

Evaluation of soil fungi producing dyes for dyeing cloth with mordanting *alum* and copper sulphate

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Abstract

Isolation of soil fungi was done to find an environmental friendly dyes for dyeing cloth. The aims of this study include isolation and identification of soil fungi from burning waste, cassava rhizosphere, and compost in Cibinong Science Center Area; and evaluation of dyes potential produced by the soil fungi in combination with *alum* and copper sulphate mordants. Thirty-six isolates of the soil fungi were successfully isolated from the burning waste, cassava rhizosphere, and compost. These include *Aspergillus*, *Colletotrichum*, *Emericella*, *Eurotium*, *Fusarium*, *Mucor*, *Paecilomyces*, *Penicillium*, *Rhizopus*, and *Trichoderma*. The soil fungi were dominated by asexual morph (77.78%) of which *Aspergillus* (3 species and 15 isolates) was found as the most common genus from the three samples. A total of 14 isolates were found potential as dye-producing fungi. These include *Aspergillus* sp. strain R-2 (1 isolate), *A. fumigatus* (3 isolates), *Penicillium* sp. strain GRC-1 (5 isolates), *Penicillium* sp. strain R-3 (1 isolate), *Penicillium* sp. strain RC-1 (3 isolates), *Trichoderma harzianum* (1 isolate); and *Monascus purpureus* (1 isolate; as control). Color intensity produced by fungal dyes in combination with *alum* and copper sulphate as pre-mordanting agents is higher than *alum* and copper sulphate as post-mordanting agents.

Keywords: *alum*, copper sulphate, dye, mordant, soil fungi

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Introduction

Rapid development of textiles industry has caused high environmental pollution due to high use of synthetic dye. Therefore, natural dye obtained from various organisms is promising solution to replace utilization of the synthetic dye. Natural materials, such as plants and animals have long been used as the natural dye in the textiles production (Sivakumar et al., 2009). However, utilization of plants and animals is in contradiction with conservation of plants and animals. Therefore, exploration on another alternative sources of natural dye exploration is necessary.

One group of organism which is promising as a source of natural dye producer is soil fungi. Various dyes from soil fungi can be produced using fermentation at large scale. Several species were reported as dye-producing fungi such as brownish red dye by *Aspergillus* sp. (Anchanadevi, 2014), red dye by *Fusarium fujikuroi* (Studt et al., 2012), red dye by *Isaria farinosa* (Velmurugan et al., 2010), *Monascus purpureus* (Yuliana and Apriyani, 2018) and *Penicillium* sp. NIOM-02 (Dhale and Vijay-Raj, 2009), and yellow dye by *Trichoderma* sp. (Gupta et al., 2013; Anchanadevi, 2014). Natural dye requires chemicals (mordants) in the form of metal salts to produce af-

finity between fabrics material (cotton, silk, and wool) and the dye (Vankar et al., 2009). Metal ions from mordant agent acts as electron acceptor to form a coordination bond with dye molecules as an electron donor. $KAl(SO_4)_2 \cdot 12H_2O$ (*alum*), $K_2Cr_2O_7$, $SnCl_2 \cdot 2H_2O$, $CuSO_4 \cdot 5H_2O$, and $FeSO_4$ are commonly used as mordanting agents for textiles colorants.

Many activities such as cultivation, mushroom cultivation, and composting are carried out in the Cibinong Science Center (CSC) – LIPI. Plant litter and rest of the medium that has been used for growing mushrooms (sawdust) mixed with soil will be end up in landfills, hereinafter garbage is burned. A similar environment with a different activity will certainly affect the presence of soil fungi or fungi producing dye.

In this study, we isolate and identify dye-producing soil fungi from three groups of sample collected from Cibinong Science Center (CSC) – LIPI, such as cassava rhizosphere, burning waste, and compost. The performance of the soil fungal dyes is evaluated in combination with *alum* and copper sulphate.

Methods

Soil Sampling

Three groups of the following soil samples were collected at the Cibinong Science Center (CSC), Cibinong: 1) cassava rhizosphere, 2) burning waste, and 3) compost. Three samples (250 g from each group were randomly collected and then mixed together. Each soil groups was further air dried at room temperature for 3-5 days. Dried soil was sieved using a flour sieving to homogenize and separate large-sized soil particles.

