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Research Article

Screening of Natural Dyes from Selected Fungal Species

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Abstract: Some fungi are observed as effective pigments. Its importance in the production of natural pigments has grown significantly. The pigment-producing fungi were developed and evaluated for application in dyeing cotton fabric. In the research, five fungal strains were identified as *Aspergillus terreus S10, Talaromyces atroroseus WW5A3, Penicillium oxalicum WW3A4 (DG), WW5C2* and *WW31DG*. These strains were incubated for 21 days under static and non-static conditions using MSM and PDB media. Under liquid state fermentation conditions, the production of the pigments by the fungus was improved by altering temperatures (25-35 °C) and pH (4.5-6.5). *T. atroroseus WW5A3* showed pinkish color, *A. terrreus S10* displayed yellow color, *P. oxalicum WW3A4 (DG)* presented yellow-greenish, *WW5C2* exhibited light yellow color and *WW31DG* demonstrated greenish color. The results showed the maximum percentage absorbance of *T. atroroteus WW5A3* showed 90.36 % at 600 nm, *A. terrreus S10* showed 88 % at 500 nm, *P. oxalicum WW35A4* showed 46.04 % at 550 nm, *WW5C2* showed 59.60 % at 550 nm, and *WW31DG* showed 81.9 % at 550 nm. The natural fungal pigments were tested against bacterial pathogens to check the antibacterial activity. The results indicated that *S. aureus* and *E. coli* exhibited antibacterial activity in terms of maximum zone of inhibition. In conclusion, out of five pigments producing fungi, *Aspergillus terreus S10* and *Talaromyces atroroseus* produced maximum pigment and highest percentage absorbance under liquid state fermentation conditions. Potential applications in the textile and leather industries have been discovered as a result of this research.

Keywords: Cotton fabric, ecofriendly, natural pigments, characterization, antibacterial activity, dyeing

1. INTRODUCTION

Modern times heighten the demand for prompt industrialization that has compelled the immediate formulation to use artificial colorants in the field of food, medicine leather, and other industrial areas nevertheless of their cancer-causing, immune-oppressive, and hazardous environmental effects. To attain these objectives researchers are discovering natural pigments from microbial resources as a substitute for artificial dyes [1]. Researchers revealed that environment-adapted microbial colorants from microbes are better than artificial dyes because of their fast growth, cool handling, and important roles in transcriptional and intracellular signaling. Furthermore, their applications in the food and cosmetic industry are due to their assembly and easiness of big-scale production [2]. Microbes such as fungi and bacteria deliver the availability of naturally derived pigments [3]. Synthetic dyes lead to the production of industrial effluents that are considered toxins and exhibited numerous natural issues and therapeutic problems [4]. Phycocyanins extracted from thermophilic blue-green bacteria and fungi were verbalized to use in makeup mostly an eye-shadows and lipstick shades were manufactured in the industry by using both fungi and bacteria [5]. Many filamentous fungi are used in the production of natural pigments as they are eco-friendly to the environment and less hazardous. Toxic effluents from industries utilizing different colors posed threat to the environment and caused serious health issues [6]. Natural colors in food manufacturers

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are associated with several advantages and endless therapeutic diseases [7]. Naturally, fungi produce colored pigments, secondary metabolites such as flavonoid and tannin are familiar as pigments with extraordinary pharmaceutical importance. Many filamentous fungi including *Aspergillus sp., Penicillium sp., Paecilomyces sp.,* and *Monascus purpureus* can be utilized in the extraction of colored pigments. *Thermomyces* are utilized to extract red color and found huge industrial applications [8]. Pigments produced by fungal species showed improved dyeing ability at acidic (5 pH) [9]. Shade created from *Talaromyces* under 4.5 - 5 pH to direct antacid settings (pH 4.5 and 6.0) [10]. Colors from fungal species, become steadier at extreme temperatures [11]. *A. Flavus* shade appeared 93 % to 96 % steadiness on cotton and silk fabric at 26 °C [12]. The colors extracted by *Talaromyces sp*. had plentiful benefits in the food, textile, and cosmetic industry [13]. Filamentous Fungi create a wide assortment of bio-colorants and have secondary metabolites like melanins, phenazines, flavins, carotenoids, quinones, violacein, indigo, and monascins [14]. Fungi species are curiously good for the environment and give attractive natural colors, few fungal species are good to produce consistent colorants and they have carotenoids present in them as secondary compounds [15]. These filamentous fungi have been distinguished as they have anti-bacterial activity against Gram-positive and Gram-negative microbes.

