

Coloring Properties Assessment of Dyes Produced by Mixed *Aspergillus* and *Paecilomyces*

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Abstract. 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 dyed 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 0.0023 g without mordant and the color was 82A purple-violet, while alum gave the optimum mass of the fungal dye adsorbed of 0.0013 g, 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 using the fungal dye without mordant was 0.0036 g and the color was 88A violet, while alum gave the fungal dye reduced of 0.0018 g, which with 88C violet color. The obtained result showed dyes produced by mixed fungi could dye cotton cloth and resistant to detergent solution.

1. Introduction

The increasing awareness of health and pollution hazards of chemical dye stuffs has led to a resurgence of interest in natural dyes. Natural dyes are derived from plants, animals, and microbes. Microbes can be used as an alternative source of natural dyes to develop eco-friendly dye production technologies for textile industries. Microbial dyes have meaningful advantages over animals and plants dyes. These proliferate very fast and are capable of growing in the cheap culture medium, independence from weather conditions and colors of different shades [1].

Fungi are reported to be potential color producer [2]. Various natural dyes from fungi were reported. These include yellow dyes by *Aspergillus sydowii* (CML2967), two isolates of *A. aureolatus* (CML2964 and E.4.1), two isolates of *A. keveii* (CML2968 and ONI75), three isolates of *Penicillium flavigenum* (CML2965; E.2.7 and 3.1.a) and *P. chermesinum* (CML2966) [3]; and red dyes by *Paecilomyces farinosus* (*Isaria farinosa*) [4]. Previous studies confirmed that fungal dye exhibit non-toxic and biodegradability [5].

Natural dyes, however, still face a huge disadvantage against synthetic dyes. Dyeing of textile with natural dyes usually involves issues of limited shade range and lower fastness properties of the dyed



cloths. These problems have been overcome applying a pretreatment to the textile with mordants. Mordants help in the binding of dyes to the cloth by forming a chemical bridge from dye to fiber thus improving the staining ability of a dye with increasing its fastness properties [6]. Selection of mordants is important in natural dyeing processes. Shahid et al. [7] informed that mordants can increase the depth of shade or drastically alter the final color of the dyed cloth.

The present study aimed to evaluate the potential application of the dyes produced by mixed *Aspergillus* and *Paecilomyces* as natural dyes in the coloring of cotton cloth.

2. Materials and Methods

Chemical materials used in this study includes alum ($\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, CaCO_3 , $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, glucose, H_3BO_3 , KCl , $\text{K}_2\text{Cr}_2\text{O}_7$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (monohydrate), NaH_2PO_4 , NaNO_3 , $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, *Potato Dextrose Agar* (PDA) and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, while other materials used are beaker glass, cotton cloth, detergent, Erlenmeyer, measuring cup, muslin cloth, Petri dish, stove and test tube. *Aspergillus* and *Paecilomyces* were obtained from the Laboratory of Environmental Microbiology, Research Center for Biology, Indonesian Institute of Sciences (LIPI). The research was conducted in the Laboratory of Environmental Microbiology, Research Center for Biology, LIPI, Cibinong.

2.1. Inoculation process

Mixed fungi consisting of *Aspergillus* and *Paecilomyces* was used for this study. The mixed fungi were grown in Petri dishes containing PDA medium and incubated at room temperature (27-28°C) for 5 days. It was then printed with a straw of pop ice (10 mm) for further inoculation. Five mycelial prints of the mixed fungi [8] were inoculated into Erlenmeyer flask containing mineral salts-glucose medium [9] and then incubated at room temperature (27-28°C) in stationary cultures for 4 weeks. After the incubation period, the mycelium was harvested and the dye was filtered with a muslin cloth for further assay.