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Isolation and Identification of Fungi

Fungal isolation was carried out using dilution method (Sharma et al., 2010) with modification. Dilution of 10^{-3} and 10^{-4} were used to isolate soil fungi. A total of 0.2 mL suspension of each dilution was spread on Potato Dextrose Agar (PDA) (Pronadisa, Conda) medium containing 200 mg/L of chloramphenicol. The PDA medium was incubated for three days at room temperature. Each treatment was repeated three times. Single colony of the fungi on each Petri dish was transferred into new PDA medium and incubated for 1-4 weeks. Single isolate of each fungus was morphologically identified according to the manual described by Domsch et al. (1980). One additional isolate from personal collection, namely *Monascus purpureus*, was included in this study.

Inoculum Preparation

Spore suspension of each fungal isolate was prepared as inoculum for the fermentation of fungal dye. Six milliliters of sterile distilled water were added onto the colony of 6-8 days old fungal cultures and then scrapped using needle on the agar tubes under aseptic conditions.

Solid-state Fermentation

Rice IR-42 was prepared by soaking in water for 24 hours before drained. A total of 25 g of the dried rice was inserted into the glass jar and then autoclaved at 121 °C for 15 minutes. This fermentation medium was cooled at room temperature. The fermentation medium was separately inoculated with each the fungal isolate spore suspension (one agar tube/one glass jar) and then incubated at room temperature for 1 month.

Extraction of Dye

In solid-state fermentation, fungi will penetrate into solid substrate and dyes are released onto the surface. The fungal dye was extracted according to the procedure described by Yuliana and Apriyani (2018) with modification as follow: 25 gram of the fermented rice were extracted with 250 ml of sterile distilled water, shaken at 100 rpm for 6 hours, and then allowed to stand for 15 min, and filtered through muslin cloth.

Dyeing Procedure

To remove the wax and impurities, cloth used for dyeing was firstly washed with detergent, rinsed with water, and dried. The muslin cloth samples (0.27 g) were treated with different dye extracts obtained from the soil fungi with ratio of cloth sample:dye extract (1:30 w/v). The dyeing process was carried out at 90 °C for 5 minutes and left overnight (modified from Jothi, 2008). The colors exhibited by the cloth were identified according to the Royal Horticultural Society color chart (The Royal Horticultural Society, 1966).

Pre-mordanting

Before dyeing, the cloth samples was treated with or without pre-mordanting using each 1 % of alum or copper sulphate with ratio of cloth sample:mordant (1 : 30 w/v). The mordanting process was carried out according to the procedure described by Jothi (2008) with modification.

Cloth samples were soaked in mordanting solution at 90 °C for 5 minutes, followed by separately squeezing and dyeing the cloth with different dye extracts from the soil fungi at 90 °C for 5 minutes and the samples were left overnight. The cloth samples were further rinsed with water and dried at room temperature.

Post-mordanting

This process was conducted according to the procedure described by Jothi (2008) with modification. The cloth samples were separately dyed with different dye extracts of soil fungi. Dyeing process was carried out at 90 °C for 5 minutes and the samples were left overnight. Dyed samples were taken out without rinsed with water, and then squeezed. The samples were treated with each 1 % of copper sulphate or alum with ratio of cloth sample:mordant (1:30 w/v). The mordanting process was carried out at 90 °C for 5 minutes. All samples were dried at room temperature.

Results

Isolation and Identification of Fungi

A total of 36 fungal isolates were obtained from burning waste (6 genera and 4 determined species), cassava rhizosphere (6 genera and 3 determined species), and compost (4 genera and 4 determined species) (Tab. 1). Two genera belong to Ascomycota, two genera belong to Zygomycota, and the remaining isolates belong to asexual morph. The fungal isolates from burning waste sample include *Emericella*, *Eurotium* (*E. chevalieri*), *Aspergillus* (*A. fumigatus*, *A. niger*), *Penicillium*, *Trichoderma* (*T. harzianum*), and *Rhizopus*. While on cassava rhizosphere include *Emericella*, *Aspergillus* (*A. fumigatus*, *A. niger*, *A. terreus*), *Colletotrichum*, *Fusarium*, *Penicillium*, and *Mucor*; and on compost include *Emericella*, *Aspergillus* (*A. fumigatus*, *A. niger*, *A. terreus*), *Paecilomyces* (*P. variotii*), and *Penicillium*. Among them, four isolates, namely *Emericella* sp., *A. fumigatus*, *A. niger*, and *Penicillium* sp. strain GRC-1 were found in all samples.

