are discharged into the waste streams irrespective of the substrate involved in dyeing (Kanth S.V *et al.*, 2009). It is estimated that 1 to 5% of the dye applied in the leather dyeing remains in the effluent (Puntener A.G *et al.*, 2000). Furthermore, the undesirable pollution associated with colors and dyes in wastewater, especially azo and metal complexes, may result in reduced water re-oxygenation capacity, acute and chronic toxicities, and difficulties in water treatment by biological methods, in addition to preventing the reuse of the water in other process steps (Piccin J.S *et al.*, 2016).

**AIM:**

To synthesize and extract microbial pigments from fungi isolated from coconut (*Cocos nucifera*) sample and its application in fabric dyeing.

**OBJECTIVES**

- To isolate various fungal species from spoiled coconut sample.
- To identify the morphology of the isolated fungi using lactophenol cotton blue (LPCB) staining method.
- To cultivate the fungi isolates in liquid medium for pigment production.
- To extract the fungal pigments using organic solvents.
- To characterize the isolated pigments through UV visible spectroscopy.
- To incorporate the synthesized dyes in cotton fabrics and its dyeing.
- To evaluate the color fastness under different conditions.

## REVIEW OF LITERATURE

To feed a growing population, new food ingredients must be produced from sustainable sources immediately. Functional proteins for food bio-manufacturing of structured products can be easily produced by filamentous fungi from inexpensive agricultural waste. Nevertheless, nothing is known about the gelling properties of fungal proteins or how they might be used in additive bio-manufacturing (Lauren Doyle *et al.*, 2025).

The intricacy of synthetic color, pollution, and the growing expense of raw materials have made natural pigments more important. Natural pigments are less allergenic and have antibacterial qualities as compared to synthetic pigments. Microbial pigments can be readily obtained in low-cost culture conditions, manufactured in large quantities, and bacteria can produce a variety of colorful pigments. It is now urgently necessary to find new sources of natural pigments to replace synthetic ones in culinary applications; yet, the volatility of these compounds is sometimes cited as one of the barriers that limit their use. Through a regulated release technique, encapsulation offers the best option for protecting natural dyes (Mwaheb M.A *et al.*, 2024).

Pigments of fungal origin have aroused increasing interest in the food dye and cosmetics industries since the global demand for natural dyes has grown. Endophytic microorganisms are a source of bioactive compounds, and Amazonian plant species can harbor fungi with a wide range of biotechnological applications. Popularly known in Brazil as crajiru, *Fridericia chica* is a medicinal plant that produces a red pigment (Dorothy Ivila de Melo Pereira *et al.*, 2024).

Pigments and dyes have the power to beautify the world. They have been in use for a long time and have numerous uses in the paper, textile, food, and artifact industries. As a result, a great deal of study has been done for creating natural colorants from natural sources. There are several benefits to employing natural colorants, including the fact that they are safe, easy to obtain from renewable resources, soft, glossy, and aesthetically pleasing. They pose no health risks and present no disposal issues. Colorants can be found in abundance in India. An estimated

500 different types of colorant producing plants exist in India, from which colorants are readily harvested (S. Yadav *et al.*, 2023).

Colorants are widely used in various industries, including food, cosmetics, pharmaceuticals, textiles, paints, papers, inks, and photography. They are categorized based on their solubility into dyes and pigments. Naturally derived colorants offer several advantages over synthetic alternatives, as they are generally less likely to cause allergies or other health issues. Moreover, their use in the food and pharmaceutical industries is beneficial due to their nutritional and health-promoting properties, with many exhibiting anti-oxidant activity or contributing to vitamin production. While plants, animals, insects, and microorganisms serve as sources of natural colorants, microbial pigments are preferred due to their higher yield, stability and cost-effective production (Wail Elkhateeb and Ghoson Daba, 2023).

Natural pigments and colorants have seen a substantial increase in use over the last few decades due to their eco-friendly and safe properties. Currently, customer preferences for more natural products are driving the substitution of natural pigments for synthetic colorants. Filamentous fungi, particularly ascomycetes fungi (*Monascus*, *Fusarium*, *Penicillium* and *Aspergillus*), have been shown to produce secondary metabolites containing a wide variety of pigments, including  $\beta$ -carotene, melanins, azaphilones, quinines, flavins, ankaflavin, monascin, anthraquinone, and naphthoquinone. These pigments produce a variety of colors and tints, including yellow, orange, red, green, purple, brown, and blue. Additionally, these pigments have a broad spectrum of pharmacological activities, including immunomodulatory, anti-cancer, antioxidant, antibacterial and anti-proliferative activities (Maria Afroz Toma *et al.*, 2023).

Microbial pigments are valuable in the food industry due to their stability and bioactivity. *Aspergillus ustus* (DBFL05) produces a bright brown, water-soluble polyketone pigment, stable under sunlight, pH 2-10, and below 100°C but sensitive to certain metal ions. Composed of pyrroprazine diketone and dianthrone, it exhibits anti-bacterial and anti-oxidant properties. It is non-toxic to plants and has low toxicity to brine shrimp, making it a promising, safe, and stable natural pigment (Minghui Zhou *et al.*, 2023).

There is a growing demand for colorants of natural origin in the food, pharmaceutical, cosmetic and textile sectors. Previously, our group has screened a fungal species from forest soil, identified as *Penicillium europium*. The isolated fungus transformed the longifolene into various metabolites, of which 12 were isolated in pure form, with potential to be utilized in the perfumery industry (Aejaz Abdullatif Khan *et al.*, 2021).