For the better growth of fungal pigments, the optimal carbon source, moderate pH, temperature, and availability of light source is very necessary. It has been detailed that fungal species can develop and create colors in the submerged state containing glucose, fructose, and dextrose [16]. Hydrogen molecules exceptionally play important role in the production of eco-friendly pigments. The development of color under 5.5 and 6.5 pH was maintained for two strains of *Pycnoporus* [17]. The calculations [18] showed the most noteworthy value of yellow color was gotten with 6.5 pH while the greatest biomass concentration was measured at pH values of 8 for *Talaromyces* sp. [19].

Temperature is the main element that influences colors and other metabolites generation by organisms. Research showed the impacts of temperature on cell development, color generation by a different number of organisms demonstrating that the ideal temperature ranges from 25 to 32 °C. Fungi also respond to light during development for metabolites generation. The impact of light on color generation is very important for the production of fungal pigments. It was observed that in production of pigment from *M. ruber* repressed color production when plates are covered and cannot coordinate with light [20]. Consequently, the extractions of pigments from these filamentous fungi are being used in many applications [21]. The main objective of this research was to replace artificial dyes with natural ones.

2. MATERIAL AND METHODS

2.1 Isolation and Collection of Fungi

Fungal strains were collected from the Biology Lab of Lahore Garrison University. The cultures were preserved at -80 °C, the fungal strains were refreshed by using PDA media. For isolation, textile and lignocellulosic waste were collected and stored in sterilized plastic bags. By using the serial dilution method samples were collected and 1 mL was dissolved in 10 mL of water in a test tube. After that (0-1 mL) will spread onto the PDA plates with the sterile spreader. All PDA plates were kept in an incubator at 28 °C for 3-4 days. Diverse fungal colonies showed up on PDA plates from which cultures of the fungi were acquired by transferring them onto new PDA plates and after that kept once more in an incubator at 28 °C for 3-4 days [22].

2.2. Qualitative Analysis of Fungal Dyes

2.2.1 Preparation of Media

PDA is a solid medium comprising the extract of 300 g skinned potato, 2.5 g glucose, 15 g agar. All PDA plates were incubated at 28 °C for 3-4 days. Fungal colonies grew on PDA plates and were placed in an incubator at 28 °C for 3-4 days [23].

2.2.2 Cultivation of Fungi on appropriate Medium for Optimum Color Production

For the cultivation of fungi, two media were used i.e. Mineral Salt Media (MSM) and Potato Dextrose Broth (PDB). MSM was prepared using the following salts i.e., dextrose, K_2HPO_4-5.0 g,

KH_2PO_4-5.0 g, $CaCl_2$-0.1 g, $MgSO_4 7H_2O$-0.5 g, $FeSO_4 7H_2$ O-0.01 g, $ZnSO_4.7H_2O$-0.01 g, $MnSO_4H_2O$ -0.03 g. All these salts were dissolved in 1000 mL of distilled water (Figure 4). Two sets of flasks were placed in an incubator, in dark, under static conditions for 4-6 weeks until the bulk of pigments appeared.

2.3 Extraction of Pigments from Fungi

The isolation of fungal supernatant was caused by utilizing a sterile muslin cloth after the incubation period. The residue was ground by mortar and pestle. Later, 2 ml water was added and incubated at 130 rpm for 60 min at 28 °C [24].

2.4 Quantitative Characterization of Natural Pigments

The soluble compounds from the fungal extract and culture filtrates of different fungal isolates were subjected to UV–Visible spectrophotometer. Compounds that were present in water extracts were subjected to a wavelength scan from 400 nm to 600 nm for the determination of absorption spectra. Spectrophotometry was used for the measurement of the optical density of pigment. This was done for the determination of a better option for obtaining more concentrated pigment. The optical density (OD) was measured at 400, 450, 500, 550, and 600 nm (a wavelength which represents the absorption maximum for yellow, red, pink, greenish, and yellow-greenish pigments respectively), thus yielding the so-called yellow, pink, reddish-brown, and red pigment production [25].

