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Effect of Different Mordants on Cotton Cloth Dyed with *Aspergillus* and *Penicillium* Dyes

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Abstract–discovery of new natural dyes is important as an alternative to environmentally harmful synthetic dyes. This research was conducted using three varieties of *Aspergillus* dyes and six varieties of *Penicillium* dyes. In this study, *Aspergillus* and *Penicillium* isolates were grown in mineral salts glucose medium. Pre-mordanting on cotton cloth was conducted using different mordants, namely, alum, $CaCO_3$, $CuSO_4.5H_2O$, $FeSO_4.7H_2O$, $K_2Cr_2O_7$, and $MnSO_4.H_2O$ (monohydrate). The colour of filtrate and range of colours developed on dyed materials were measured by RHS colour chart. The results indicated that colour intensity on dyed cloth was influenced by the addition of mordants.Different shades were also obtained from the same dye filtrate using different mordants. Mordant $FeSO_4.7H_2O$ was found as the most appropriate mordant in combination with the fungal dyes. The present study describes new sources of fungal dyes, which can be used as an environmental friendly alternative for dyeing cloth.

Keyword: *Aspergillus*; cotton cloth; dyes; mordant; *Penicillium*

Introduction

Plants and animals have been known as good sources for production of natural dyes, but the inability of them to meet the world demand has led to increased interest in synthetic dyes which can pollute environment and harmful to human health. Natural dyes exhibit better biodegradability and higher compatibility with the environment than synthetic dyes (Sivakumar*et al.*, 2009).

These problems can be solved by exploiting several potential biological sources such as fungi, bacteria, and algae.Dyes production by microorganisms is very important to textile industries because microorganisms can grow rapidly, provide high productivity and its products are available throughout the year (Méndez *et al.*, 2011). Microbial dyes are often more stable and soluble than those produced by plants or animals (Gunasekaran and Poorniammal, 2008).

Aspergillus and *Penicillium*have been known as fungi secreting dyes. Various natural dyes from *Aspergillus* and *Penicillium*were reported. These include brownish red dye by *Aspergillus* sp. isolated from soil (Anchanadevi, 2014); dark brown, brown, faint reddish brown and brown dyes by*Penicilliumchrysogenum* (NRC 74), *P. italicum* (NRC E11), *P. oxalicum* (NRCM25) and *P. regulosum* (NRC 50), respectively (Atalla *et al.*, 2011);greenish blue dye by*P. fagi* isolated from beech leaves (Martinez and Ramirez, 1978);red dye by *P. marneffei* (Woo *et al.*, 2014); andyellow, red and orange dyesby *P. purpurogenum* DPUA 1275 (Santos-Ebinuma*et al.*, 2013).

Natural dyes are mostly non-substantive dyes or they have a little colouring power within themselves, and they require mordants for fixation of a dye into the fiber. Different colours and its tones can be obtained from a single dye source by addition of mordants (Sangeetha *et al.*, 2015). Satyanarayana and Chandra (2013) reported that mordants are metal salts that produce an affinity between the cloth fiber and the dye. Metal ions of mordants act as electron acceptors to electron donors to form coordination bonds with the dye molecule, making them insoluble in water. Common mordants include alum, chrome, stannous chloride, copper sulphate, ferrous sulphate, and so forth.

The aim of the present investigation is to evaluate various dyes produced by *Aspergillus* and *Penicillium* isolates using different mordants in dyeing cotton cloth. This study is expected to provide colour variations from natural dyes produced by *Aspergillus* and *Penicillium*.

Materials and Methods

Materials

Chemical materials used in this study include alum (KAI $(SO_4)_2.12H_2O$), $CaCl_2$. $2H_2O$, $CaCO_3$, $CuSO_4.5H_2O$, $FeSO_4.7H_2O$, glucose, H_3BO_3, KCl, $K_2Cr_2O_7$, $MgSO_4.7H_2O$, $MnSO_4.H_2O$ (monohydrate), NaH_2PO_4, $NaNO_3$, $Na_2MoO_4.2H_2O$, *Potato Dextrose Agar* (PDA), and $ZnSO_4.7H_2O$,while other materials used include cotton cloth, detergent, Erlenmeyer, Petri dish, stove, and test tube.