The industrial application of pigments of biological origin has been gaining strength over time, which is mainly explained by the increased interest of the consumer for products with few synthetic additives. So, the search for biomolecules from natural origin has challenged food scientists and technologists to identify, develop efficient and less consuming strategies for extraction and characterization of biopigments (Miriam S. Valenzuela-Gloria *et al.*, 2021).

The growing concern over the harmful effects of synthetic colorants on both the consumer and the environment has raised a strong interest in natural coloring alternatives. As a result the worldwide demand for colorants of natural origin is rapidly increasing in the food, cosmetic and textile sectors. Natural colorants have the capacity to be used for a variety of industrial applications, for instance, as dyes for textile and non-textile substrates such as leather, paper, within paints and coatings, in cosmetics, and in food additives. Currently, pigments and colorants produced through plants and microbes are the primary source exploited by modern industries. Among the other non-conventional sources, filamentous fungi particularly ascomycetous and basidiomycetous fungi (mushrooms), and lichens (symbiotic association of a fungus with a green alga or cyanobacterium) are known to produce an extraordinary range of colors including several chemical classes of pigments such as melanins, azaphilones, flavins, phenazines and quinines (Rishu Kalra *et al.*, 2020).

Public's demand for natural, eco-friendly and safe pigments is significantly increasing seem to be in high demand worldwide. The immense advantages of fungal pigments over other natural or synthetic pigments have opened new avenues in the market for a wide range of applications in different industries. In addition to coloring properties, other beneficial attributes

of fungal pigments, such as antimicrobial, anticancer, antioxidant and cytotoxic activity, have expanded their use in different sectors (Ajay C. Lagashetti *et al.*, 2019).

Fungi can be used as an alternative source of natural dyes for the textile industry. This research was conducted using mixed *Aspergillus* and *Paecilomyces*. The physical and chemical properties of the fungal dye were tested using 1% w/v of detergent for 15 minutes. Filtrate color and colors range developed on dye materials were measured by RHS color chart. Of six different mordants tested, alum gave a brighter color than other mordants, so alum used for further research. The optimum mass of the fungal dye was adsorbed about 0.0023g without mordant and the color was 82A purple-violet, while alum gave the optimum mass of the fungal dye adsorbed of 0.0013g, which with 82C purple-violet color. Percentage the fungal dye absorption on cotton cloth without mordant was 9.6276%, while alum gave the fungal dye absorption of 3.6068%. Cotton cloth mass reduction after soaking in a detergent solution of the dyed cotton cloth, while alum gave the fungal dye reduced of 0.0018g, which with 88C violet color. The obtained result showed dyes produced by mixed fungi could dye cotton cloth and resistant to detergent solution (Suciatmih *et al.*, 2018).

Certain species of filamentous fungi typically produce colored substances as secondary metabolites, which can be used as dyes for industrial applications, especially for products intended to be used in direct contact with the human body such as leather goods. These natural bio-dyes can be an eco-friendly alternative to synthetic dyes (mainly azo- dyes), since they are not originated from extractive activities of the environment and no hazardous chemicals are used while they are produced. Therefore, this biotechnological development for leather dyeing represents an important area to be explored and improved. However, this is a complex challenge due to the requirements of large-scale production with low cost and quality standardization with high stability and fastness (Wagner Fernando Fuck *et al.*, 2018).

Color is the prime feature of any food product as it enhances its appearance and acceptability. Several synthetic dyes are available that cause severe health problems. Hence pigments produced from natural resources are gaining worldwide popularity. In addition to this the availability of cheap raw materials, their supply throughout the year and antibacterial and

antifungal properties of some pigments have added to their value. Many lower and higher fungi are reported as potential sources of pigments like carotenoids, flavanoids, xanthophylls, anthraquinones etc. These pigments are easy to extract through microbial fermentation through Mineral-salts basal media or PDA and a variety of agro-industrial wastes serving as raw material (Meghna Shrivastava *et al.*, 2017).

Since ages, colors have been an integral part of humankind whether it belongs to foodstuff, clothing, or day-to-day living. Long back in history, various pigments are used by all races. Earlier the colors that were in use were natural in origin, but due to rise in demand mankind shifted to manufacturing of synthetic colors. With the passage of time, it has been now proved that these synthetic colors have many side effects like being immunosuppressive, carcinogenic. Due to deleterious health effects, the need for some alternative has emerged that can be used as a color. Plants, insects and other microorganisms have started taken place of synthetic colors. As there are many factors that limit the usage of plants and insects, research turned toward the microorganism. There are many fungi whose pigments are now considered as safe and economical. Fungi like *Aspergillus*, *Fusarium*, *Monascus*, *Trichoderma* and *Laetiporus* are reported to produce quinines, anthraquinones, Rubropuntamine, Rubropuntain, Ankaflavin, Monascin,  $\beta$ -carotene and many other pigments responsible for various colors, viz., red, purple, yellow, brown, orange and green. In addition to providing natural colors, these pigments possess many therapeutic applications like immuno modulators, anticancer, antioxidant, anti-proliferative. These pigments are produced as secondary metabolites by utilizing one of the pathways: polyketide, mevalonate and shikimate pathways. The pigments are fermentative products so are affected by temperature, pH, carbon source, aeration and type of fermentation (solid or submerged). There are many agencies that approve the usage of pigments for humankind. Fungi can work as cell factories for color production that is economical and human friendly (G. Mukherjee *et al.*, 2017).

