Production of textile reddish brown dyes by fungi

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ABSTRACT

Eleven fungal strains were tested for their ability to produce brown and reddish brown textile dyes using H-acid (1-naphthol-8-amino-3, 6-disulfonic acid) as a dye precursor in the fermentation medium. All tested fungal strains exhibited high ability to produce dyes varying in both dye color (brown to reddish brown) and fastness properties to washing, perspiration and UV light. The produced dyes were subjected to further analysis for quantitative determination of dye components for investigation of their inter-relations as well as their role in dye color and stability.

Keywords: fastness, fermentation, fungi, H-acid, perspiration, reddish brown dye, textile dye and washing

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INTRODUCTION

The dyestuff industry is suffering from increase in costs of feedstock and energy for dye synthesis, and they are under increasing pressure to minimize the damage to the environment. The industries are continuously looking for cheaper, more environmental friendly routes to existing dyes (Nelson *et al.*, 2002).

Until the latter half of the 19th century when synthetic dyes were discovered, all dyes were natural products from vegetables or animal origin except a few mineral colors and were extracted from the roots, stems, leaves, flowers and fruits of various dye plants and dried bodies of certain insects and shellfish (Singh, 2000).

The production and evaluation of microbial pigments as textile colorants is currently being investigated (Hamlyn, 1998). Fungi are more ecological interesting source of pigments, since some fungal species are rich in stable colorants such as anthraquinone (Gill and Steglich, 1987; Raisanen, 2001).

Many fungi produce pigments during their growth as intermediate metabolites such as *Aspergillus, Fusarium* and *Trichoderma*. Pigment production by fungi owing to the genetic background of the species and taken into consideration as a taxonomic feature.

Recently, there have been a revival of interest in natural dyes throughout the world as some synthetic dyes are being banned by the western countries due to their toxic, carcinogenic and polluting nature (Singh, 2000).

The aim of the present investigation is to select the most potent fungal isolate in producing brown and reddish brown dyes as well as the isolation, purification and structure elucidation of the dye components.

MATERIALS AND METHODS

Fungal isolates

Eleven fungal isolates were used in this study, they were *Acrostalagmus* sp. (NRC 90), *Alternaria alternata* (NRC 117), *Alternaria* sp. (NRC 97), *Aspergillus niger* (NRC 95), *Bisporomyces* sp. (NRC 63), *Cunninghamella* (NRC 188), *Penicillium chrysogenum* (NRC 74), *Penicillium italicum* (NRC E11), *Penicillium oxalicum* (NRC M25), *Penicillium regulosum* (NRC 50), *Phymatotrichum* sp. (NRC 151). The test fungi used were kindly provided from Culture Collection of the Department of Natural and Microbial Products Chemistry at the National Research Centre (NRC), Dokki, Cairo, Egypt.

Cultivation medium

Czapek Dox agar medium with the following composition was used as cultivation medium, $NaNO_3$, 2.0 g, K_2HPO_4, 1.0 g, KCl, 0.5 g, $MgSO_4 \cdot 7H_2O$, 0.5 g, $FeSO_4 \cdot 7H_2O$, 0.001 g, sucrose, 20 g, agar, 20 g. The pH was adjusted at 6.5 – 7.0.

Dye intermediate

The dye intermediate used in this research point was H-acid (1-naphthol-8-amino-3,6-disulfonic acid) which was kindly provided from Isma Dye Company, Kafr El-Dawwar, Egypt.

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Fermentation medium

Modified Czapek-Dox medium was used as a fermentation medium for dye production. One gram of H-acid was added to 1 L of fermentation medium. The level of carbon source was reduced to 10 g/L of fermentation medium.

Chromatography materials

Paper chromatograms Whatman No.1 for determination of single dye compounds (qualitative) and Whatman No.3 for preparative isolation and quantitative determination of single dye compounds.

Cellulose for column chromatography, Loba Company, was used for isolation and purification of dye compounds.

Isolation and purification of dye components

Column chromatography was used for isolation and purification of single dye components. Cellulose for column was used in this point. Crude dye material was concentrated and applied to the top of the column. Elution was done by distilled water, fractions were collected and concentrated. These fractions were reapplied separately to a cellulose column several times till obtaining pure compounds.

GC/MS of the single dye compounds

GC/MS spectrometer Finnigan mat SSQ 7000, digital DEC 3000 was used, capillary GC conditions as above were employed using DB-5 column. Injection volume was 1.0 µL, flow rate 1mL/min., injection mode (splitless). Significant MS operating parameters: ionization voltage 70 ev, injector temperature (220 °C), scan mass range (50 – 500 µ), with a temperature program 50 °C/5 min., 50 – 250 °C.