Fungi

Three isolates of *Aspergillus*, namely *A. terreus*, *Aspergillus*sp. strain 1, and *Aspergillus* sp. strain 2 (sexual morph: *Emericella* sp.), and six strains of *Penicillium*, such as *Penicillium* sp. strain 604, *Penicillium*sp. strain 720, *Penicillium* sp. strain 1, *Penicillium*sp. strain 3a, *Penicillium*sp. strain 3b, and *Penicillium*sp. strain 4uhb were used in this study.

Inoculum preparation

Each fungal isolate was grown separately in Petri dishes containing PDA medium and incubated at room temperature (27-28°C) for 5 days. Each fungal isolate was then printed with a straw of pop ice (10 mm) for further inoculation.

Liquid fermentation

Five mycelial prints of *Aspergillus* or *Penicillium*were inoculated separately into Erlenmeyer flask containing mineral salts-glucose medium (Baker and Tatum, 1998). Composition of the mineral salts-glucose medium is described as follows (in ppm): $NaNO_3$, 848; KCl, 300; $MgSO_4.7H_2O$, 165; NaH_2PO_4, 100; $CaC1_2.2H_2O$, 40; H_3BO_3, 5.7; $FeSO_4.7H_2O$, 5.0; $ZnSO_4.7H_2O$, 4.4; $MnSO_4.H_2O$ (monohydrate), 3.1; $Na_2MoO_4.2H_2O$, 2.5; $CuSO_4.5H_2O$, 0.4; and glucose, 20,000. Mineral salts and glucose solutions were separately autoclaved, and combined after cooling in culture flasks in a sterile laminar flow hood. The Erlenmeyer containing fungal isolates were incubated at room temperature (27-28^o C) in stationary cultures for 4 weeks.

Harvesting of dyes

Aspergillus and*Penicillium* cultures will form heavy mycelial mats floating within 2 weeks, and begin elaborating dyes that leaked into the medium. After 4 weeks incubation period, the mycelium was harvested, and the dye was filtered in a muslin cloth for further assay.

Dyeing of cotton cloth

Dyeing of cotton cloth with dyes produced by *Aspergillus* and *Penicillium*was carried out in three stages as follows: 1) washing, 2) mordanting, and 3) dyeing.

Washing

To remove the wax and impurities, cotton cloth used for dyeing was first washed with detergent, rinsed with water, and then dried.

Mordanting

Mordantingwas conducted in three stages of dyeing: pre-mordanting, simultaneous mordanting, and post-mordanting. Pre-mordanting step was used due to producing brighter colour appearance in the previous study. $CaCO_3$, $CuSO_4.5H_2O$, $FeSO_4.7H_2O$,KAI $(SO_4)_2.12H_2O$, $K_2Cr_2O_7$, and $MnSO_4.H_2O$ (monohydrate) were used as the mordant. Before colouring process, samples of cotton cloth (5 cm × 5 cm or 0.27 g) were treated with or without pre-mordanting using 1 % of each mordant with ratio of 1: 30 (material : liquor). Mordanting process was carried out at 90º C for 5 minutes. The samples was further detached, squeezed and dyed with the different dyes extracted separately from *Aspergillus* and *Penicillium* liquid inoculums.

Dyeing

Cotton cloth samples were treated with the different dyes extracted from *Aspergillus* or *Penicillium* with ratio of material to liquor is 1 : 30. Dyeing process was carried out at 90ºC for 5 minutes and left overnight (Jothi, 2008 with modification). After dyeing process, the dyed clothes was rinsed with water and dried at room temperature (27-28^{o} C). The colour that appears on the cotton clothwas measured with the RHS colour chart (Anonymous, 1966).