Filamentous fungi, including the Ascomycetes *Monascus*, *Fusarium*, *Penicillium* and *Neurospora*, are being explored as novel sources of natural pigments with biological functionality for food, feed and cosmetic applications. Such edible fungi can be used in

biorefineries for the production of ethanol, animal feed and pigments from waste sources (Gmoster *et al.*, 2017).

The dye is a colored 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 (Anjana Devi *et al.*, 2015).

There is a growing demand of eco-friendly/ non-toxic dyes especially for food colors and child textiles. Natural dye producing microbes offer a viable alternative to natural vegetable and harmful synthetic dyes. Fungi are reported as potential biological source of natural pigments like anthraquinones (Iffat Sayyed *et al.*, 2015).

The natural fungal pigment is extracted from the thermophilic fungi-based species, namely *Thermomyces*, purified and characterized using Fourier transform infrared spectra, and subjected for the dyeing process. An experiment using Box and Behnken is designed with three levels and three variables using pH, temperature and time as independent variables and with wash fastness, rubbing fastness, light fastness and bacterial reduction (%) as dependent variables, and the conditions optimized. Regression equations are obtained to analyze the fastness properties and bacterial reduction (%), and the optimum conditions for dyeing the silk fabric by fungal pigmentation is 2% ovm at 60°C for 30 min, maintaining a pH of 3 (Parthiban Manickam and G Thilagavthi, 2015).

## **MATERIALS AND METHODS**

### **1) SAMPLE COLLECTION**

A fresh coconut was taken and placed in a dark environment. It was incubated for 5 days and the fungal growth was observed. The spoiled coconut sample was chosen as the source of fungal isolation.

### **2) ISOLATION OF FUNGI**

The fungal sample was isolated by culturing in media such as Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA). After the preparation of the media, it was poured onto the sterilized Petri plates and allows it to solidify. The organic samples were spread on PDA plates and incubate it at 28°C for 5-7 days.

### **3) IDENTIFICATION OF FUNGI**

The grown fungal colonies were identified by using Lacto Phenol cotton blue (LPCB) staining. A drop of ethanol was placed on a clean glass slide. A loopful of isolated fungal colony was smeared on glass slide. Then, a drop of Lacto phenol Cotton Blue stain was added to the slide. Place a coverslip over the slide and remove the excess stain using blotting paper. The fungal morphology was identified by observing the slide under microscope.

### **4) SCREENING FOR PIGMENT PRODUCTION**

By observing the fungal colonies for the visible pigment production, these colonies were transferred to fresh Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) plates for sub-culturing to ensure purity. The pigment producing fungi was inoculated into Potato Dextrose Broth (PDB) and Sabouraud Dextrose Broth (SDB) in 250ml conical flasks. The broth culture was incubated on rotatory shaker at 120rpm for 7-10 days.

## **5) PIGMENT EXTRACTION**

The pigments were extracted from biomass using solvent - methanol. Methanol was commonly used solvent for pigment extraction. The fungal biomass was crushed using mortar and pestle and solvent. The cells were ultrasonicated for 10 minutes to facilitate the cell lysis process for the release of pigment to the solvent. The extracted pigments were concentrated by applying heat to evaporate the solvent mixture and kept for storage.

## **6) PREPARATION OF NATURAL DYE**

The extracted pigment-solvent mixture was used in preparation of natural dye. It was mixed with mordant (Sodium carbonate). Mordant was added to improve adherence to cotton fabric.

## **7) APPLICATION ON FABRICS**

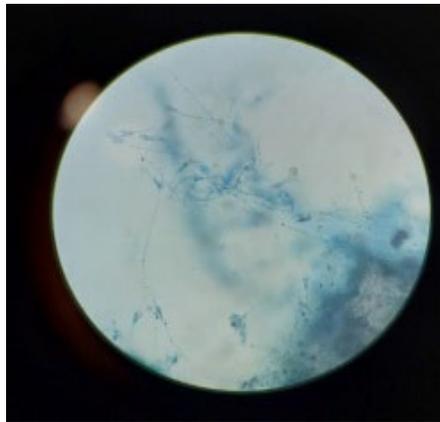
The fabric was pre-treated by soaking them in a mordant solution for one hour. The dye was applied by dipping the fabric into the pigment solution and let it to dry. The dye was tested for its color fastness by exposing fabric to sunlight, water and wash with detergents. The dyed fabric was first washed with tap water for few seconds. Then, another set of dyed fabric was washed with commercial detergent for few seconds. Finally, the dyed fabric was exposed to sunlight for 1-2 days.

## RESULTS

This section presents the experimental findings on the extraction of pigments from fungal species. The results include observations on fungal growth, pigment yield, solvent extraction efficiency, spectrophotometric analysis and application on the cotton fabric.

### Isolation and identification of pigment producing fungi

The fungal colonies were isolated from coconut sample. The isolated fungi were cultured in a medium. The fungal culture was identified by the Lacto Phenol Cotton Blue staining as *Trichophyton rubrum* and *Aspergillus*.