2.2. Coloring process

Before dyeing, cotton cloth was washed with detergent, rinsed with water and then dried [8]. The cotton cloth mordanting process was carried out using pre-mordanting technique [8]. To assess the most appropriate mordant for the fungal dye, six different mordants such as CaCO_3 , $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$, $\text{K}_2\text{Cr}_2\text{O}_7$, and $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ were tested. Samples of cotton cloth (4 cm × 4 cm or 0.24 g) were treated with or without pre-mordanting using 1.2% of each mordant with a ratio of 1: 30 (material: liquor). The mordanting process was carried out at 90° C for 30 minutes. The samples were then squeezed and air dried at room temperature. Mordant which produces the best color on the cotton cloth was used for further research. As a control treatment, cotton cloth with code A was weighed and the base color recorded. Cotton cloth was then dipped in the fungal dye solution for 30 minutes at 90° C, lifted and dried under the sun. The dried cotton cloth was weighed again and the color recorded. Finally, the resistance of the cotton cloth was tested by soaking it in detergent 1% solution for 15 minutes, removed, dried and weighed again. As a pre-mordanting treatment, cotton cloth with code B was soaked in mordant alum solution for 30 minutes at 90° C, removed, dried and weighed until the mass was constant. The dried cotton cloth was treated like for coloring without mordant (control treatment). The color of cotton cloth after dyeing from each treatment was determined with the RHS color chart [10]. Each treatment was done with three repetitions.

2.3. Dye uptake by cotton cloth

Dye uptake by cotton cloth was measured both with the adsorption and percentage absorption of dyes by cotton cloths. The adsorption of dye on cotton cloth can be determined by determining the mass of cotton cloth before and after staining [11], while percentage absorption of dye by cotton cloth was calculated by measuring the optical density of the dye solution samples at a wavelength of 530 nm [12 modified]. Percentage absorption of dye (%) was calculated using the following equation:

$$\frac{\text{OD before dyeing} - \text{OD after dyeing}}{\text{OD before dyeing}} \times 100 \quad (1)$$

3. Results and Discussion

Mixed *Aspergillus* and *Paecilomyces* in the present study produced pigmentation in mineral salts glucose medium. The dye color produced by the fungi was 187 A greyed-purple (**Figure 1**). Several studies of dyes produced by fungi reported that *Aspergillus niger* produced brown dyes [13]; yellow dyes were produced by *A. terreus* [14], *A. calidoustus* 4BV13 [15], *A. versicolor* [16] and *Aspergillus* sp. [17]; *Aspergillus* sp. produced green dyes [18]; *Eurotium* spp. (asexual morph: *Aspergillus*) produced yellow and red dyes [19]; some strains of *Aspergillus* (*A. glaucus* and *A. repens*) produced yellow and red dyes [20]; *E. nidulans* (asexual morph: *Aspergillus*) produced reddish brown dyes [4]; and red dyes by *Paecilomyces sinclairii* [21]. Pigments or dyes are generally produced in the cell cytoplasm as a response to disadvantageous environmental conditions, such as nutrient limitation and this process is controlled by a complex regulatory network [22].

PH for the medium in this study before inoculation was 6, however, pH before dyeing (after culturing) for the fungi became 5.7. Hence the dye liquor became more acidic in nature. This result is in accordance with the dyes produced by *Trichoderma* [12]. It has been reported that many kinds of fungi have more acidic pH optima during submerged culture for dyes production [23].

Mordants application of natural dyes produced by the fungi generated various shades of cotton cloth (**Table 1**). The fungal dye can stain cotton cloth from 155 D white into 88A & C violet, 87 C violet, and 201 A, B & D grey. Satyanarayana and Chandra [24] informed that this is presumably due to the influence of the chemical content available on mordant materials, namely the existence of Al^{3+} from $\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$, Ca^{2+} from CaCO_3 , Cu^{2+} from $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, Fe^{2+} from $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, K^+ from $\text{K}_2\text{Cr}_2\text{O}_7$ and Mn^{2+} from $\text{MnSO}_4 \cdot \text{H}_2\text{O}$. Mordants play a very important role in imparting color to the cotton cloth in the form of the metal complex formation. Previous studies found that *A. niger* produced a reddish brown color on wool [13] and yellow colors on silk, cotton, and silk-cotton [25]; and *Aspergillus* sp. produced green colors on cotton and silk [18]. The fungi can then be used as the source of natural dyes for various purpose, viz. dyeing of yarns and dyeing of cloths [26].

The intensity of color produced on the dyed cotton cloth using the fungal dye without mordant (88 C violet) and with mordant alum (87 C violet) was found brighter than that obtained for other mordants, so mordant alum used for further research (**Table 1**). The dyed cotton cloth using the fungal dye without mordant was brighter than the dyed cotton cloth using the fungal dye added with mordant alum. Satyanarayana and Chandra [24] informed that this is presumably due to the influence of Al^{3+} from $\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$.