Results and Discussion

Variation of colours produced by *A. terreus*, *Aspergillus*sp. strain 1, and *Aspergillus* sp. strain 2 on mineral salts glucose medium are presented in Tables 1, 2 and 3; and Figure 1. The colours produced by these three *Aspergillus* isolates were greyed-orange (168 B) and red (40 A). Similarcolours were also reported from other *Aspergillus* species such as dark red and orange-red dyes by*A. glaucus* and *Aspergillus* sp., respectively (Malik *et al*., 2012), brown dye by *A. niger* NRC 95 (Atalla *et al*., 2011), red dye by *Aspergillus* sp. (Anchana devi, 2014), and red dye by *Emericella* (sexual morph of *Aspergillus*) (Velmurugan *et al*., 2010).

In addition, more diverse colour variation was found from *Penicillium* isolates grown on mineral salts glucose medium (Tables 1, 4, 5; Figures 2, 3). Six different dyes, viz, 45 B red, 33 A orange-red, 168 C greyed-orange, 169 B greyed-orange, 168 B greyed-orange, and 187 A greyed-purple, were produced by *Penicillium*isolates.Previous studies also found similar dyes produced by *Penicillium*such as chromophore greenish-yellow and orange-yellow by *P. caseifulvum* (Suhr *et al*., 2002), atrovenetin yellowby *P. herquei* (Robinson *et al*., 1992), red dye by *P . marneffei* (Woo *et al*., 2014), mitorubrin (yellow) and mitorubrinol (orange-red) by *P. purpurogenum* (Buchi *et al*., 1965), arpink red (dark red)by *P. oxalicum* var. *armeniaca* (Sardaryan *et al*., 2004), red-orange and red by *P. pseudostromaticum* (Mahesh *et al*., 2014), and purpurogenone red by *P. purpurogenum* (Qui*et al*., 2010).

Table 1. Variation of dyes produced by *Aspergillus* and *Penicillium* isolates

Aspergillus	Dyes produced by *Aspergillus*	*Penicillium*	Dyes produced by *Penicillium*
Aspergillus terreus	168 B Greyed-orange	*Penicillium*sp. strain 604	45 B Red
Aspergillus sp. strain 1	40 A Red	*Penicillium* sp. strain 720	33 A Orange-red
Aspergillus sp. strain 2	168 B Greyed-orange	*Penicillium*sp. strain 1	168 C Greyed-orange
		*Penicillium*sp. strain 3a	169 B Greyed-orange
		*Penicillium*sp. strain 3b	168 B Greyed-orange
		*Penicillium*sp. strain 4uhb	187 A Greyed-purple

Application of mordants to the natural dyes produced by *Aspergillus* isolates generated various shades on cotton cloth (Tables 2, 3). All dyes can colouring cotton cloth from 155 D white into 154 C & D, and 145 D yellow-green, 165 D greyed-orange, 161 B, 160 A, B & C greyed-yellow, 156 D greyed-white, 199 C greyed-brown, and159 C yellow-white. Previous studies also found that brown colour on wool was produced by *A. niger* (Atalla *et al*., 2011) and the *A. niger* dye added with mordanting potassium dichromate produces yellow colour on cotton, silk, and the mix of silk and cotton (Anchanadevi, 2014).

Figure 1. *Aspergillus* dyes;(1)*Aspergillus*sp. strain 2, (2)*Aspergillus*sp. strain 1, (3)*A. terreus*,(4) mineral saltsglucose medium.

Figure 2. *Penicillium* dyes; (1)*Penicillium*sp. strain604, (2)*Penicillium*sp. strain 720, (3)*Penicillium*sp. strain 1,(4)mineral salts glucose medium.

Figure 3. *Penicillium* dyes;(1) *Penicillium*sp. strain3a, (2)*Penicillium*sp. strain 3b, (3)*Penicillium*sp. strain 4uhb,(4) mineral salts glucose medium.