*Trichophyton rubrum*



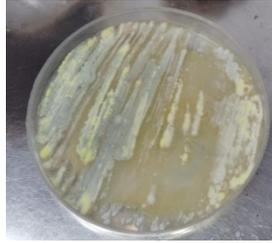
*Aspergillus* species

### Fungal growth and Pigment production

*T. rubrum* produced yellow pigments in both solid (PDA) and liquid (PDB) media after 7-10 days of incubation at 28°C. *Aspergillus* species exhibited green and black pigments depending on the strain. Maximum pigment production was observed on day 7 for *T. rubrum* and day 5-7 for *Aspergillus* species.



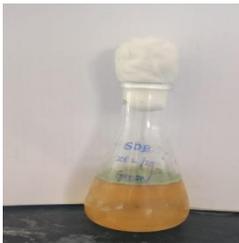
SDA plate with green colonies



PDA plate with yellow colonies



PDA plate with green colonies



SD Broth with green colonies



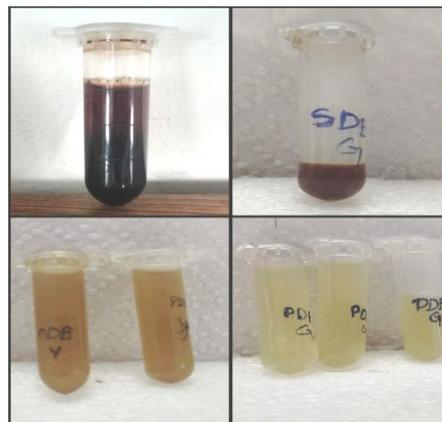
PD Broth with yellow colonies



PD Broth with green colonies

## Pigment Extraction

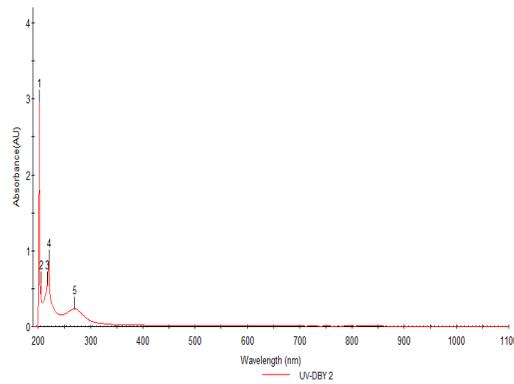
Pigment extraction was performed using solvent - methanol. Methanol was used due to its ability to dissolve both polar and non-polar pigments, and its relative high volatility, allowing for easy removal after extraction.



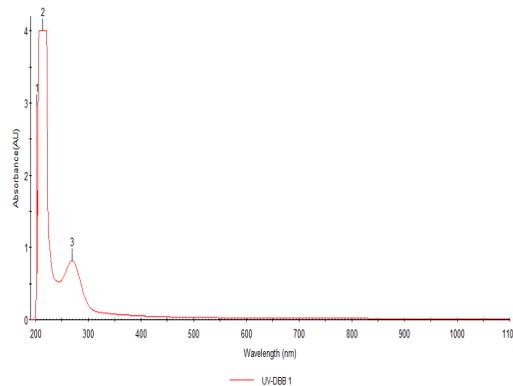
Extracted dye with methanol

## Spectrophotometric Analysis

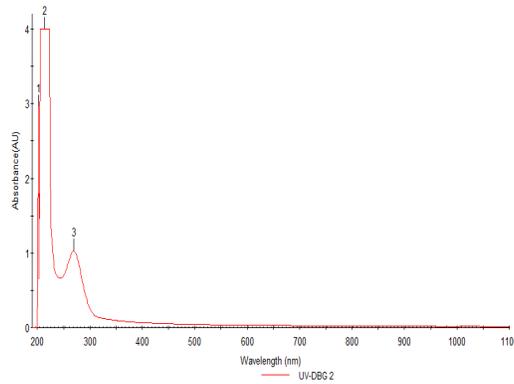
The extracted pigments were analyzed using a UV- Vis spectrophotometer. UV spectrophotometer was used to detect, characterize and quantify the pigments produced by fungi through analyzing their absorbance of ultraviolet and visible light. Pigment extraction was carried out using solvents (methanol) yielding highest pigment concentration from *T. rubrum* and *Aspergillus* species. Spectrophotometric analysis showed that *T. rubrum* pigments had maximum absorbance ( $\lambda$  max) at 268.7 nm, indicating Anthraquinone- like pigment, while *Aspergillus* pigments showed peak at 268.7 nm, suggesting Chlorophyll derivatives and melanin-like compounds.



a) UV DBY (Yellow)



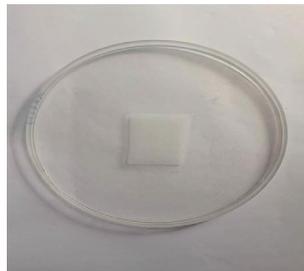
b) UV DBB (Brown)



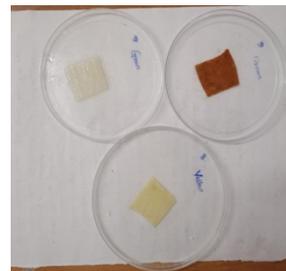
c) UV DBG (Green)

## Application Potential

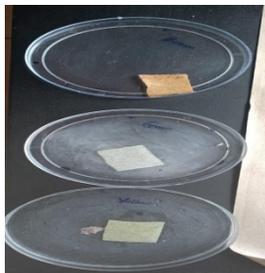
The extracted pigments were tested for textile dyeing on cotton fabric. Mordanting with Sodium carbonate improved dye uptake. The pigments exhibited moderate colorfastness to washing and light exposure.