Only 14 isolates (38.9%) of 36 isolates of the isolated soil fungi; and one isolate of the collection (*M. purpureus*) evaluated, producing dyes with the resulting colors orange-white 159 A, yellow 4A, orange-red 30 C, red 41 A, and green 139 A (Tab. 1; Fig. 1 & 2). Four of five color types were produced by 14 isolates (38.9%), viz, orange-white 159 A (*A. fumigatus*, *Penicillium* sp. strain GRC-1, and *Penicillium* sp. strain R-3), yellow 4A (*Penicillium* sp. strain RC-1), orange-red 30 C (*Aspergillus* sp. strain R-2), and green 139 A (*Trichoderma harzianum*); and one color type viz, red 41 A was produced by *M. purpureus* as control. Unless *M. purpureus* (Ascomycotina), the isolated fungi that producing dyes are included in Deuteromycotina. *Aspergillus fumigatus* and *Penicillium* sp. strain GRC-1 were isolated from burning waste, cassava rhizosphere, and compost, while *Aspergillus* sp. strain R-2 and *Penicillium* sp. strain R-3 isolated only from cassava rhizosphere. *Penicillium* sp. strain RC-1 was isolated from cassava rhizosphere and compost, while *T. harzianum* isolated only from burning waste.

Table 1. Number of soil fungal isolates and dye-producing fungi in this study

No.	Fungi	Burning waste (isolate)	Cassava rhizosphere (isolate)	Compost (isolate)	Color type
Ascomycota					
1.	<i>Emericella</i> sp.	2	1	1	-
2.	<i>Eurotium chevalieri</i> Mangin	1	-	-	-
3.	<i>Monascus purpureus</i> Went				Red 41 A
Asexual morph					
4.	<i>A. fumigatus</i> Fresen.	1	1	1	Orange-white 159 A
5.	<i>A. niger</i> Tiegh.	1	1	1	-
6.	<i>A. terreus</i> Thom	-	1	2	-
7.	<i>Aspergillus</i> sp. strain G-1	1	-	-	-
8.	<i>Aspergillus</i> sp. strain G-2	1	-	-	-
9.	<i>Aspergillus</i> sp. strain R-1	-	3	-	-
10.	<i>Aspergillus</i> sp. strain R-2	-	1	-	Orange-red 30 C
11.	<i>Colletotrichum</i> sp.	-	1	-	-
12.	<i>Fusarium</i> sp.	-	1	-	-
13.	<i>Paecilomyces variotti</i> Bainier	-	-	1	-
14.	<i>Penicillium</i> sp. strain GRC-1	1	1	3	Orange-white 159 A
15.	<i>Penicillium</i> sp. strain R-3	-	1	-	Orange-white 159 A
16.	<i>Penicillium</i> sp. strain RC-1	-	1	2	Yellow 4A
17.	<i>Trichoderma harzianum</i> Rifai	1	-	-	Green 139 A
18.	Unidentified	-	1	-	-
Zygomycota					
19.	<i>Mucor</i> sp.	-	1	-	-
20.	<i>Rhizopus</i> sp.	1	-	-	-
	Total	10	15	11	

Table 2. Characteristics of fungal dyed-clothes treated with pre and post-mordanting copper sulphate

No.	Fungi/A fungal dye with the water solvent	The dyed cloth		
		Pre-mordanting	Post-mordanting	Without mordanting
1.	<i>A. fumigatus</i> (3 isolates)/Orange-white 159 A	Grayed-yellow 161 C	Grayed-white 156 D	Grayed-white 156 D
2.	<i>Aspergillus</i> sp. strain R-2 (1 isolate)/Orange-red 30 C	Grayed-orange 165 C	Grayed-orange 164 D	Grayed-orange 164 D
3.	<i>Monascus purpureus</i> /Red 41 A	Red 39 C	Red 38 D	Red 38 D
4.	<i>Penicillium</i> sp. strain GRC-1 (5 isolates)/ Orange-white 159 A	Grayed-orange 164 B	Grayed-orange 164 D	Grayed-orange 164 D
5.	<i>Penicillium</i> sp. strain R-3 (1 isolate)/Orange-white 159 A	Yellow-orange 19 B	Red 37 C	Yellow-orange 18 C
6.	<i>Penicillium</i> sp. strain RC-1 (2 isolates)/Yellow 4A	Yellow 13 A	Yellow 10 C	Yellow 10 C
7.	<i>T. harzianum</i> (1 isolate)/Green 139 A	Orange-white 159 A	Orange-white 159 C	Orange-white 159 C
8.	Mordanting copper sulphate	White 155 C		