Table 2. Colour variation on cloth stained with *Aspergillus* dyes using different mordants

No	Fungus	Dyes variation	Dyed cotton cloth			
			$CaCO_3$	$CuSO_4.5H_2O$	$FeSO_4.7H_2O$	Without mordanting
1.	*Aspergillus terreus*	168 B Greyed-orange	154 C Yellow-green	154 C Yellow-green	160 A Greyed-yellow	154 D Yellow-green
2.	*Aspergillus* sp. strain 1	40 A Red	165 D Greyed-orange	145 D Yellow-green	199 C Greyed-brown	160 B Greyed-yellow
3.	*Aspergillus* sp. strain 2	168 B Greyed-orange	159 C Yellow-white	160 C Greyed-yellow	161 B Greyed-yellow	156 D Greyed-white
4.	Mordanting		155 D White	156 D Greyed-white	20 D Yellow-orange	155 D White

Table 3. Colour variation on cloth stained with *Aspergillus* dyes using different mordants

No	Fungus	Dyes variation	Dyed cotton cloth			
			$K_2Cr_2O_7$	$MnSO_4$.H2O (monohydrate)	Alum	Without mordanting
1.	*Aspergillus terreus*	168 B Greyed-orange	154 C Yellow-green	154 C Yellow-green	154 C Yellow-green	154 D Yellow-green
2.	*Aspergillus* sp. strain 1	40 A Red	156 D Greyed-white	156 D Greyed-white	156 D Greyed-white	160 B Greyed-yellow
3.	*Aspergillus* sp. strain 2	168 B Greyed-orange	156 D Greyed-white	156 D Greyed-white	160 C Greyed-yellow	156 D Greyed-white
4.	Mordanting		157 A Green-white	155 B White	155 D White	155 D White

Mordants application on *Penicillium*dyes also produced different shades on cotton cloth (Tables 4, 5). The *Penicllium* dyes can stain cotton cloth from 155 D white into 76 C violet, 160 A & D; 161 A, B & D; 162 B greyed-yellow, 163 B & D; 164 C and 165 D greyed-orange, 156 D greyed-white, 199 D greyed-brown, 157 A green-white, 145 C yellow-green, 26 D orange, 159 B orange-white, 201 D grey, 159 C yellow-white, and 155 C white. The study of dyeing cloth with a *Penicillium* dye reported that *Penicillium* spp. grown in a liquid medium produces a reddish brown colour on wool (Atalla *et al*., 2011).

Table 4. Colour variation on cloth stained with *Penicillium*dyes using different mordants

No	Fungus	Dyes variation	Dyed cotton cloth			
			$CaCO_3$	$CuSO_4.5H_2O$	$FeSO_4.7H_2O$	Without mordanting
1.	*Penicillium*sp. strain 604	45 B Red	76 C Violet	76 C Violet	26 D Orange	76 C Violet
2.	*Penicillium* sp.	30 A	161 D	161 D	165 D	161 D

	strain 720	Orange-red	Greyed-yellow	Greyed-yellow	Greyed-orange	Greyed-yellow
3.	*Penicillium*sp. strain 1	162 A Greyed-yellow	156 D Greyed-white	145 C Yellow-green	163 B Greyed-orange	163 D Greyed-orange
4.	*Penicillium*sp. strain 3a	165 B Greyed-orange	161 B Greyed-yellow	161 A Greyed-yellow	199 D Greyed-brown	159 C Yellow-white
5.	*Penicillium*sp. strain 3b	163 A Greyed-orange	156 D Greyed-white	155 C White	159 B Orange-white	155 C White
6.	*Penicillium*-4uhb	187 A Greyed-purple	199 D Greyed brown	201 D Grey	199 D Greyed-brown	201 D Grey
7.	Mordanting		155 D White	156 D Greyed-white	20 D Yellow-orange	155 D White