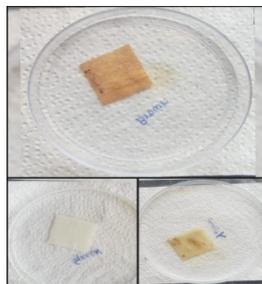
a) Control



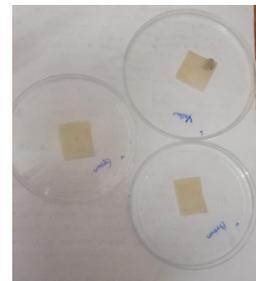
b) Fabric with dye and mordant



c) Fabric washed with water



d) Fabric washed with detergent



e) Fabric exposed to sunlight

## DISCUSSION

In this present study fungal sample was collected from spoiled coconut. The collected sample was cultured using PDA and SDA plates, incubated at room temperature for one week. After incubation the plates were observed its macroscopic observation based on its color of the colony and spores formation. In microscopic observation using Lacto phenol cotton blue staining identified types of spores and spores arrangement, it was identified as *Aspergillus* and *Trichophyton* (Johnson L.F and Manka Karol, 1961).

Microscopic view of fungal strains by slide culture method. Cultivation, extraction and purification of fungal pigments: Four fungal strains IDP1, IDP2, IDP3 and IDP4 were isolated which produced four different natural water soluble pigments- IDR, IDY, IDG and IDBr respectively; maximum absorbance and pigment yield are tabulated. UV analysis of the compound at 340nm except for one (IDG), which is at 371nm the presence of highly conjugated compound. All four isolates of fungi can tolerate lower temperature but the production of pigment can be observed at 30-32°C (Dikshit Rashmi and Tallapragada Padmavathi, 2013).

The four potent isolates were identified as *Penicillium* spp. based on the morphological characteristics. The shades of the pigment extracted from fungus exhibited a marked difference in the color component and pigment concentration. Maximum production of pigment was observed in the IDP3 *Penicillium* spp. However, production in IDP1, IDP2 and IDP4 was similar. Most probably the pigments belong to the Polyphenolic group (Flavenoids) (Iffat Sayyed *et al.*, 2015).

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 (Anchanna Devi *et al*, 2015).

Spectrophotometric analysis showed that *T. rubrum* pigments had maximum absorbance ( $\lambda$  max) at 268.7 nm, indicating Anthraquinone- like pigment, while *Aspergillus* pigments showed peak at 268.7 nm, suggesting Chlorophyll derivatives and melanin-like compounds.

The functional assessment of these pigments in textile dyeing demonstrated the strong fabric adherence, moderate colorfastness to washing, and improved dye retention upon mordanting with Sodium carbonate. These attributes underscore their viability as sustainable alternatives to synthetic dyes. The findings also suggest their potential use in pharmaceutical, cosmetic, and food industries, given their natural origin and lower environmental impact compared to synthetic colorants.

Despite promising results, further investigations are warranted to optimize pigment yield through metabolic engineering, enhance stability via microencapsulation, and evaluate scalability for commercial applications. Additionally, toxicology assessment and regulatory compliance studies will be crucial for their safe deployment in consumer products.

## SUMMARY

The present study focused on the extraction of pigment from fungal isolates of spoiled coconut sample. The fresh coconut was incubated for 5 days in dark and moisture place. The grown culture was isolated and cultured in Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) plates. After 5-7 days of incubation, the visible color producing fungal colonies (yellow and green colony) were sub-cultured for pigment production using Potato Dextrose Broth and Sabouraud Dextrose Broth incubated at 28°C for 5-10 days. The fungal morphology was observed by Lacto Phenol Cotton Blue (LPCB) staining method and the strains were identified as *Trichophyton rubrum* and *Aspergillus* sp.

After reaching the desired level of growth, the cells were lysed using crushing and ultrasonication. Pigment extraction was carried out using solvents (methanol) yielding highest pigment concentration from *T. rubrum* and *Aspergillus* species. Spectrophotometric analysis showed that *T. rubrum* pigments had maximum absorbance ( $\lambda$  max) at 268.7 nm, indicating Anthraquinone-like pigment, while *Aspergillus* pigments showed peak at 268.7 nm, suggesting Chlorophyll derivatives and melanin-like compounds.

The extracted pigments were successfully tested for textile dyeing, showing good dye uptake and moderate colorfastness when applied to cotton fabric with mordant (Sodium carbonate). The colorfastness was tested by washing with water, detergents and exposed to sunlight.

## CONCLUSION

This study provides a comprehensive approach to fungal pigment extraction, demonstrating the feasibility of utilizing *T. rubrum* and *Aspergillus* species as bio-resource platforms for natural dye production. By leveraging their bio-compatibility, eco-friendliness, and versatile applications, fungal pigments have the potential to revolutionizing the natural colorant industry, aligning with the global shift toward sustainable and bio-based alternatives.

Based on this study, we conclude that this pigment is non-toxic, and it can be used as food colorant and additive. Thus our research has shown that this pigment can be used as food, feed, colorant and as essential nutrient. Microbes producing pigments could contribute to biotechnology and add value to the food, feed and pharmaceutical industries. The present work provides an overview of the recent advances and perspectives on some natural dyes and pigments.