Evaluation of Dye from the Soil Fungi

Characteristics of clothes colored with five kinds of dyes from the soil fungi and *M. purpureus* (treated with copper sulphate and *alum* at pre-mordanting and post-mordanting processes) are shown in table 2 and 3. Color range of white cloth (155 D) after treated with fungal dyes following pre- and post-mordanting with *alum* and copper sulphate includes grayed-yellow (161 C & D), grayed-orange (164 B, C & D and 165 C & D), grayed-white (156 D), yellow (10 C, 11 A, and 13 A), yellow-orange (18 C & B and 19 B), orange-white (159 A, B, and C), and red (37 C, 38 C & D, and 39 C). The color of clothes treated with only copper sulphate or *alum* was white (155 C) and white (155 D), respectively.

Color changes were found during application of fungal dyes from white cloth treated with mordanting agents (Tab. 2 & 3). Orange-white dye 159 A (*A. fumigatus*, *Penicillium* sp. strain GRC-1, and *Penicillium* sp. strain R-3); orange-red 30 C (*Aspergillus* sp. strain R-2); and green 139 A (*T. harzianum*) treated with pre- and post-mordanting agents exhibited different colors on the clothes viz grayed-yellow, grayed-white, grayed-orange,

yellow-orange, and red; grayed-orange; and orange-white (Fig. 3). Slight changes were found on the dyes produced by *M. purpureus* and *Penicillium* sp. strain RC-1. The red dye 41 A produced by *M. purpureus* showed slight changes into red (38 C, 38 D, and 39 C) after treated with pre- and post-mordanting agents (Fig. 4). The yellow dye 4 A produced by *Penicillium* sp. strain RC-1 also exhibited slight color changes into yellow (10 C, 11 A, and 13 A) (Fig. 5).

This study showed that addition of pre-mordanting copper sulphate and *alum* to the fungal dyes produced the different final colors on the clothes. For example, after treated with pre-mordanting copper sulphate, orange-white dye 159 A produced by *A. fumigatus* and *Penicillium* sp. strain GRC-1 changed into grayed-yellow 161 C and grayed-orange 164 B, respectively. In addition, pre-mordanting *alum* also changed the orange-white dye 159 A produced by *A. fumigatus* and *Penicillium* sp. strain GRC-1 into grayed-yellow 161 D and grayed-orange 164 C, respectively. In contrast, post-mordants treatment by copper sulphate or *alum* did not produce the different final colors on the clothes (Tab. 2 & 3).