Table 5. Colour variation on cloth stained with *Penicillium*dyes using different mordants

No	Fungus	Dyes variation	Dyed cotton cloth			
			$K_2Cr_2O_7$	$MnSO_4.H_2O$ (monohydrate)	Alum	Without mordanting
1.	*Penicillium*sp. strain 604	45 B Red	76 C Violet	76 C Violet	76 C Violet	76 C Violet
2.	*Penicillium* sp. strain 720	30 A Orange-red	161 D Greyed-yellow	161 D Greyed-yellow	161 D Greyed-yellow	161 D Greyed-yellow
3.	*Penicillium*sp. strain 1	162 A Greyed-yellow	160 D Greyed-yellow	160 D Greyed-yellow	160 A Greyed-yellow	163 D Greyed-orange
4.	*Penicillium*sp. strain 3a	165 B Greyed-orange	161 B Greyed-yellow	164 C Greyed-orange	162 B Greyed-yellow	159 C Yellow-white
5.	*Penicillium*sp. strain 3b	163 A Greyed-orange	157 A Green-white	157 A Green-white	160 A Greyed-yellow	155 C White
6.	*Penicillium*sp. strain 4uhb	187 A Greyed-purple	201 D Grey	201 D Grey	201 D Grey	201 D Grey
7.	Mordanting		157 A Green-white	155 B White	155 D White	155 D White

Cotton is composed of glucoside units. It can exhibit a coordinative and intermolecular hydrogen bonding with the mordants agent and dye (Onal, 1996). This study showed that staining cotton cloth with *Aspergillus* and *Penicillium* dyes produced different shades of the colour. The characteristics of colour shadings depend on the mordants type used during the dyeing process (Tables 2, 3, 4, 5). Mordantsare generally used for fixing the dye to the cloth. Unmordanted cotton cloth showed decrease in the intensity of colour than mordanted cotton cloth, especially on *A. terreus* (Figure 4) and *Penicillium*sp. strain 3b dyes.

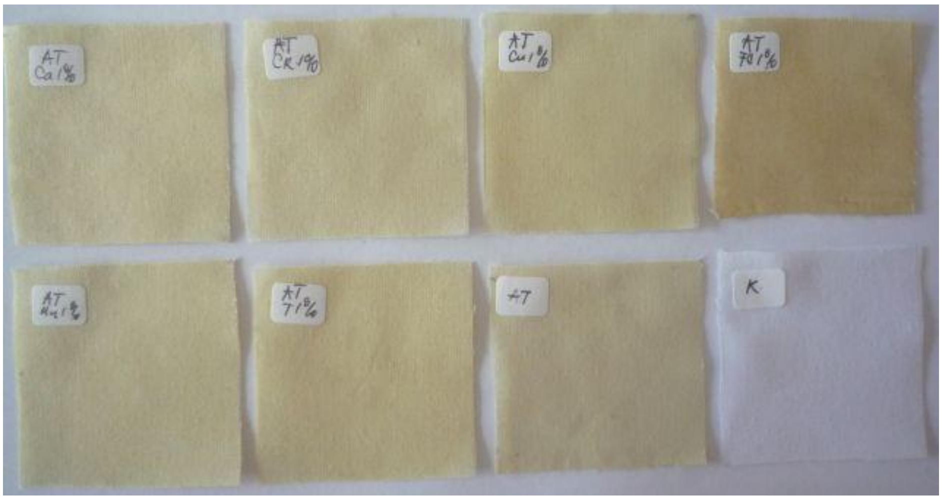

Figure 4. Cotton cloths dyed with *A. terreus* dye using different mordants; Ca = $CaCO_3$, Cr = $K_2CR_2O_7$, Cu = $CuSO_4.5H_2O$, Fe = $FeSO_4.7H_2O$, $MnSO_4.H_2O$, T = alum, K = control.