## REFERENCE

- Aejaz Abdullatif Khan, Ali Mohamed Alshabi, Yahya S. Alqahtani, Awad Mohammed Alqahtani, R.S. Bennur, Ibrahim Ahmed Shaikh, Uday M. Muddapur, S.M. Shakeel Iqbal, Tasneem Mohammed, Areej Dawoud, Sunil S. More & Muazzam Sheriff Maqbul (2021). Extraction and identification of fungal pigment from *Penicillium europium* using different spectral studies. J. of King Saud University ; 33. doi: 10.1016/j.jksus.2021.101437.
- Ajay C. Legashetti, Laurent Duffose, Sanjay K. Singh & Paras N. Singh (2019). Microorganisms.7.604. doi: 10.3390/microorganisms7120604.
- Ana Abad, Jimena Victoria Ferna'ndez-Molina, Josebabikandi, Andoni Rami'rez, Javier Margareto, Javier Sendino, Fernandolushiernando, Josepon'to, Javiergaraizar & Aitorrementeria (2010). What makes *Aspergillus fumigates* a successful pathogen. Genes.
- Anchana Devi & Aishwarya (2014). Extraction of Natural Dyes from Fungus- An Alternate for Textile Dyeing. J Nat Sci Res; 4(7): 1-6. 2225-0921.
- Arora S (2014). Textile dyes: Its impact on the environment and its treatment. J. Bioremediat. Biodegrad; 5:1. doi: 10.4172/2155-6199.1000e146.
- Atalla M, El-Khrisy E, Youssef Y & Mohammed A (2011). Production of textile reddish b
- Babitha S, C.R. Soccol & A. Pandey (2006). Jackfruit seed- a novel substrate for the production of *Monascus* pigments through solid state fermentation. Food Technology and Biotechnology. 44(4): p.465-71.
- Berdy J. (2005). Bioactive microbial metabolites. J Antibiot. 58:1-26.
- rown dyes by fungi. Malaysian Journal of Microbiology, 7(1), 33-40.
- Brakhage Aaas V. (2011). Fungal secondary metabolites - strategies to activate silent gene

clusters. *Fungal Genet Biol.* 48:15-22.

Capozzi V, M. Fragasso, R. Romaniello, C. Berbegal, P. Russo & G. Spano (2017). Spontaneous Food Fermentations and Potential Risks for Human Health. *Fermentation.* 3(4):p .49.

Caro Y, Anamale L, Fouillaud M, Laurent P, Petit T, Duffose L. (2012) Natural hydroxyanthraquinoid pigments as potent food grade colorants: an overview. *Nat Prod Bioprospect*; 2:174-93.

Celestino JDR, De Carvalho LE, Lima MDP, Lima AM, Ogusku MM & De Souza JVB (2014). *Process Biochem.* 49:569-575

Cho, Y. J., Park, J. P., Hwang, H. J., Kim, S. W., Choi, J. W., & Yun, J. W. (2002). Production of red pigment by submerged culture of *Paecilomyces sinclairii*. *Letters in Applied Microbiology*, 35(3), 195-202. doi: 10.1046/j.1472-765X.2002.01168.x.

Cobanoglu Sandyazici A (2022). Isolation, characterization and antibiofilm activity of pigments synthesized by *Rhodococcus* sp. SC1. *Curr. Microbiol.* 79:15.

Dikshit Rashmi & Tallapragada Padmavathi (2013). Exploring *Monascus anguineus* as a potential natural source for pigment production. *Int. Res. J. Biol. Sci.*, 2: 59-67.

Dorothy Ivila de Melo Pereira, Raiana Silveira Gurgel, Anne Terezinha Fernanded de Souza, Rosiane Rodriques Matias, Lucas de Souza Falcao, Fransico Celio Maia Chaves, Gilvan Ferreira da Silva, Jose Gregorio Martinez, Rudi Emerson de Lima Procopio, Cleiton Fantin & Patricia Melchionna Albuquerque (2024). *J. Fungi* ; 10(1). 77. doi: 10.3390/jof10010077.

Downham A., Collins P (2000). Coloring our foods in the last and next millennium. *Int. J. Food Sci. Technol*; 35:5-22. doi: 10.1046/j.1365-2621.2000.00373.x.

Duffose L, Fouillaud M, Caro Y, Mapari Sameer AS & Sutthiwong N (2014). Filamentous fungi are large-scale producers of pigments and colorants for the food industry. *Curr Opin Biotechnol*; 26:56-61

Duffose L. (2018). Red colorants from filamentous fungi: Are they ready for the food industry? *J. Food Compos. Anal.*, 69, pp.156-161. 10.1016/j.jfca.2017.11.002.

Duffose L (2006). Microbial food grade pigments. *Food Technol Biotechnol*; 44: 313-321.

Dziezak, J.D. (1987). Applications of food colorants. *Food technology*. 41,78-80.

Feng Y, Y. Shao & F. Chen (2012). *Monascus* pigments. *Applied Microbiology and Biotechnology*. 96(6):p.1421-40.

Ferreira JA, Mahboubi A, Lennartsson PR & Taherzadeh MJ (2016). Waste biorefineries using filamentous ascomycetes fungi: present status and future prospects. *Bioresour Technol.*;215:334-45.

FoodDive, 2021. Why Are Natural Colors Poised to Pop in 2021? 591608. Pigments concomitant with the bioactive red bikaverin and derivatives thereof produced by *Fusarium oxysporum*. *Biotechnol. Prog.*, 35 (2019). 10.1002/btpr.2738.