2.5 Analysis of Color on Cotton Fabric

To analyze the fabric properties, rate retention of the dyed cotton cloth, percentage absorption was calculated on UV spectrophotometer at 400 nm to 600 nm and the percentage absorption was calculated by measuring OD of concentrated pigment before dipping the cotton cloth and again OD was calculated after absorption of pigment by cotton cloth. The final percentage absorption was calculated by the given formula [26]:

$$Percentage\ absorption = \frac{OD\ before\ dyeing - OD\ after\ dyeing}{OD\ before\ dyeing} \times 100$$

2.6 Antibacterial Activity of Fungal Pigments

The bacterial suspension was made of *E. coli* and *S. aureus* which was arranged by taking ordinary saline sterilized test tube. Bacterial colonies were exchanged with the assistance of a wire loop into the test tube. The circle utilized was sterilized by warming. The test tube was swirled well with the assistance of a syringe. For the making of media, the L-Agar and L-broth were utilized. These media were included in distilled water containing jar and shake well with the assistance of a stirrer, the volume raised to 500 ml at that point closed the carafe with the assistance of a cotton plug and secured with an aluminum thwart, and autoclaved for 15 minutes at temperature 121 °C and weight 15 lb / $inch^2$. A loop full of *E. coli* and *S. aureus* bacteria were inoculated into a nutrient broth and incubated on a shaker at 28 °C for a period of 16–18 h. After incubation, 100 µL of new bacterial cultures were immunized separately onto the agar plates by the spread plate method.

3. RESULTS

3.1 Optimization of Fungal Pigments

Two different media PDB and MSM media were used for the production of pigments. In the case of submerged fermentation where the flasks were incubated at rotary conditions, the MSM showed color production for all five fungi (Fig. 1). However, *A. terreus S10, T. atroroseus WW5A3* and *WW5C2* secreted pinkish, dark, and light yellowish colors, respectively, in PDB. Moreover, the impact of incubation conditions (rotating and/or static) was observed. On the other hand, in static, and dark conditions all five fungi showed color in a liquid state under MSM media and in the case of PDB media, except *WW35A4* all fungal strains produced color. *T. atroroseus WW5A3* appeared pinkish color and *A. terreus* S10 showed yellow color. The greenish-yellow color was advertised by *WW31DG.* Beneath 25 °C to 35 °C (Fig. 3) *WW5C2* created light yellow color, whereas *WW35A4* displayed a light pale-yellow color in solid-state and fluid state maturation (Fig. 4) in 4.5 to 6.5 pH as described in (Fig. 2).

3.2. Analysis of Fungal Pigments

The different fungal isolates were subjected to UV–Visible spectrophotometer [27]. The *T. atroroseus WW5A3* showed pinkish color with a strong absorbance in the near UV region with a distinctive peak at about 600 nm as shown in (Fig. 5). However, in the case of *A. terreus* (Fig. 6) showed yellow color, *P. oxalicum* showed Pale yellow, *WW31DG* showed yellow-greenish and *WW5C2* showed light yellow bio-colorants, a strong peak at ~ 500 and ~ 550 nm, respectively, was observed. However, they may have secondary metabolites like carotenoids, melanins, azaphilones, and polyketide that showed that specific color and also possess resistance to natural variables (drying up, high temperatures, irradiations, and photo-oxidation).

3.3 Application of Cotton Dyeing using Fungal Pigments

The extracted pigments were further used for dyeing the cotton cloth. For dyeing ferrous sulphate was used as a mordant. The capacity of extracted colors was evaluated on cotton cloth which weighed around 10 g. For detecting any change in color on the cotton, control was also held as shown in Fig. 7. It was observed that the pinkish, green, and yellow colors, exhibited an undeviating shade on cotton. The % OD was taken before dyeing and after dyeing to check the absorbance of color in cotton fabric and to check colorfastness on fabric. The overall process of pigment production from fungi is shown in Fig. 8.

3.4 Study of Antibacterial Activity of Fungal Pigments Against Gram-Positive Bacteria by Agar Well Diffusion Method

The antibacterial activity of fungal pigments against two bacterial strains was assessed by the Agar Well Diffusion method. The extracts exhibited a varying degree of antibacterial activity at 100 mg/mL against gram-positive and gram-negative bacteria. The result described that *S. aureus* and *E. coli* are susceptible to fungal pigments as described in

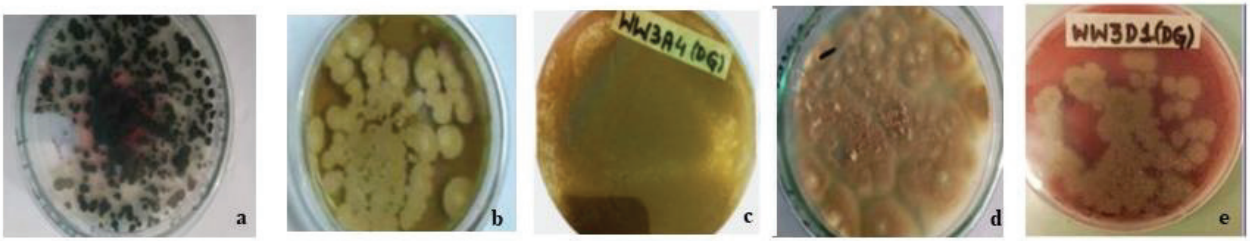

Fig. 1. Results of Solid Sate fermentation (a) *T. atroroseus WW5A3* (b) *A. terreus S10* (c) *P. oxalicum WW3A4* d) *WW5C2* (e) *WW31DG*