Unmordanted cotton cloth stained with dyes from *Aspergillus* sp. strain 1 and *Penicillium*sp. strain 1 produced bright sufficient colours. Application of mordants to the *Aspergillus* sp. strain 1 and *Penicillium*sp. strain 1 dyes caused the colour of cotton cloth became fade (Tables 2, 3, 4, 5). The unmordanted cotton cloth stained with *Aspergillus* sp. strain 1 dye produced 160 B greyed-yellow colouron the cotton cloth, however, application of mordants alum, $K_2Cr_2O_7$, and $MnSO_4.H_2O$ to fungal dye causing the colour of cotton cloth became 156 D greyed-white. The unmordanted cotton cloth stained with *Penicillium*sp. strain 1 dye produced 163 D greyed-orange colour, however, application of mordants $CaCO_3$, $K_2Cr_2O_7$, and $MnSO_4.H_2O$ causing the colour of cotton cloth became 156 D greyed-white, 160 D greyed-yellow, and 160 D greyed-yellow, respectively (Figure 5). This evidence showed that metal complex formation for these dyes did not improve the colour intensity on the cotton cloth.

Figure 5. Cotton cloth dyed with *Penicillium*sp. strain 1 dye using different mordants;Ca = $CaCO_3$, Cr = $K_2CR_2O_7$, Cu = $CuSO_4.5H_2O$, Fe = $FeSO_4.7H_2O$, $MnSO_4.H_2O$, T = alum, K = control.

Dyed cotton cloth using dyes from *Penicillium*sp. strain 604 and *Penicillium*sp. strain 720 treated with mordants did not show difference with the dyed cotton cloth without mordanting. Distinct colour to the cotton cloth stained with *Penicillium*sp. strain 604 and *Penicillium*sp. strain 720 dyes was found when $FeSO_4.7H_2O$ was applied as mordant. Similar colour was also found in the cotton cloth stained with *Aspergillus* sp. strain 2 dye treated with $K_2Cr_2O_7$ and $MnSO_4.H_2O$, with the cotton cloth stained without mordanting. Another similar colour was also found between the cotton cloth stained using *Penicillium*sp. strain 4uhb dye treated with mordants (except $CaCO_3$ and $FeSO_47H_2O$) and the cotton cloth without mordanting. These data showed that metal complex formation for these natural dyes did not improve the colour on the cotton cloth.

Different types of mordant added to the same natural dye produced by *Aspergillus* spp. or *Penicillium* spp. may drastically alter the colour and influence the shades of the final cotton cloth colour which may be a desired effect or an unwanted result. Similar result reported that different shades obtained from natural dye extracted from the flowers of *Tecomastans*when applyingdifferent mordants like copper sulphate, ferrous sulphate, ferric chloride, potassium dichromate, myrobolon, and cow dung (Chandra Mohan *et al*., 2012).Uddin (2014) also noted that depending on the metal character of mordants, the complex formation not only strengthens dye fixation on the substrate but also changes the colour of dyeing.

