Optimization of Dyes Production by Mixed *Aspergillus* and *Paecilomyces* and Its Coloring on Cotton Cloth

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Optimization of Dyes Production by Mixed *Aspergillus* and *Paecilomyces* and Its Coloring on Cotton Cloth

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Abstract. The present study aimed to optimize dyes of mixed *Aspergillus* and *Paecilomyces* and to evaluate dyes of the fungi in coloring cotton cloth. Different initial pH values (3, 5, 7, and 9), temperatures (24, 27, and 30°C), carbon sources (lactose, glucose, and sucrose,) and nitrogen sources (monosodium glutamate, potassium nitrate, sodium nitrate, and yeast extract) of the medium related to dyes and biomass production; and they coloring on cotton cloth were analysed. The optimum culture conditions for the dyes production by the mixed fungi was achieved at pH 9 (4.074 ± 0.0621 UA/L), a temperature of 24°C (4.4145 ± 0.1530 UA/L), with sucrose (4.1503 ± 0.0711 UA/L) as a carbon source, and sodium nitrate (4.0730 ± 0.0459 UA/L) as a nitrogen source, while for the maximum biomass production was obtained at pH 5 (3.7303 ± 0.1432 g/L), a temperature of 30°C (4.2997 ± 0.0372 g/L), with sucrose (2.965 ± 0.5431 g/L) as a carbon source, and monosodium glutamate (4.2697 ± 0.2843 g/L) as a nitrogen source. Culture conditions generated various shades on cotton cloth dyed with the fungal dyes. The intensity of color produced on the dyed cotton cloth by the fungal dyes was in line with the concentration of the dyes.

1. Introduction

The increasing attention over the eventual harmful effects of synthetic dyes on both the consumer and environment has led to preferential interest in natural dyeing alternatives. Natural dyes for fabric dyeing exhibit biodegradable, eco-friendly, and higher compatibility with the environmental than synthetic dyes [1]. Several organisms, such as algae, animals, bacteria, fungi and plants, are capable of synthesizing natural dyes, but filamentous fungi stand out for their potential to produce large amounts of dyes in small spaces [2]. Fungi are also rich in stable colorants such as anthraquinone [3].

In the previous study, greyed-purple dyes produced by mixed *Aspergillus* and *Paecilomyces* succesfully stained cotton cloth [4]. The fungi were isolated from soil collected at the Cibinong Science Center (CSC), Cibinong, they produced dyes at room temperature (27-28 °C) in mineral salt glucose medium. However, the effect of culture parameters on dyes production was unknown.

Tudor et al. [5] informed that the production of dyes by fungi is affected by the type of nutrients such as carbon and nitrogen as well as some environmental factors such as the initial pH and temperature. Several studies reported the optimization of culture conditions for dyes production by fungi. The highest yield of the *Monascus* yellow dyes was obtained at pH 5 [6], while the incubation temperature of 28°C gave the maximum pigmentation in a submerged culture for *Alternaria alternata*, *Curvularia lunata*, and *Trichoderma virens* [3]. When used separately, rhamnose and peptone increased the production of sclerotiorin yield from *Penicillium sclerotiorum* 2AVG [7].

The main aims of this work were (1) to optimize dyes of mixed *Aspergillus* and *Paecilomyces* and (2) to evaluate dyes of the fungi in coloring cotton cloth.

2. Materials and Methods

Chemical materials used in this study include alum ($KAl(SO_4)_2.12H_2O$), $CaCl_2.2H_2O$, $CaCO_3$, $CuSO_4.5H_2O$, $FeSO_4.7H_2O$, glucose, H_3BO_3, KCl, KNO_3, lactose, $MgSO_4.7H_2O$, Monosodium glutamate, $MnSO_4.H_2O$ (monohydrate), NaH_2PO_4, $NaNO_3$, $Na_2MoO_4.2H_2O$, *Potato Dextrose Agar* (PDA), sucrose, yeast extract and $ZnSO_4.7H_2O$ (all these substances had analytical grade and were obtained by Sigma-Aldrich, USA), while other materials used were beaker glass, cotton cloth, Erlenmeyer, measuring cup, muslin cloth, Petri dish, stove, and test tube.