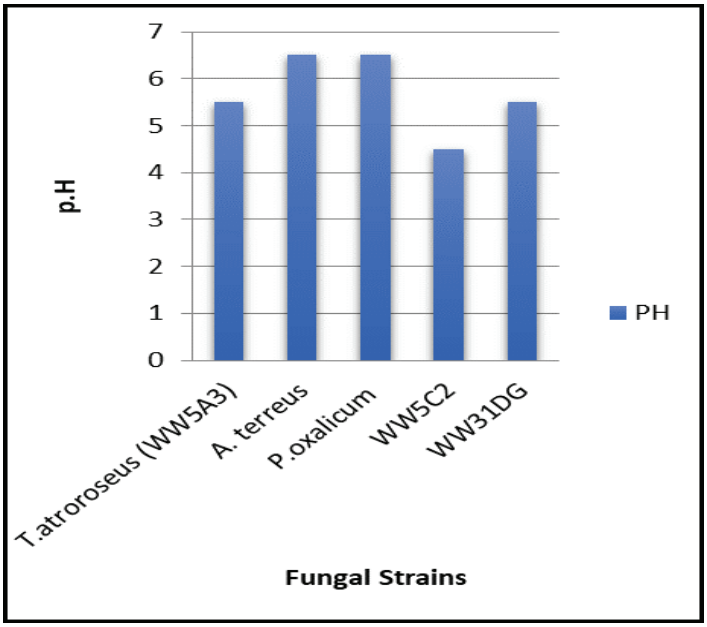

Fig. 2. Optimization of *T. atroroseus, A. terreus, P. oxalicum, WW5C2 and WW31DG* on different pH ranges

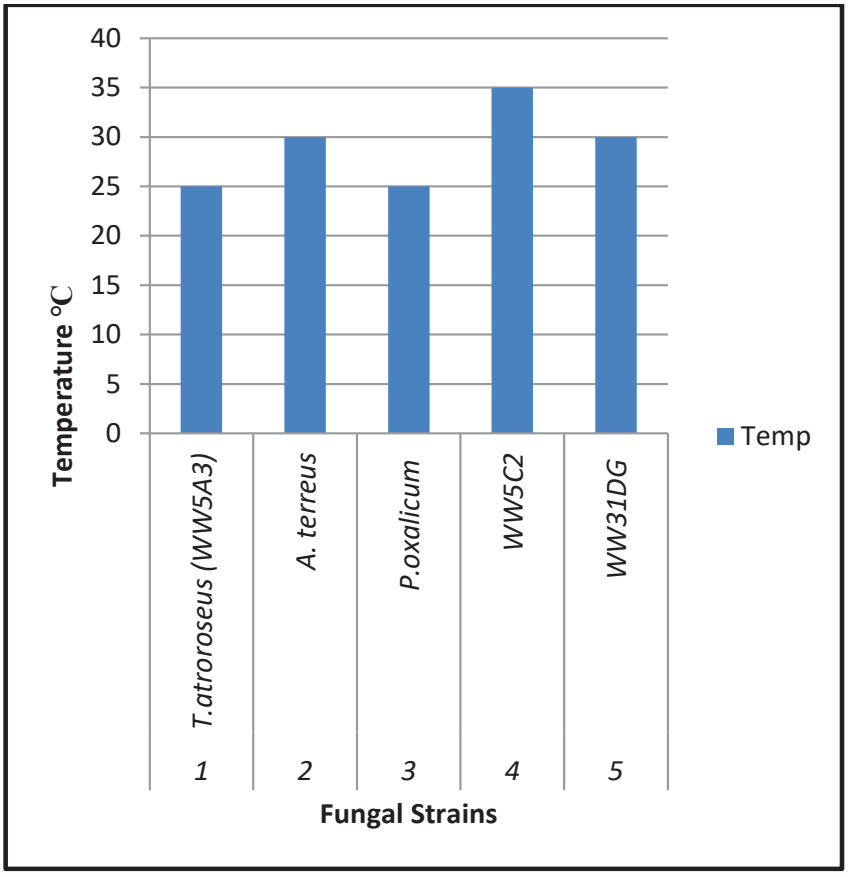

Fig. 3. Optimization of different fungal strains on different temperatures ranges