Based on the variation in colour intensity on dyed cotton cloth treated with pre-mordanting process using alum, $CaCO_3$, $K_2Cr_2O_7$, $CuSO_4.5H_2O$, $FeSO_4.7H_2O$, and $MnSO_4.H_2O$, the sequence of colour from highest to lowest were $FeSO_4.7H_2O$>$CuSO_4.5H_2O$ or alum > $CaCO_3$ > $MnSO_4.H_2O$ or $K_2Cr_2O_7$. The colour intensity gradually decline from $FeSO_4.7H_2O$ to $MnSO_4.H_2O$ or $K_2Cr_2O_7$. Application of mordant $FeSO_4.7H_2O$ to the *Aspergillus* and *Penicillium* dyes produce darker or brighter colour to the cotton cloth than application of the other mordants. Previous study also found that addition of pre-mordanting$FeSO_4$ in natural dyes (onion extracts) produced darker final colour (Uddin, 2014). Application of alum as mordant agent produced stronger colour intensity to five fungal natural dyes from *A. terreus*, *Aspergillus* sp. strain 2, *Penicillium*sp. strain 1, *Penicillium*sp. strain 3a, and *Penicillium* sp. strain 3b. Application of $CuSO_4.5H_2O$ to the dyes produced by five isolates, namely *A. terreus*, *Aspergillus* sp. strain 1, *Aspergillus* sp. strain 2, *Penicillium*sp. strain 1, and *Penicillium*sp. strain 3a also produced stronger colour intensity to the cotton cloth. In addition, mordant $CaCO_3$ produces stronger colour to the cotton cloth stained by dyes from four isolates, namely *A. terreus*, *Aspergillus* sp. strain 1, *Penicillium*sp. strain 3a, and *Penicillium*sp. strain 4uhb. $K_2Cr_2O_7$ and $MnSO_4.H_2O$mordanting agents produced stronger colour on the cotton cloth stained by three dyes, namely *A. terreus*, *Penicillium*sp. strain 3a, and *Penicillium*sp. strain 3b.

Mordants play very important role in imparting colour to the cotton cloth in the form of metal complex formation which results in increase of dye uptake. This is attributed to the fact that the metal ions of mordants act as electron acceptors from groups of dye donating electron to form co-ordination bonds with the dye molecule, making them insoluble in water (Mongkholrattanasit*et al*., 2011). Kamel*et al*. (2009) also reported that colour strength are dependent on the metal salt used. There was tendency of Fe make a strong coordination, therefore, enhances the interaction between fiber and the dye, resulting in a greater depth of shades(Jothi, 2008; Tripathi*et al*., 2015). For example, $FeSO_4.7H_2O$has the ability of forming coordination complexes. Functional group as hydroxy on the cotton cloth can occupy the unoccupied sites on metal ion interaction with the dye. Thus, a tenary complex is formed by the metal salt on which one site is with the cotton fiber and the other site is with the dye (Mongkholrattanasit*et al*., 2011) (Figure 6). The current study aslo showed that the cotton cloth mordanted with $FeSO_4.7H_2O$ showed comparatively a good colour yield (Figures 4, 5).

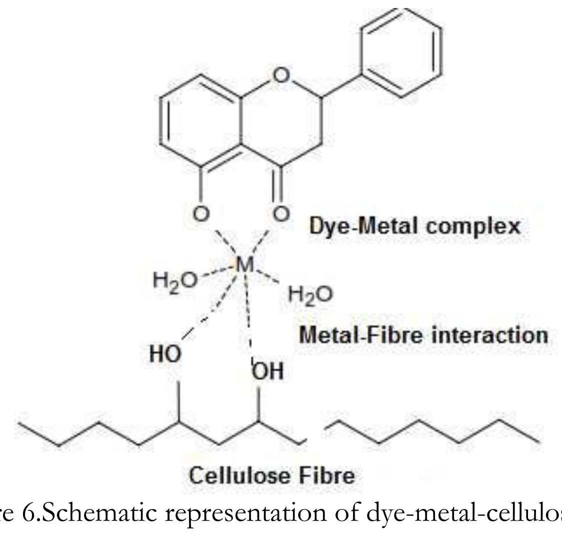

Figure 6.Schematic representation of dye-metal-cellulose fibre interaction (from Kechi *et al*., 2013).

Conclusions

This study showed that nine selected fungal isolates, namely *A. terreus*, *Aspergillus* sp. strain 1, *Aspergillus* sp. strain 2 (sexual morph: *Emericella*), *Penicillium*sp. strain 604, *Penicillium*sp. strain 720, *Penicillium*sp. strain 1, *Penicillium*sp. strain 3a, *Penicillium*sp. strain 3b, and *Penicillium*sp. strain 4uhb produced natural dyes that can be used to dye textile.Cotton cloth mordanted with $FeSO_4.7H_2O$exhibited comparatively a good colour yield.

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