2.1. Inoculation preparation

Mixed *Aspergillus* and *Paecilomyces* was used for this study. The mixed fungi was inoculated in Petri dishes containing PDA medium and incubated at room temperature (27°C) for 5 days [4]. With a straw of pop ice (10 mm), it was then printed for further assay.

2.2. Effect of carbon and nitrogen sources on growth and dyes production

To evaluate the effect of carbon and nitrogen sources on growth and dyes production, the mineral salt glucose medium [8] was prepared with modified carbon and nitrogen sources by inoculating five mycelial prints of the mixed fungi into Erlenmeyer flask containing the medium. The carbohydrates evaluated were glucose (as control of a carbon source), lactose and sucrose, while the nitrogen sources analysed were KNO_3, monosodium glutamate, $NaNO_3$ (as control of a nitrogen source) and yeast extract. The Erlenmeyers containing the fungi were incubated at room temperature (27-28^o C) in static conditions for 4 weeks. The experiments were performed in triplicate.

2.3. Effect of temperature and pH on growth and dyes production

The effect of culture conditions like different temperatures (24, 27,and 30^{o}C) and initial pH values (3, 5, 7, and 9) on growth and dyes production was studied separately by inoculating five mycelial prints of the mixed fungi into Erlenmeyer flask containing mineral salt sucrose medium ($NaNO_3$ as a nitrogen source and sucrose as a carbon source) due to its potentiality as the best medium for dyes production. The Erlenmeyers containing the mixed fungi were incubated at room temperature (27-28^o C) in stationary cultures for 4 weeks. The experiments were conducted in triplicate. At the end of the incubation period for pH treatment, dyes were measured for pH values.

2.4. Biomass estimation

The medium for culturing of the mixed fungi was centrifuged at 8,500 rpm for 20 min and the supernatant fluid was filtered through a muslin cloth [9 modified]. The mycelia biomass yield was estimated by washing with deionized water and dried at 60°C for 48 h [6 with modification]. The biomass concentration was expressed as mycelia dry weight per unit volume of culture medium.

2.5. Extracellular dyes

The dyes were quantified in a spectrophotometer (Shimadzu). The concentration of extracellular dyes was estimated by measuring the absorbance of filtrates at 530 nm [4]. The dyes produced by the mixed fungi were expressed in optical density units (UA530).

2.6. Coloring of cotton cloth

Cotton cloth samples (4 cm x 4 cm or 0.127 g) were treated with filtrate of the fungi with ratio of material to liquor is 1 : 30 w/v [4]. Dyeing process was carried out at 90^{o}C for 30 minutes and left over night [10]. After dyeing process, the dyed cotton cloths was rinsed with water and dried at room temperature. The color that appears on the cotton cloth was analysed using the RHS color chart [11].

3. Results and Discussion

3.1. Effect of carbon sources on the dyes production and growth of mycelium

Ogbonna [12] informed that organic carbon sources are the main sources of carbon and energy for growth and dyes production by fungi. The effect of different carbon sources at a concentration of 20 g/L on cell growth and dyes production was investigated in flask cultures. As shown in Figure 1 and 2, the mixed fungi cells used almost all of the substrates tested as carbon sources for cell growth and dyes production. Among these, sucrose proved to be better than other carbon sources in terms of both the growth (2.965 ± 0.5431 g/L) and dyes production (4.1503 ± 0.0711 UA/L). The carbon source of lactose gave lower yield than sucrose and glucose. It is estimated that the fungi are easier to perform the metabolism of sucrose than any other carbon sources tested in this study. The ease of using sucrose as a carbon source allows for the growth and formation of cell biomass from the fungi so that the cell can optimally excrete the dyes. The result is in agreement with Velmurugan et al. [13] who reported the production of dyes and biomass by *Isaria farinose*; and Bhattacharyya and Jha [14] for the optimum growth and production of active metabolites by *Aspergillus* strain TSF 146. In other researches, however, glucose (as control of carbon source) is a superior substrate proved to be better than other carbon sources in terms of both the growth and dyes production by *Monascus purpureus* [15] and *Aspergillus terreus* KMBF1501 [16].