Francis, F.J. (1987). Lesser-known food colorants. *Food technology*. 41, 62-68.

Francis, F.J. (1989). Food colorants: Anthocyanins. *Critical Review in Food Science*. 28, 273-313.

Fuck W.F, Marcilio N.R, Bordignon S, Gutterres M (2011). The influence of chromium supplied by tanning and wet finishing processes on the formation of Cr(VI) in leather. *Braz. J. Chem. Eng.* 28(2), 221-228.

Geiser DM (2009). Sexual structures in *Aspergillus*: morphology, importance and genomics. Medical Mycology. 49 Suppl 1 (S1): S70-6. doi: 10.1080/13693780802139859.

Gmoser R, Jorge A. Ferreira, Patrik R. Lennartsson & Mohammad J. Taherzadeh (2017). Filamentous ascomycetes fungi as a source of natural pigments. Fungal Biol Biotechnol; 4:4. doi: 10.1186/s40694-017-0033-2.

Gunasekaran S & Poorniammal R (2008). Optimization of fermentation conditions for red pigment production from *Penicillium* sp. under submerged cultivation. Afr J Biotechnol; 7:1894-1898.

Gunjan Mukherjee, Tulika Mishra & Sunnil K. Deshmukh (2017). Fungal Pigments: An Overview. Developments in Fungal Biology and Applied Mycology; doi: 10.1007/978-10-4768-8-26.

Iffat Sayyad & Devipriya R. Majumder (2015). Pigment Production from Fungi. Int. J. Curr. Microbiol. App. Sci. 2 pp. 103-109.2319-7706.

Indra Arulselvi, P., Umamaheswari, S., Ranandkumar, S. G., Karthik, C., & Jayakrishna, C. (2014). Screening of yellow pigment producing bacterial isolates from various eco-climate areas and analysis of the carotenoid produced produced by the isolate. Journal of Food Processing & Technology, 5(1), 1-4. doi: 10.4172/2157-7110.1000292.

Jiang Y, Li HB, Chen F, Hyde KD (2005). Production potential of water soluble *Monascus* red pigments by a newly isolated *Penicillium* sp. J Agric Technol 1:113-126.

Johnson L.K & Manka Karol (1961). A Modification of Warcup's Soil Plate Method for Isolating Soil Fungi. Soil Science. 92(2). 79-84.

Kalra R, X.A. Conlan, M. Goel (2020). Fungi as a potential source of pigments: harnessing

filamentous fungi. *Front. Chem*; 8: 369. 10.3389/fchem.2020.00369.

Kanth S.V, Venba R, Jayakumar G.C, Chandrababu N.K (2009). Kinetics of leather dyeing pretreated with enzymes: role of acid protease. *Bioresour. Technol.* 100(8), 2430-2435.

Kim, Y. E., Matter, I. A., Lee, N., Jung, M., Lee, Y.C., Choi, S.A., Lee, S.Y., Kim, J.R., & Oh, Y.K., (2020). Enhancement of astaxanthin production by *Haematococcus pluvialis* using magnesium aminoclay nanoparticles,. *Bioresource Technology*, 307, 123270. doi: 10.1016/j.biortech.2020.123270.

Lagashetti AC, Duffose L, Singh SK & Singh PN (2019). Fungal pigments and their prospects in different industries. *Microorganisms*. 7:604.

Lauren Doyle, Suvro Talukdar, Youling L. Xiong, Akinbode Adedeji & Tyler J. Barzee (2025). Evaluation of the Gelation Characteristics and Printability of Edible Filamentous Fungi Flours and Protein Extracts. *Foods*. 14(6), 923; doi: 10.3390/foods14060923.

Lauro, G.J. (1991). A primer on natural colors. *Cereal Foods World* 36, 949-953.

Lebeau J, Petit T, Clerc P, Duffose L & Caro Y (2019). Isolation of two novel purple naphthoquinone pigments concomitant with the bioactive red bikaverin and derivatives thereof produced by *Fusarium oxysporum*. *Biotechnol. Prog*; 35. 10.1002/btpr.2738.

Lin L, Xu J (2020). *J Fungi*. 6(4). 280

Mai Ali Mwaheb, Yasmeen A. Hasanien, Amira G. Zaki, Alaa S. Abdel-Razek & Laila R. Abd Al Halim (2024). *Fusarium verticillioides* pigment: production, response surface optimization, gamma irradiation and encapsulation studies. *BMC Biotechnology*. 24:84. doi:10.1186/s12896-024-00909-7.

Malik K, Tokkas J, Goyal S (2012). Microbial pigments: a review. *Int J Microbial Res*

Technol.;1:361-5.

Maria Afroz Toma, Hasibur Rahman, Saydar Rahman, Mohammad Arif, K.H.M. Nazmul Hussain Nazir & Laurent Dufosse (2023). J. Fungi; 9(4), 454. doi: 10.3390/jof9040454.

Meghna Shrivastava, Madhavi Tiwari & Dr. Ashish Saraf (2017). Pigment Extraction Methods from Fungi for Industrial Applications. IJARSE. 6(10). 2319-8351.

Michael R. McGinnis & Stephen K. Tyring (2003). Introduction to Mycology (4<sup>th</sup> ed.). In Medical microbiology, Elsevier. NBK8125.