Fig. 4. Production of fungal pigments under liquid-state fermentation (a) *T. atroroseus WW5A3* (b) *A. terreus S10*, (c) *P. oxalicum WW5A4* (d) *WW5C2* and (e) *WW31DG* (left to right)

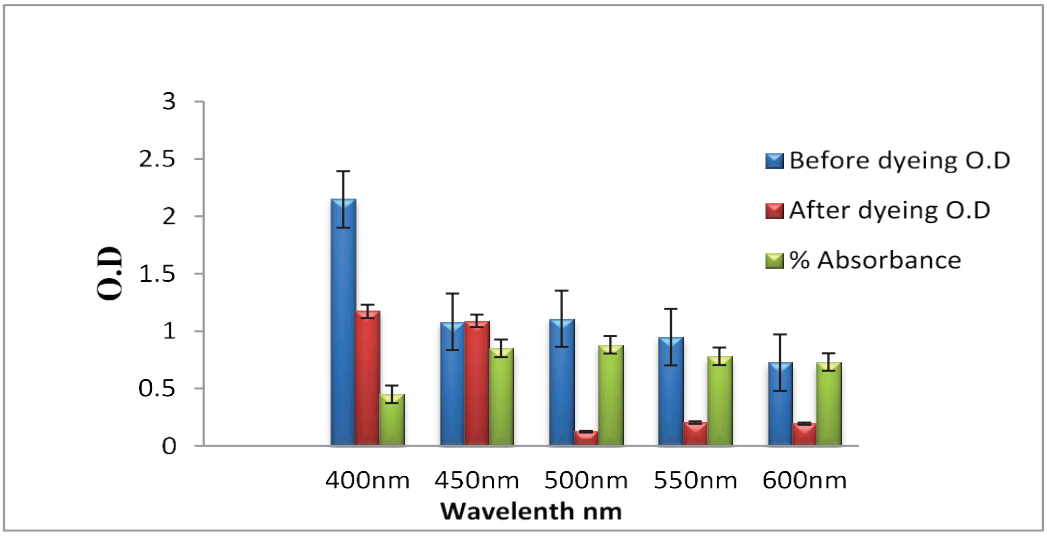

Fig. 5. Results of *T. atroroseus WW5A3* before and after OD at a different wavelength

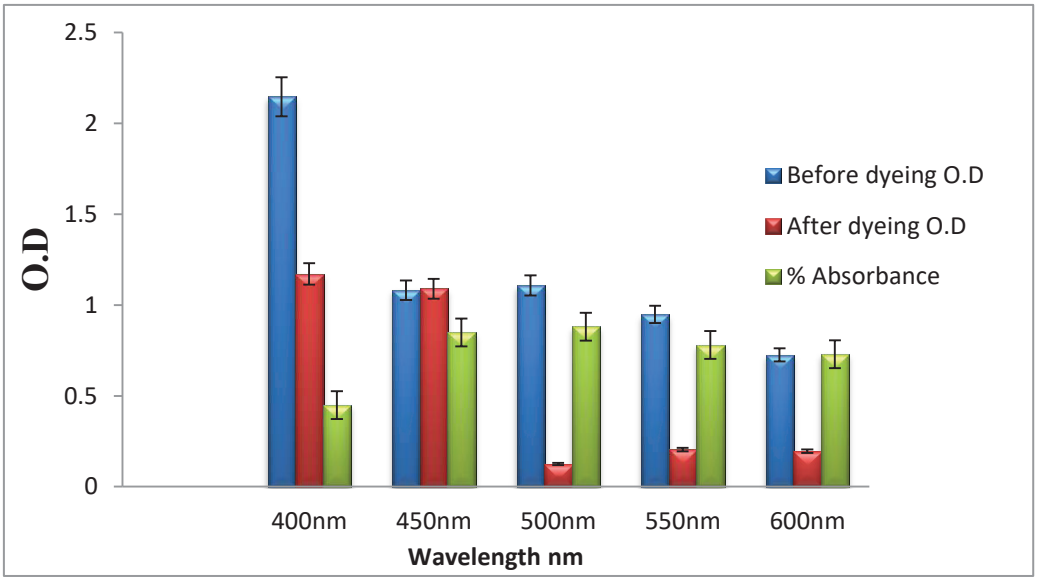

Fig. 6. Results of *Aspergillus terreus* S10 before and after OD at a different wavelength