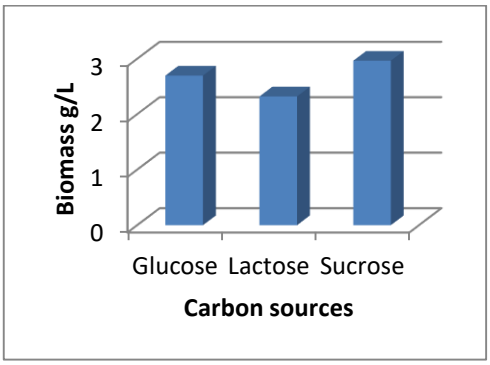

Figure 1. Effect of carbon sources on the biomass production of mixed *Aspergillus* and *Paecilomyces*

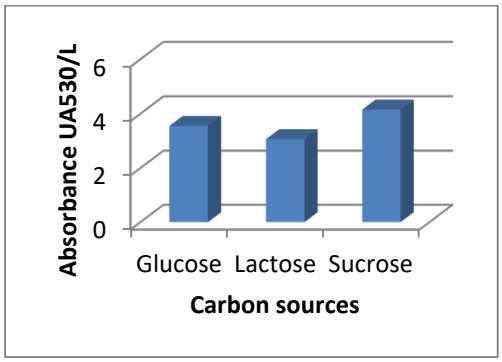

Figure 2. Effect of carbon sources on the absorbance of mixed *Aspergillus* and *Paecilomyces* dyes

3.2. Effect of nitrogen sources on the dyes production and growth of mycelium

Figure 3 and 4 show the growth and dyes production of the mixed fungi in the liquid medium with various nitrogen sources. Of the four nitrogen sources examined in this study, $NaNO_3$ (as control of a nitrogen source) gave the highest dyes production of 4.073 ± 0.0459 UA/L, while the maximum mycelia growth (4.2697 ± 0.2843 g/L) was obtained in monosodium glutamate. The result is in accordance with Akilandeswari and Pradeep [16] who informed that $NaNO_3$ favors the production of dyes and biomass by *Aspergillus terreus* KMBF1501; and Dey et al. [9] for the dyes production of *Pezicula* sp. BDF9/1. Other researches, however, informed that the most suitable nitrogen source was yeast extract for the production of natural dyes of yellow, orange, and red in the submerged culture of *Talaromyces purpurogenus* (formerly *Penicillium purpurogenum*) [17]; tryptone for the dyes production of *M. purpureus* [15]; and glysine for the mycelium growth of *Pezicula* sp. BDF9/1 [9]. A nitrogen source is required by various species of fungi for the growth and synthesis of both primary and secondary metabolites [12]. Nitrogen can be available as monosodium glutamate, yeast extract, nitrate, or in organic components, such as amino acids and proteins. The removal of nitrogen in the growth medium greatly influences the growth of fungi.

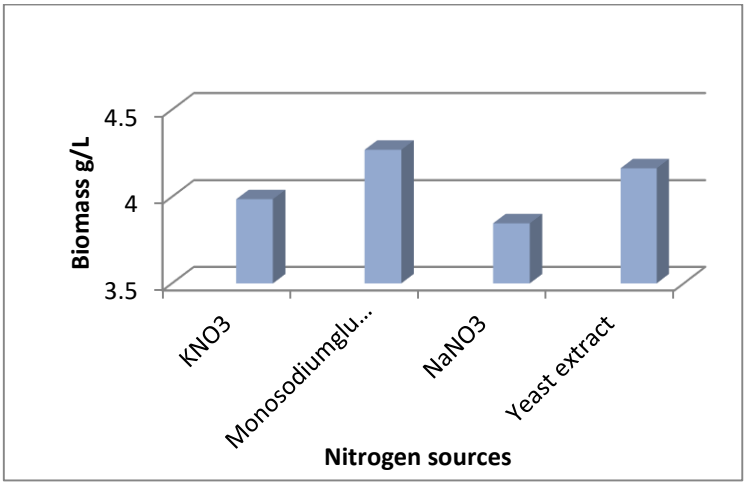

Figure 3. Effect of nitrogen sources on the biomass production of mixed *Aspergillus* and *Paecilomyces*