Minghui Zhou, Yajun Chen, Xue Fang, Li Wu & Yan Zhang (2023). Isolation and identification of pigment-producing filamentous fungus DBFL05 and its pigment characteristics and chemical structure. Journal of food; 21(1): 374-385.

Miriam S. Valenzuela-Gloria, Nagamani Balagurusamy, Monica L. Chavez-Gonzalez, Oscar Aguilar, Ayerim Hernandez-Almanza & Crisobal N. Aguilar (2021). Molecular Characterization of Fungal Pigments. J. Fungi; 7(5). 326. doi: 10.3390/jof7050326.

Nagia F& El-Mohamedy R (2007). Dyeing of wool with natural anthraquinone dyes from *Fusarium oxysporum*. Dyes and Pigments, 75(3), 550-555.

Nimnoi P & S. Lumyong (2011). Improving Solid- State Fermentation of *Monascus purpureus* on Agricultural Products for Pigment Production. Food and Bioprocess Technology. 4(8):p. 1384-90.

Numan M, Bashir S, Mumtaz R, Tayyab S, Rehman NU, Khan AL, *et al.* (2018). Therapeutic applications of bacterial pigments: a review of current status and future opportunities. 3 Biotech. 8:207.

Nystrom M, Graham NAJ, Lokrantz J & Norstrom AV (2008). Coral Reefs. 27. 795-809.

Oliver Blechert, Hailin Zheng, Xiaohul Zang, Qiong Wang & Weida Liu (2019). Influence of the cultivation medium and pH on the pigmentation of *Trichopyton rubrum*. PLoS One; 14(9): e0222333. doi: 10.1371/journal.pone.0222333.

Page C (2001). What are leather dyes today? The relationship between the dye structure and its performance properties, XXVI IULTCS Congress Proceedings, Cape Town.

Parthiban Manickam & G Thilagavathi (2015). A natural fungal extract for improving dyeability and antibacterial activity of silk fabric. Journal of Industrial Textiles; 44(5). 769-780. doi: 10.1177/1528083713516662.

Piccin J.S, Gomes C.S, Mella B, Gutterres M (2016). Color removal from real leather dyeing effluent using tannery waste as an adsorbent. J. Environ. Chem. Eng. 4(1), 1061-1067.

Puntener A.G; Leather dyes (2000). In: Colorants for Non-Textile Applications. Elsevier Science, Amsterdam, ed. 1, ISBN 9780-444-82888-0, 478-484.

Rao M.P.N., Xiao M., Li W.J (2017). Fungal and bacterial pigments: Secondary metabolites with wide application. Front. Microbiol; 8:1113. doi: 10.3389/fmicb.2017.01113.

Rishu Kalra, Xavier A. Conlan & Mayurika Goel (2020). Fungi as a Potential Source of Pigments: Harnessing Filamentous Fungi. Front. Chem; 8. doi: 10.3389/fchem.2020.00369.

Shailendra Yadav, Kanha Singh Tiwari, Chitrasen Gupta, Mahendra Kumar Tiwari, Arbaj Khan & Sankatha P. Sonkar (2022). A brief review on natural dyes, pigments: Recent advances and future perspectives. Results in Chemistry. doi: 10.1016/j.rechem.2022.100733.

Shrivastava M, Saraf A, Shukla K.K & Sharma D (2017). Isolation of Bioactive Metabolites Producing Fungal Strains from Milk Products and Milk Industries of Raipur District. World Journal of Pharmacy and Pharmaceutical Sciences. 6(9), 1652-1658.

Subhasree R, P. D. Babu, R. Vidyalakshmi & V.C. Mohan (2011). Effect of carbon and nitrogen sources on stimulation of pigment production by *Monascus purpureus* on jack fruit seeds. Intl J of Microbiological Res. 2(2): p.184-87.

Suciatmih, Nurfianti & S. V Magfirani (2018). Coloring Properties Assessment of Dyes Produced by Mixed *Aspergillus* and *Paecilomyces*. IOP Publishing; 166. doi: 10.1088/1755-1315/166/1/012023.

Venil CK, Duffose L & Renuka Devi P (2020). Bacterial pigments: sustainable compounds with market potential for pharma and food industry. Front. Sustain. Food Syst. 4:100.

Wagner Fernando Fuck, Adriano Brandelli & Mariliz Gutterres (2018). Leather Dyeing with Biodyes from Filamentous Fungi. JALCA; 113. 55(51). 3308- 3638.

Wail Elkhateeb & Ghoson Daba (2023). Fungal Pigments: Their Diversity, Chemistry, Food and Non-Food Applications. Appl. Microbiol. 3, 735-751. doi: 10.3390/applmicrobiol3030051.

Wang Y, V. Herdegen, X. Li & J.U. Repke (2018). Numerical study and evaluation of solid-liquid extraction of Montan wax in stirred tanks on different scales. Separation and Purification Technology. 204:p. 90-97.

## APPENDIX

### MEDIA COMPOSITION

#### 1) Potato Dextrose Agar

Diced Potatoes	- 20g
Dextrose	- 2g
Agar	- 1.5g
Distilled Water	- 100ml

#### 2) Sabouraud Dextrose Agar

Dextrose	- 4g
Peptone	- 1g
Agar	- 1.75g
Distilled Water	- 100ml

### REAGENT

#### Lacto Phenol Cotton Blue Stain

Lactic acid	- 200ml
Phenol	- 20g
Glycerol	- 40ml
Distilled Water	- 20ml
Cotton Blue	- 2ml