Table 1. The color variations of cotton cloth dyed with the fungal dye using different mordants

Mordant	Cotton cloth color
Without mordant	88A violet
CaCO_3	87 C violet
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	201 B grey
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	201 A grey
$\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	88C violet
$\text{K}_2\text{Cr}_2\text{O}_7$	201 D grey
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	201 A grey



Figure 1. Fungal dyes. Mineral salts glucose medium (right) and mixed *Aspergillus* and *Paecilomyces* (left)

The magnitude of dyes adsorption and absorption by cotton cloth treated without mordant can be seen in **Table 2**, **Table 3** and **Figure 2**. The mass of dyestuff adsorbed on cotton cloth without mordant was 0.0023 ± 0.0016 g (**Table 2**) and percentage dye absorption by cotton cloth without mordant was 9.6276 ± 0.2524 % (**Table 3**). The fungal dye was able to change the cotton cloth color from 155 D white to 82A purple-violet. The adsorption and absorption processes are possible because the OH group of cellulose present in the cotton cloth fiber is capable of forming hydrogen bonds with OH or other polar groups of the dyestuff. This bond is generally easy to break because it is not so strong [11]. Therefore, to clarify the color of the cotton cloth and to strengthen the bond between the dye and the cotton cloth it is necessary to add mordant in the staining process.

Table 2. Adsorption of the fungal dye on cotton cloth without and with mordant alum (n = 3)

Code	Cotton cloth mass before dyeing (g)	Cotton cloth mass after dyeing (g)	Average dye adsorption(g)	Cotton cloth color
A	0.2234	0.2257	0.0023 ± 0.0016	82A purple-violet
	Cotton cloth mass + mordant before dyeing (g)	Cotton cloth mass + mordant after dyeing (g)	0.0013 ± 0.0006	
B	0.2096	0.2109		82C Purple-violet

Table 3. Absorption of the fungal dye by cotton cloth without and with mordant alum (n=3)

Code	Treatment	OD before dyeing	OD after dyeing	% Average dye absorption
A	Without mordant	4.8565	4.388933	9.6276 ± 0.2524
B	Mordant alum	4.8565	4.68133	3.6068 ± 0.2062

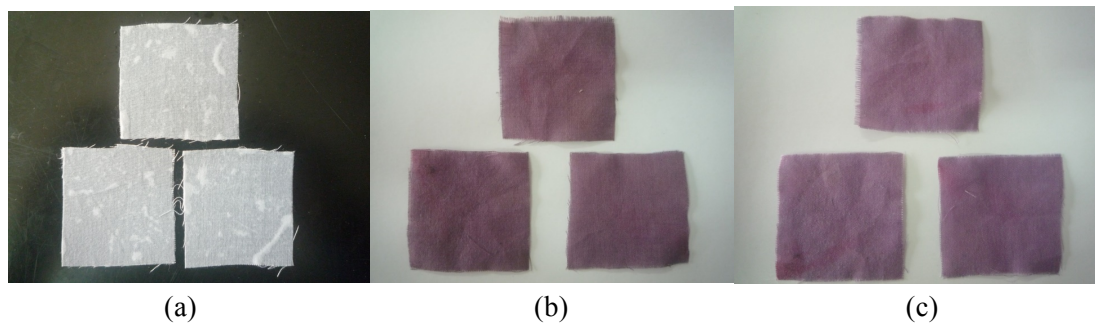


Figure 2. Dyed cotton cloth using the fungal dye without mordant alum. Cotton cloth before dyeing (a); cotton cloth dyed with the fungal dye without mordant alum (b) and cotton cloth after soaked with detergent 1% (c)