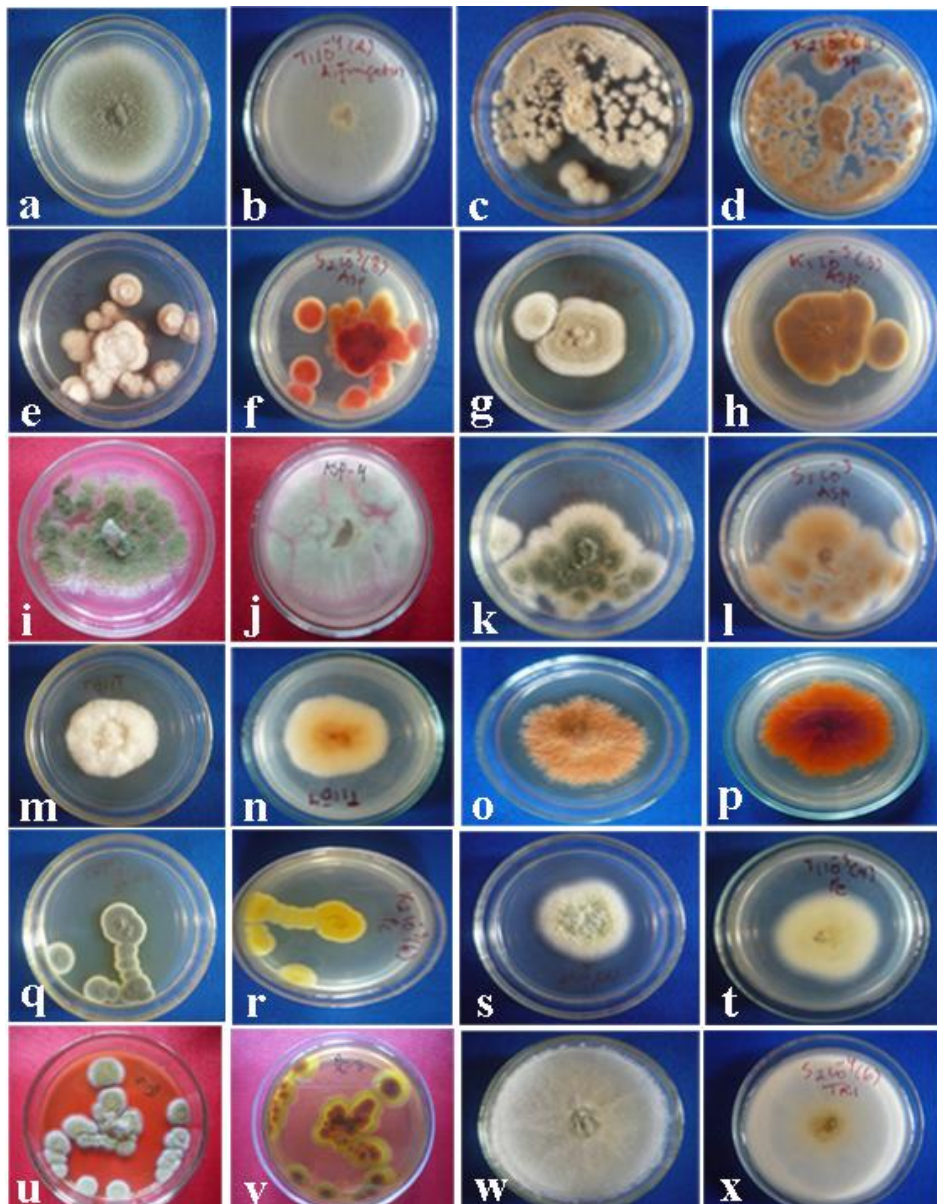


Figure 1. The upper and reverse surface of the fungal colonies producing dye. a-b. *Aspergillus fumigatus*, c-d. *Aspergillus terreus*, e-f. *Aspergillus* sp. strain G-1, g-h. *Aspergillus* sp. strain R-1, i-j. *Aspergillus* sp. strain R-2, k-l. *Emericella* sp., m-n. *Fusarium* sp., o-p. *Monascus purpureus*, q-r. *Penicillium* sp. strain GRC-1, s-t. *Penicillium* sp. strain R-3, u-v. *Penicillium* sp. strain RC-1, and w-x. *Trichoderma harzianum*

Discussions

Several soil fungal genera have been known for their ability in producing dyes (Dhale and Vijay-Raj, 2009; Velmurugan et al., 2010; Gupta et al., 2013; Anchanadevi, 2014). The soil fungi obtained from this study were dominated by asexual morph fungal isolates (77.78%), followed by Ascomycota (13.89%) and Zygomycota (5.56%) (Tab.1). Domination of taxa belonging to the asexual morph within soil fungi community was also reported in several regions (Karkun et al., 2012). The important factor affecting domination of asexual morph fungi in the soil environment is possibly their capability in producing large amounts of conidia (Swier et al., 2011). The conidia can be spread by wind to a long distance and

resist to environmental stress until their germination stage. Among the asexual morph fungi, members of *Aspergillus*, *Fusarium*, *Penicillium*, and *Trichoderma* are common in various substrates (Karkun et al., 2012). This study also found that *Aspergillus* and *Penicillium* are dominant within fungal community from burning waste, cassava rhizosphere, and compost. Both genera are well known as cosmopolitan group, found in almost all type of habitats all over the world.