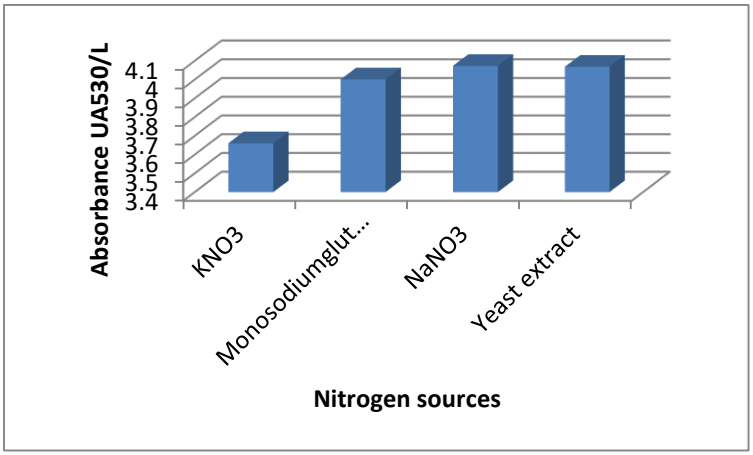

Figure 4. Effect of nitrogen sources on the absorbance of mixed *Aspergillus* and *Paecilomyces* dyes

3.3. Effect of temperature on the dyes production and growth of mycelium

To test the optimal temperature for biomass and dyes production, the mixed fungi was cultivated in mineral salt sucrose medium (sucrose as a carbon source and $NaNO_3$ as a nitrogen source) at various temperatures (24, 27, and 30°C). Of all the temperatures tested, a temperature of 30ºC produced the highest biomass production (4.2997 ± 0.0372 g/L), while the maximum dyes production (4.4145 ± 0.1530 UA/L) was achieved at 24ºC (Figure 5 & 6). Temperature is another important factor as it influences the metabolic activity of fungi and subsequently, their growth. The result is similar to Gunasekaran and Poorniammal finding[18] who reported that a temperature of 30ºC favors for biomass production of *Penicillium* sp. Other research findings showed that at 25°C, *Fusarium solani* LCPANCF01 gave the maximum growth and bioactive metabolite production [19]. Generally, it is known that temperature affects the membrane fluidity so it will affect the uptake of nutrients and excretion of products by microorganisms [12].

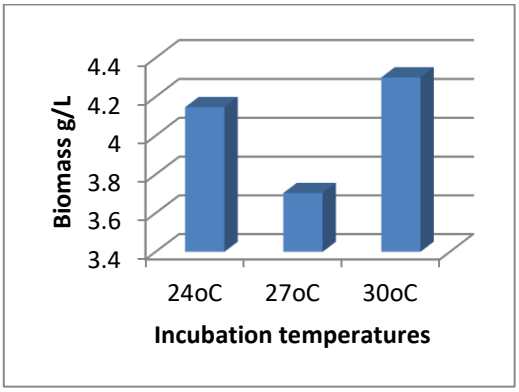

Figure 5. Effect of incubation temperatures on the biomass production of mixed *Aspergillus* and *Paecilomyces*

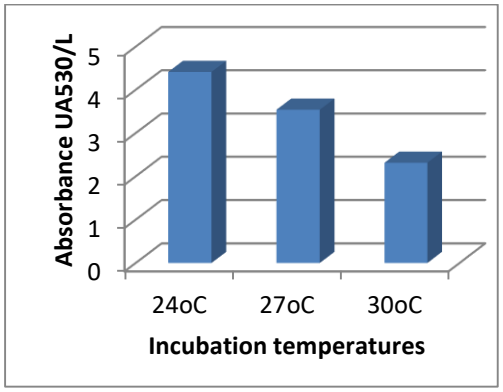

Figure 6. Effect of incubation temperatures on the absorbance of mixed *Aspergillus* and *Paecilomyces* dyes

3.4. Effect of pH on the dyes production and growth of mycelium

The mixed fungi was cultivated at different initial pH values (3, 5, 7, and 9) in mineral salt sucrose medium (sucrose as a carbon source and $NaNO_3$ as a nitrogen source). The results indicated that biomass and dyes production were affected by initial pH of the medium (Table 1). The highest biomass production (3.7303 ± 0.1432 g/L) was observed at pH 5, while the maximum dye production (4.0740 ± 0.0621 UA/L) was achieved when initial pH of culture medium set at pH 9. The result is in accordance with Dey et al. [9] who reported the production of biomass by *Pezicula* sp. BDF 9/1; and Gunasekaran and Poorniammal [18] on the dyes production by *Penicillium* sp. Ogbonna [12] informed that the pH of growth medium affects most aspects of the production process such as the cellular metabolism and nutrient absorption and utilization by the organism. No growth and dyes production were observed at pH 3. The pH of culture medium is one of the determining factor for the metabolism and hence for the biosynthesis of secondary metabolites [19]. PH 3 may interfere with cell membrane function, cell morphology and structure, salt solubility, substrate ion state, absorption of various nutrients so that growth and biosynthesis of the product is not formed [9]. PH for the medium in this study before inoculation was 5, 7, and 9 respectively, however pH before dyeing (after culturing) for the fungi became 7.89, 6.15, and 7.46 respectively. Tudor et al. [5] reported that fungi tend to alter the pH on the medium to create their own favorable condition by selective uptake and exchange of ions.