Dyed cotton cloth using the fungal dye added with mordant alum can be seen in **Figure 3**. The fungal dye was able to change the cotton cloth color from 155D white to 82C purple-violet. Cotton cloth dyed using the fungal dye added with mordant alum gave more faded color (82C purple-violet) than cotton cloth without mordant alum (82A purple-violet) (**Figure 2** and **3**). This is presumably due to the influence of the chemical content available on the mordant material (the existence of Al^{3+} from $\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$). This result is also in accordance with the result of calculating the dyestuff mass adsorbed on cotton cloth, and the result of percentage dyestuff absorption by cotton cloth. The mass of dyestuff adsorbed on cotton cloth with mordant alum (0.0013 ± 0.0006 g) (**Table 2**) was smaller than the mass of dyestuff adsorbed on cotton cloth without mordant alum (0.0023 ± 0.0016) (**Table 2**). Percentage absorption of the dye by cotton cloth treated with mordant alum (3.6068 ± 0.2062 %) (**Table 3**) was also smaller than percentage absorption of the dye by cotton cloth without mordant alum (9.6276 ± 0.2524 %) (**Table 3**). The results indicated that mordant has not provided more dye sites and bears high color depth of shades as compared to those without mordant so that the dyed cotton cloth using the fungal dye does not require the addition of mordant.

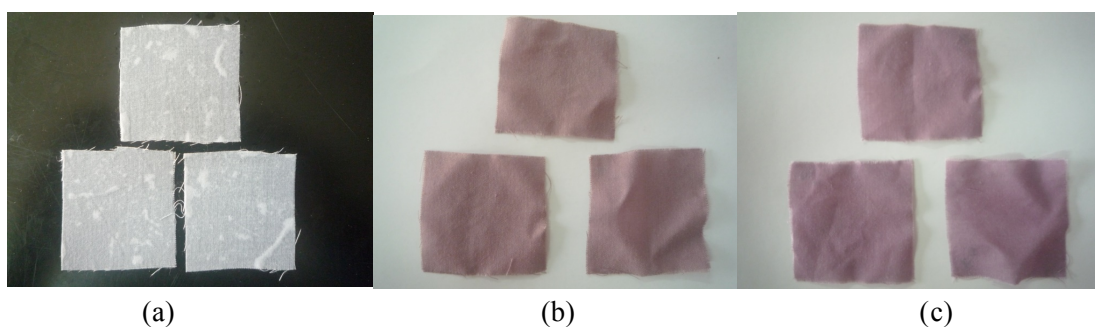


Figure 3. Cotton cloth dyed using the fungal dye added with mordant alum. Cotton cloth before dyeing (a); cotton cloth dyed using the fungal dye added with mordant alum (b) and cotton cloth after soaked with detergent 1% (c)

The mechanism of binding on the dyed cotton cloth in the presence of mordant is described in **Figure 4**. The ability of both the dyestuff adsorption and the dyestuff absorption in each cotton cloth in the presence of mordant have to be increased due to functional group as hydroxy on the cotton cloth can occupy the unoccupied sites on metal ion interaction with the dye so that a ternary complex is formed by the metal salt on which one site is with the cotton cloth fiber and the other site is with the dye [27]. In this study, however, both the dyestuff adsorption and the dyestuff absorption by cotton cloths treated with mordant alum was smaller than both the dyestuff adsorption and the dyestuff absorption by cotton cloths without mordant alum (**Table 2&3**). The dyed cotton cloth color using the

fungus dye with mordant alum was also more faded than the dyed cotton cloth color using the fungus dye without mordant alum (**Figure 2&3**). The results indicated that mordant has not provided more dye sites and bears high color depth of shades as compared to those without mordant so that the dyed cotton cloth using the fungus dye does not require the addition of mordant.

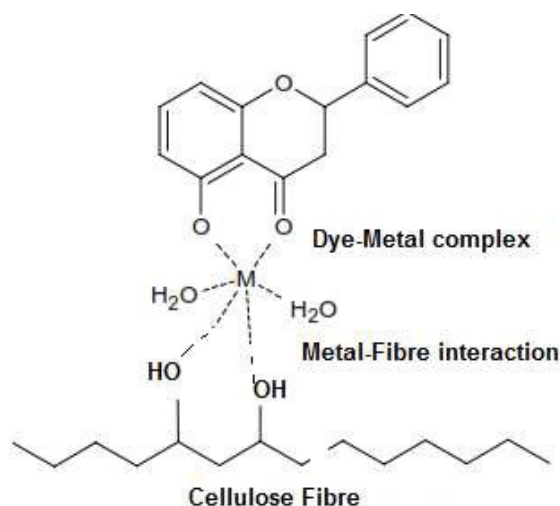


Figure 4. Schematic representation of dye-metal-cellulose fiber interaction (from Kechi et al. [28])