Among the *Aspergillus* and *Penicillium* species obtained in this study, *A. fumigatus*, *A. niger*, *Penicillium* sp. strain GRC-1, and *Emericella* sp. were found from all sample types. *Aspergillus niger* and *A. fumigatus* are

commonly found as saprobes growing on dead leaves, stored grain, compost piles, and other decaying vegetation. The spores are widespread, and are often associated with organic materials and soil (Sharma et al., 2010). Both species, including *Penicillium* spp., also commonly found on cassava rhizosphere (Arotupin and Akinyosoye, 2008; Sule and Oyeyiola, 2012; Ibrahim and Shehu, 2014); and compost (Anusuya and Geetha, 2014).

Highest diversity of soil fungal isolates from cassava rhizosphere than burning waste and compost (Tab. 1) is significant with the number of dye-producing fungi obtained from the respective samples. It is probably related

to the physical and chemical properties of the samples. The rhizosphere of cassava is the rich environment of root exudates and soil organic matters that captivated various species of the soil fungi (Raaijmakers et al., 2009), including dye-producing fungi. However, burning waste might kill some of the soil microorganisms (Sanusi, 2015). The low number and variety must have been influence by the burning. In compost, although organic matters and nutrients for fungal growth is high, the majority of fungi is, however, dominated by the fungi capable of growing on high temperature.

Table 3. Characteristics of fungal dyed-cloth treated with pre and post-mordanting alum

No.	Fungi/A fungal dye with the water solvent	The dyed cloth		
		Pre-mordanting	Post-mordanting	Without mordanting
1.	<i>A. fumigatus</i> (3 isolates)/Orange-white 159 A	Grayed-yellow 161 D	Grayed-white 156 D	Grayed-white 156 D
2.	<i>Aspergillus</i> sp. strain R-2 (1 isolate)/Orange-red 30 C	Grayed-orange 165 D	Grayed-orange 164 D	Grayed-orange 164 D
3.	<i>Monascus purpureus</i> /Red 41 A	Red 38 C	Red 38 D	Red 38 D
4.	<i>Penicillium</i> sp. strain GRC-1 (5 isolates)/Orange-white 159 A	Grayed-orange 164 C	Grayed-orange 164 D	Grayed-orange 164 D
5.	<i>Penicillium</i> sp. strain R-3 (1 isolate)/Orange-white 159 A	Yellow-orange 18 B	Yellow-orange 18 C	Yellow-orange 18 C
6.	<i>Penicillium</i> sp. strain RC-1 (2 isolates)/Yellow 4A	Yellow 11 A	Yellow 10 C	Yellow 10 C
7.	<i>T. harzianum</i> (1 isolate)/Green 139 A	Orange-white 159 B	Orange-white 159 C	Orange-white 159 C
8.	Mordant alum	White 155 D		

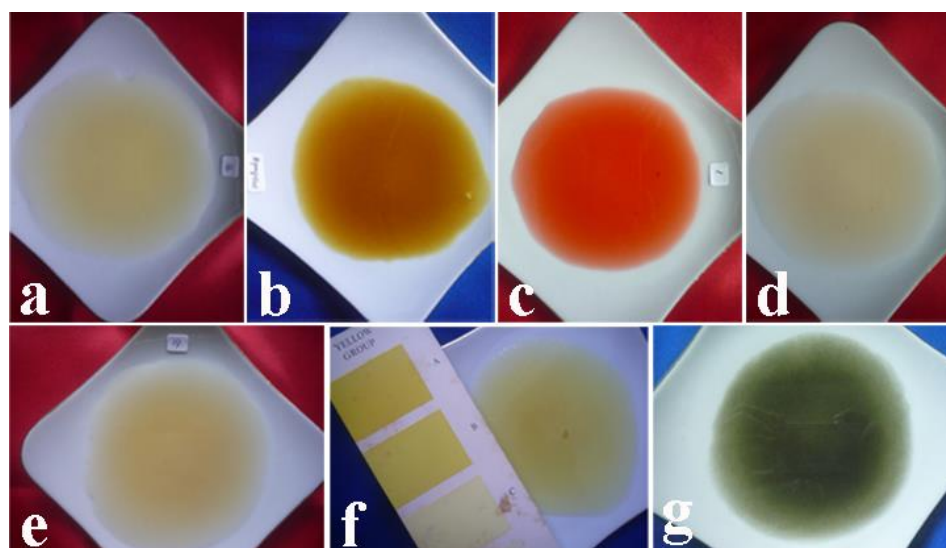


Figure 2. Fungal dyes extracted with water solvent. a. *Aspergillus fumigatus*, b. *Aspergillus* sp. strain R-2, c. *Monascus purpureus*, d. *Penicillium* sp. strain GRC-1, e. *Penicillium* sp. strain R-3, f. *Penicillium* sp. strain RC-1, g. *Trichoderma harzianum*