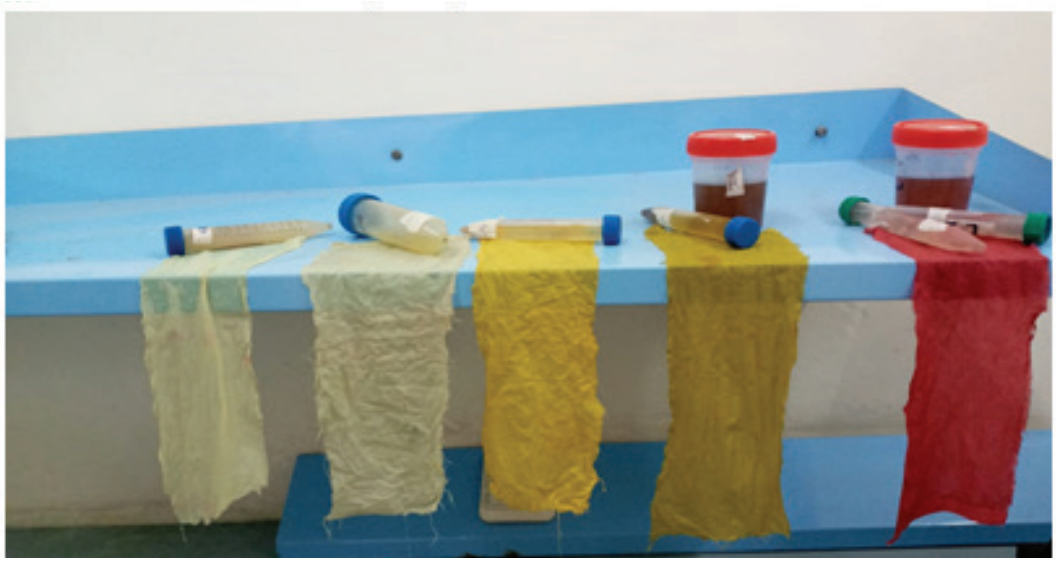

Fig. 7. Dyed cotton cloth by fungal pigments showing dyeing of cotton cloth from fungal pigments