The reduction of average mass of the dyed cotton cloth with the fungus dye without mordant alum after soaking in a detergent solution (0.0036 ± 0.0007) was greater than with mordant alum (0.0018 ± 0.0005) (**Table 4**). Similarly, water OD after the immersion of the dyed cotton cloth using the fungus dye without mordant alum (0.2240 ± 0.0283) was greater than water OD with mordant alum (0.10955 ± 0.0279) (**Table 5**). These results are in accordance with the result of discoloration of detergent 1% solution after immersion test. Detergent solution color after soaking of the dyed cotton cloth using the fungus dye with mordant alum gave more faded than detergent solution color after soaking of the dyed cotton cloth with the fungus dye without mordant alum (**Figure 5**). The results indicated that addition of mordant alum 1.2 % on the dyed cotton cloth using the fungus dyeable to strengthen the bonding of the fungus dye to the cloth. Manurung [11] informed that the OH-group of cellulose present in the dyed cotton cloth fibers using the fungus dye with mordant alum capable of forming covalent bonds with metal ions from mordant as bridges with OH or other polar groups of dyes, so that the adsorbed and absorbed dyes from the cotton cloths were not easily removed or lost. Mordants play a very important role in imparting color to the cotton cloth in the form of metal complex formation or the metal ions of mordants act as electron acceptors from groups of dye donating an electron to form coordination bonds with the dye molecule, making them insoluble in water [27]. Kamel et al. [29] also reported that color strength is dependent on the metal salt used.

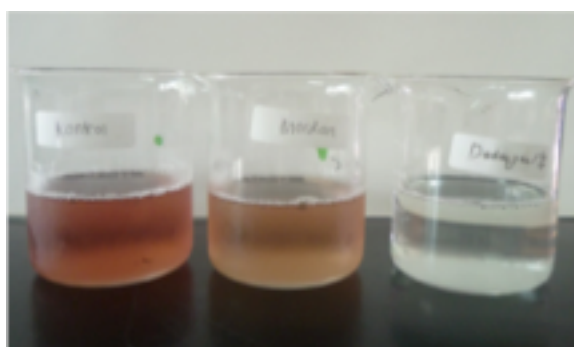
Although the reduction of average mass; and water OD after the immersion on detergent solution of the dyed cotton cloth using the fungus dye without mordant was greater than with mordant alum, but the dyed cotton cloth color using the fungus dye without mordant alum was still brighter than the dyed cotton cloth added with mordant alum (**Figure 2&3**). This result indicated that the dyed cotton cloth using the fungus dye does not require the addition of mordant. Unless for color fastness to washing, however, the results have not been tested for color fastness to perspiration; and color fastness to light. Furthermore, the fungus dye will be further tested for color fastness to perspiration and to light.

Table 4. The result of immersion test with detergent 1% to the color resistance of cotton cloths dyed with the fungal dye (n = 3)

Code	Method	Mass of cotton cloth after staining (g)	Mass of cotton cloth after detergent 1% test (g)	The reduced average cotton cloth mass (g)	Cotton cloth color
A	Without mordant	0.2257	0.2221	0.0036±0.0007	88A violet
B	Mordant alum	0.2109	0.2091	0.0018±0.0005	88C violet

Table 5. The value of water OD after dyeing of the cotton cloth stained with the fungal dye (n=3)

No	Treatment	Water OD
1.	Detergent solution 1%+ the dyed cotton cloth using the fungal dye without mordant (A)	0.224 ± 0.0283
2.	Detergent solution 1%+ the dyed cotton cloth using the fungal dye with mordant alum (B)	0.1096 ± 0.0279

**Figure 5.** Discoloration of detergent 1% solution after immersion test. Detergent solution color after soaking of the dyed cotton cloth using the fungal dye without mordant (left), detergent solution color after soaking of the dyed cotton cloth with the fungal dye added with mordant alum (middle) and detergent 1% solution colour (right)

4. Conclusion

This study showed that mixed *Aspergillus* and *Paecilomyces* produced natural dyes that can be used to dye textile. Different mordants produced different shades of color on cotton cloth. Addition of mordant alum 1.2% on the dyed cotton cloth using the fungal dyeable to strengthen the bonding of the fungal dye to the cotton cloth. Cotton cloth dyed using the fungal dye does not require the addition of mordant.

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