The current finding of *Aspergillus* sp. strain R-2 (1 isolate), *A. fumigatus* (3 isolates), *Penicillium* sp. strain GRC-1 (5 isolates), *Penicillium* sp. strain R-3 (1 isolate), *Penicillium* sp. strain RC-1 (2 isolates), *Trichoderma harzianum* (1 isolate), and *M. purpureus* (1 isolate) in producing various dyes such as grayed-yellow (161 C & D), grayed-orange (164 B, C & D and 165 C & D), grayed-white (156 D), yellow (10 C, 11 A, and 13 A), yellow-orange (18 B & C and 19 B), orange-white (159 A, B, and C) and red (37 C, 38 C & D, and 39 C), is new in Indonesia. These fungi are thus potential sources as alternative sources for environmentally safe natural dyes production in textile industry. *Aspergillus niger*, *Penicillium* spp., *Trichoderma* spp., *Fusarium* spp., and *M. purpureus* have been known for their capability in producing dyes for textile (wool, cotton, and silk) (Atalla et al., 2011; Anchanadevi, 2014).

Mapari et al. (2010) reported that fungal dye is usually produced as secondary metabolites, and generally classified as carotenoid and polyketide. The polyketide includes anthraquinone, azaphilone, hydroxy anthraquinone, naphthoquinone, and oxopolone (Mapari et al., 2010), while carotenoid contains of carotene and xanthophyll (Jaswir et al., 2011). Canthaxanthin (orange) of xanthophyll group is produced by *M. roseus* (Kirti et al., 2014), whereas β -carotene (red-orange) of carotene group is produced by *Neurospora* (Priatini, 2014). Another natural dyes from fungi includes azaphilone such as monascorubrin (orange), monascorubramine (violet-red), 12-carboxyle-monascorubramine or PP-V (violet-red), monascin (yellow), ankaflavin (yellow), and sequoiamonascin (yellow) produced by *Monascus* and *Penicillium* were also reported (Mapari et al., 2010); helminthosporin (maroon) and cynodontin (yellowish-brown) by

Curvularia (Rao et al., 2017); oxoproline group such as oreovactaene (yellow) produced by *Epicoccum nigrum*; naphthoquinone such as viopurpurin (violet-black) produced by *Aspergillus* spp.; and bikaverin (red) and nectria furone (yellow-brown) by *Fusarium* (Mapari et al., 2010).

Fungal dyes fixed with different mordanting agents and different processes produced different intensities of final color on clothes (Tab. 2 & 3). In this study, copper sulphate mordant produce higher intensity in almost all final colors the clothes than *alum* mordant, except the clothes colored by *Penicillium* sp. strain R-3 dye. This is presumably due to the influence of methal salts present in the materials of mordants, such as Cu^{2+} of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and Al^{3+} of $\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$. The metal salts serve in

direction change of the natural dye colors, depending to the type of metal salts that bind to the natural dyes (Tripathi et al., 2015). *Alum* mordant produces colors that matches with the original colors, while copper sulphate mordant would leave towards the darker color. In this study, majority of the fungal dyes added with *alum* and copper sulphate mordants at pre-mordanting produced more intense final color to the clothes than post-mordanting treatment. Post-mordanting treatment with *alum* and copper sulphate produce similar colors with the clothes without mordanting process. High intensity or older color of the natural dyes after mordanting process by copper sulphate was also reported on a flower dye obtained from *Tecoma stans* (Chandra et al., 2012).



Figure 3. Dyed clothes with natural dye from *Penicillium* sp. strain R-3 (A), *Monascus purpureus* (B), and *Penicillium* sp. strain RC-1 (C). < = pre-mordanting; > = post-mordanting; Cu = $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; T = alum; O = without mordanting

A total of 14 from 36 isolates of fungi from burning waste, cassava rhizosphere, and compost were capable in producing various natural dyes and dyed cloth such as grayed-yellow, grayed-orange, grayed-white, yellow, yellow-orange, orange-white, and red. The fungal dyes added with pre-mordanting (*alum* or copper sulphate) produce higher intensity of colors on the clothes than post-mordanting using the same mordant agents.

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