Table 1. Effect of initial pH on the biomass and absorbance of mixed *Aspergillus* and *Paecilomyces* dyes

No	Initial pH	Final pH	OD/L	Biomass (g/L)
1.	3	-	-	-
2.	5	7.89	3.8483 ± 0.0384	3.7303 ± 0.1432
3.	7	6.15	3.1862 ± 0,0889	3.5893 ± 0.1539
4.	9	7.46	4.074 ± 0.0621	3.6193 ± 0.1268

3.5. Effect of culture conditions on cotton cloth colored by the mixed fungal dyes

Culture conditions generated various shades on cotton cloth dyed with the fungal dyes (Table 2 & Figure 7). Carbon sources (glucose, lactose, and sucrose), nitrogen sources (KNO_3, monosodium glutamate, $NaNO_3$, and yeast extract), temperatures (24°C, 27°C, and 30°C), and initial pH values (5, 7, and 9) can

stain cotton cloth from white into greyed-orange, greyed-purple, purple, red-purple, violet, and yellow-orange. Colors formed on the cotton cloth added a color variation on textile dyeing.

The intensity of color produced on the dyed cotton cloth using the fungal dyes with carbon sources of glucose (violet 84 B) and sucrose (greyed-purple 186 C) was found brighter than that obtained with lactose (purple 76 C) (Table 2 & Figure 7). This result is in accordance with the result of dyes production. The dyes production with glucose (3.5533 ± 0.0675 UA/L) and sucrose (4.1503 ± 0.0711 UA/L) respectively were higher than the dyes production with lactose (3.0655 ± 0.1138 UA/L) (Figure 2). Nitrogen sources also affected the color intensity produced on the dyed cotton cloth using the fungal dyes. The intensity of color produced on the dyed cotton cloth using the fungal dyes with $NaNO_3$ (violet 84 B), yeast extract (yellow-orange 20 C), and monosodium glutamate (red-purple 70 C) as nitrogen sources was found brighter than that obtained with KNO_3 (violet 88 D) (Table 2 & Figure 7). This result is in agreement with the result of dyes production. The dyes production with $NaNO_3$ (4.0730 ± 0.0459 UA/L), yeast extract (4.0683 ± 0.0951 UA/L), and monosodium glutamate (4.001 ± 0.1400 UA/L) respectively were higher than the dye production with KNO_3 (3.6605 ± 0.0148) (Figure 4).

The color intensity produced on the dyed cotton cloth using the fungal dyes at incubation temperatures of 24ºC (greyed-orange 177 D) and 27ºC (violet 84 C) was found brighter than that obtained at 30ºC (purple 76 C) (Table 2 & Figure 7). This result is in accordance with the result of dyes production. The dyes production at 24ºC (4.4145 ± 0.1530 UA/L) and 27ºC (3.54 ± 0.0113 UA/L) respectively were higher than the dyes production at 30ºC (2.3133 ± 0.0376) (Figure 6). Initial pH also influenced the intensity of color produced on the dyed cotton cloth using the fungal dyes. The color intensity produced on the dyed cotton cloth using the fungal dyes at pH 9 (greyed-purple 186 C) as initial pH value was found brighter than that obtained at pH 5 (violet 88 D) and pH 7 (greyed-orange 177 D) respectively (Table 2 & Figure 7). This result is in agreement with the result of dyes production. The dyes production at pH 9 (4.074 ± 0.0621 UA/L) was higher than the dyes production at pH 5 (3.8483 ± 0.0384 UA/L) and pH 7 (3.1862 ± 0.0889) respectively (Table 1).