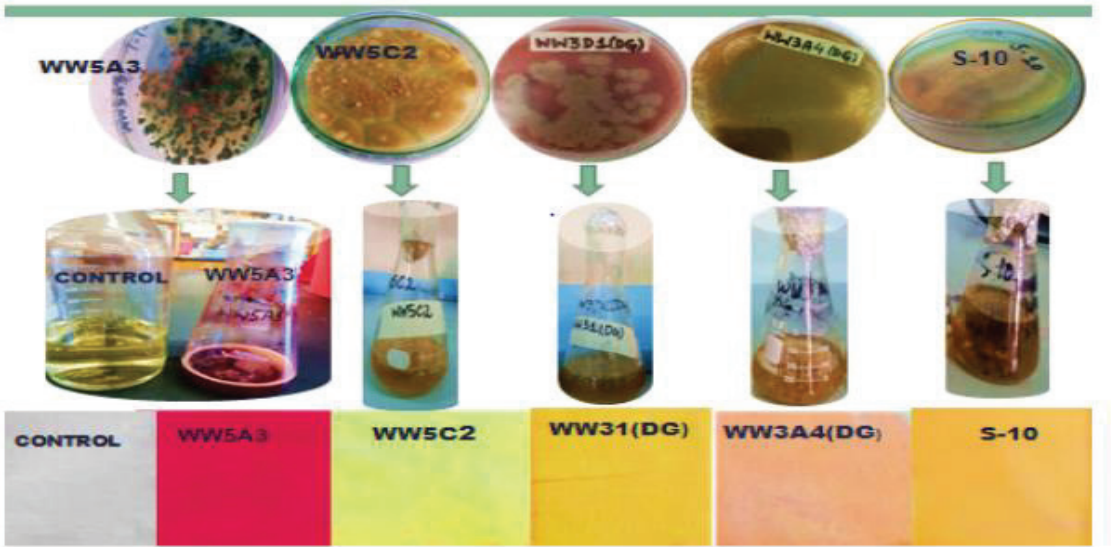

Fig. 8. The overall process of pigment production from fungi

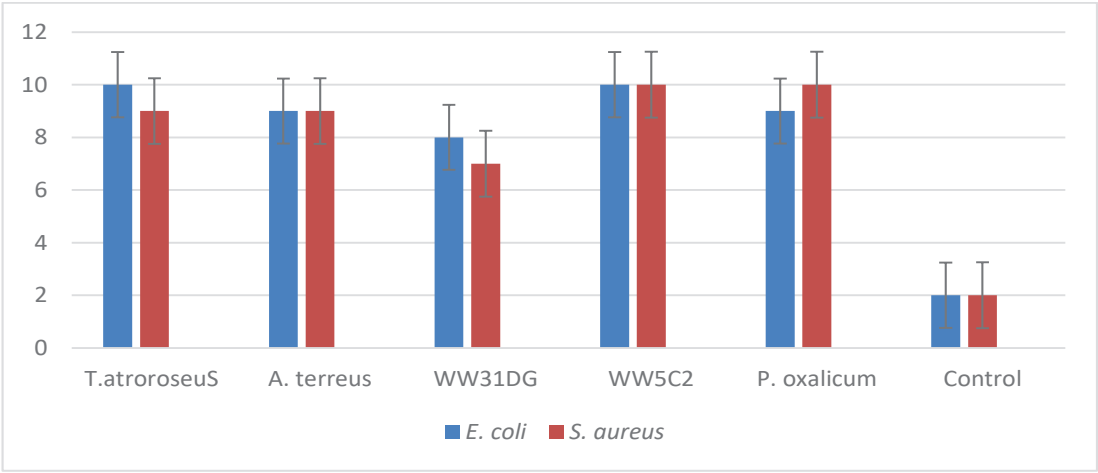

Fig. 9. Result of Zone of Inhibition for Fungal extracted pigments against strain *E. coli* and *S. aureus*

Table 1. Different fungal stains were grown on two different media that is MSM and PDA: They were grown at different temperatures and pH: on solid-state and liquid state fermentation the fungal strains showed pigments under static and non-static states.

S. No.	Fungi	Temperature	pH	Color both in solid and liquid condition	Pigments Production		Media	
					Static Condition	Non-Static condition/Dark	PDB	MSM
1.	*T. atroroseus WW5A3*	25°C	5.5	Pinkish	No	Yes	Yes	Yes
2.	*Aspergillus terreus S10*	30°C	6.5	Yellow	Yes	Yes	Yes	Yes
3.	*P. oxalicum*	25°C	6.5	Greenish Yellow	No	Yes	No	Yes
4.	*WW5C2*	35°C	4.5	Light Yellow	Yes	Yes	Yes	Yes
5.	*WW31DG*	30°C	5.5	Greenish	No	Yes	No	Yes

Table 2. Results of Zone inhibition of fungal extracted pigments against *E. coli* and *S. aureus*

Bacterial Strains	*T. atroroseus* WW5A3	*A. terreus* S10	*P. oxalicum* WW3A4 (DG)	WW5C2	WW31DG	Control
Gram Negative *E. coli*	10	9	8	10	9	2
Gram Positive *S. aureus*	9	9	7	10	10	2

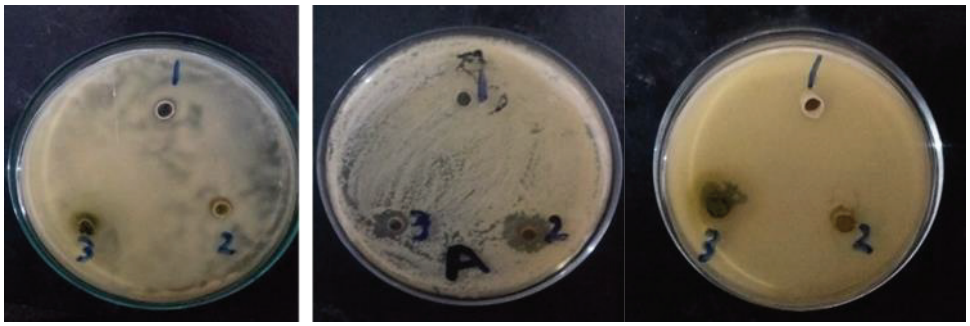

Fig. 10. Results of Antibacterial Activity against *E. coli* by Agar Well Diffusion Method

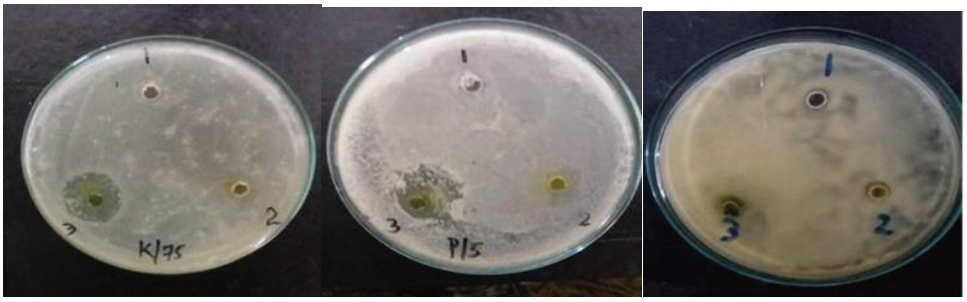

Fig. 11. Results of Antibacterial Activity against S. aureus by Agar Well Diffusion Method

(Fig. 9) and (Table 2). The antibacterial activity against *E. coli* and *S. aureus* is shown in Fig. 10 and Fig.11.