Table 2. Effect of culture conditions on cotton cloth color dyed with mixed *Aspergillus* and *Paecilomyces* dyes

Culture condition	Dyed cotton cloth color			
Carbon sources	Glucose	Lactose		Sucrose
	Violet 84 B	Purple 76 C		Greyed-purple 186 C
Nitrogen sources	KNO_3	Monosodium glutamate	$NaNO_3$	Yeast extrct
	Violet 88 D	Red-purple 70 C	Violet 84 B	Yellow-orange 20 C
Incubation temperatures	24ºC	27ºC		30ºC
	Greyed-orange 177 D	Violet 84 C		Purple 76 C
Initial pH values	5	7		9
	Violet 88 D	Greyed-orange 177 D		Greyed-purple 186 C

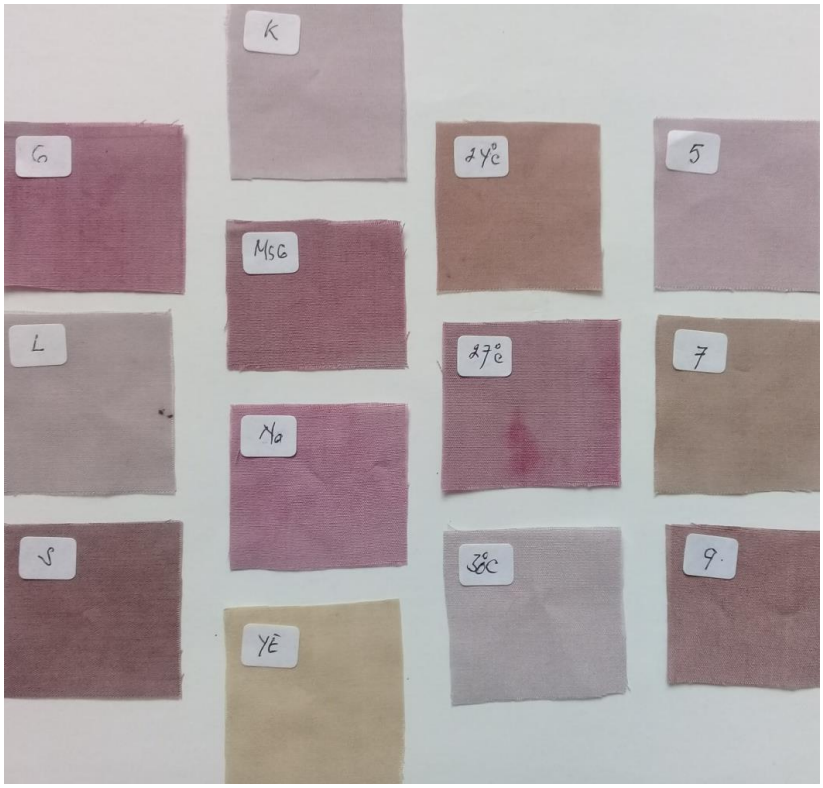

Figure 7. Dyed cotton cloth by mixed *Aspergillus* and *Paecilomyces* dyes produced with different culture conditions. G = glucose, L = lactose, & S = sucrose; K = KNO_3, MSG = monosodium glutamate, Na = $NaNO_3$, & YE = yeast extract; 24ºC, 27ºC, & 30ºC = incubation temperatures; 5, 7, & 9 = initial pH values

4. Conclusion

Optimization of initial pH of the medium, incubation temperature, carbon and nitrogen sources greatly influenced the dyes and biomass production by mixed *Aspergillus* and *Paecilomyces*. Among the evaluated conditions, the highest dyes production was obtained at pH 9, 24°C, $NaNO_3$ and sucrose, while the maximal biomass production was achieved at pH 5, 30°C, monosodium glutamate, and sucrose. The intensity of color on the dyed cotton cloth using the fungal dyes with glucose and sucrose as carbon sources was brighter than that obtained with lactose. The color intensity on the dyed cotton cloth using the fungal dyes with $NaNO_3$, yeast extract, and monosodium glutamate as nitrogen sources was brighter than that achieved with KNO_3. The intensity of color on the dyed cotton cloth using the fungal dyes at 24ºC and 27ºC as incubation temperatures was brighter than that obtained at 30ºC. The color intensity on the dyed cotton cloth using the fungal dyes at initial pH of 9 was brighter than that achieved for pH 5 and 7.

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