4. DISCUSSION

An effort was made to improve an eco-friendly process for the formation of pigments. This was attained by fungi and its utilization in cotton dyeing. It was observed that *T. atroroteus* and A. terreus showed a bulk of production of pinkish and yellow color pigments under MSM media while *WW31DG* showed good production of greenish-yellow pigment under both media. While *WW5C2* and *WW35A4* showed pale yellow color under MSM media. All these pigments presented a double advantage by giving the bulk of natural pigments that minimize the use of artificial dyes in several industries. Various parameters, such as the pH of the culture medium and temperature, promote the growth of fungal strains, resulting in the good synthesis of natural colors from fungus. The temperature ranges of 25 to 35 °C and the pH range of 4.5 to 5.5 were shown to be optimal for fungus growth and pigment synthesis. For both mycelial development and pigment formation, the pH of the media fluctuates. The optimum pH and temperature of the fungal strain were 4.5-5.5 and 25 ± 5 °C were observed for growth and secondary metabolite production. A pale yellow (537– 540 nm); neutral arrangement, violet (529–536 nm) and acidic arrangement, ruddy (500–507 nm) [28]. The samples were exposed to a wavelength scan from 400 nm to 600 nm to determine the maximum

absorbance of chemicals present in water extraction. The greenish and pinkish color exhibited as a solid absorber inside the UV area, with a typical absorption crest observed around 550 and 600 nm. In addition, the pinkish color showed a strong absorbance in the near UV area, with a prominent peak at nearly 600 nm. However, substantial peaks at 500 and 550 nm were seen in the case of yellow-greenish and light-yellow bio-colorants, respectively. Due to the proximity of a large conjugated framework, which induces absorbance at longer wavelengths, higher absorbance values were observed. Shade broken down within the water is effectively particle pulls in the water particles and make cotton retains water well. Shades broken up within the water are effectively bound and express their tint. The absorbance of *A. terrreus* is 88 % under 500 nm of wavelength, *T. atroroteus* showed 90.36 % of absorbance under 600 nm of wavelength, *WW31DG* showed 81.9 % of absorbance under 550 nm of wavelength, *WW5C2* showed 59.60 % of absorbance under 550 nm of wavelength and *WW35A4* showed 44.04 % of absorbance under 550 nm of wavelength. The results of the study advocated that the fungal extracts were effective against tested human pathogens i.e. gram-negative *Escherichia coli*. The results indicated that fungal pigments were more effective against gram-negative bacterial strain *E. coli* showed a 10 mm zone opposite to *T. atroroteus*, 9 mm against *A. terreus,* 8 mm adverse *WW31DG,* 10 mm *WW5C2*, and 9 mm differing *WW5A4.* Streptomycin served as control which showed 2 mm ± 0 zones of inhibition respectively. Unique organisms were isolated from the samples. These fungal species are safe to use due to all of the safety precautions taken during the generation process. There are only a few ideas about how to use bio-colors from organisms for cotton coloring.

5. CONCLUSION

Fungi are rich in natural pigments hence we can extract natural dyes from them and use them in industries as an alternative to artificial dyes. Natural colors are non-toxic, non-polluting, and less health dangerous. Additionally, their antioxidant and antimicrobial nature encourage includes their positive impacts. Fungal colors have a few focal points over the plant and animal-based colors as organisms are fast in developing colors and have the potential of being standardized commercially. Efforts have been made to synthesize fungal colors to be utilized within the leather and textile industry. The extracted shades can be tried for their better utilization in industries such as cloth or leather dying, makeup, food colorant, and pharmaceuticals industries, etc. Due to its natural pigments, it is considered eco-friendly. Within the show work, efforts have been made to screen and extricate certain color-creating species of organisms and optimize their yielding conditions for the most extreme generation of colors which can be utilized productively in industries.

6. CONFLICT OF INTEREST

The authors declared no conflict of interest

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