Determination of λ max of the dye components

Quantitative determination of single dye compounds was performed using standard curves of separate compounds which was determined by UV/VS spectrophotometry. Pure compounds were dissolved separately in distilled water and scanned for detection of λ max. using Perkin Elmer Double Beam Spectrophotometer.

Dyeing experiment

Lab scale dyeing experiments were carried out on wool fabrics using the produced dyes without mordanting (Gulrajani *et al.*, 1992). The dye bath was prepared with the produced dye at a liquor ratio 50:1 and with 1 g/L amphoteric leveling agent (albegal B), pH was adjusted to pH 3.0. Dyeing was started at 50 °C for ten min and then the dye bath temperature was raised to boil over 30 min and the dyeing continued for 45 min. After dyeing the temperature was lowered to 60 °C then the dye samples were rinsed and washed off in an aqueous solution 2 g/L non-ionic detergent (Hostapal CV) at 60 °C using a liquor ratio 50:1 for 30 min then rinsed and dried.

Color strength

The reflectance values of the dyed fabrics were measured using a Data Color SF 600 + Relative color strengths (K/S values) were determined using the Kubelka-Munk equation (Teli *et al.*, 2000).

$$K/S = \frac{(1-R)^2}{2R}$$

where R is the decimal fraction of the reflectance of dyed fabric, K is the absorption coefficient, and S is the scattering coefficient.

Fastness testing

The dyed samples were tested according to ISO standard methods (Bradford, 1990). The specific tests were as follows: ISO 105-C02 (1989), color fastness to washing; ISO 105-E04 (1989), color fastness to perspiration; and ISO 105-B02 (1988), color fastness to light (carbon arc).

Color fastness to washing

The color fastness to washing was determined according to the test method ISO 105-C02 (1989) the composite specimens were sewed between two pieces of bleached cotton and wool fabrics.

The composite specimens were immersed into an aqueous solution containing 5 g/L soap non-ionic detergent, at liquor ratio 50:1. The bath was thermostatically adjusted to 50 °C. The test was run for 45 min at 42 rpm, samples were then removed, rinsed twice with occasional hand squeezing, then dried. Evaluation of the wash fastness was established using the Grey-scale for color change.

Color fastness to light

The light fastness test was carried out in accordance with test method ISO 105-B02 using carbon arc lamp, continuous light for 35 h using ATLAS FADE-O METER MODEL 18-F (Bradford, 1990).

Evaluation of color fastness to light was determined using the Blue-scale for color change.

Color fastness to perspiration

Two artificial perspiration solutions were made as follows according to test method ISO 105-E04 (1989) (Bradford, 1990):

1-Acidic solution:

L-Histidine monohydrochloride monohydrate (0.5 g), sodium chloride (5 g), sodium dihydrogen orthophosphate dihydrate (2.5 g) were dissolved in 1 L distilled water.

Finally the pH was adjusted to 5.5 by NaOH solution (0.1 N).

2- Alkaline solution:

L-Histidine monohydrochloride monohydrate (0.5 g), sodium chloride (5 g), disodium hydrogen orthophosphate dehydrate (2.5 g), were dissolved in 1 L distilled water. Finally the pH was adjusted to 8.0 by NaOH solution (0.1 N).

The colored specimens (4 x 5 cm) were sewed between two pieces of uncolored specimens (cotton and wool) to form composite specimen. The composite samples were then immersed for (15-30 min) in each solution with occasional agitation and squeezing to insure complete wetting. The test specimens were placed between two glass or plastic plates under a force of about 4-5 kg. The plates containing the composite specimens were then held vertical in the oven at 37 °C $\pm$ 2 for 4 h.

The effect on the color of the test specimen was expressed and defined by reference to Grey-scale for color change.

RESULTS AND DISCUSSION

Inoculated fermentation media were incubated on a shaking incubator for 21 days at 27 $\pm$ 2 °C, elaboration of dyes was detected by *Acrostalagmus* sp. (NRC 90), *Alternaria alternata* (NRC 117), *Alternaria* sp. (NRC 97), *Aspergillus niger* (NRC 95), *Bisporomyces* sp. (NRC 63), *Cunninghamella* (NRC 188), *Penicillium chrysogenum* (NRC 74), *Penicillium italicum* (NRC E11), *Penicillium oxalicum* (NRC M25), *Penicillium regulosum* (NRC 50) and *Phymatotrichum* sp. (NRC 151) during the second week of the fermentation process (Table 1). The produced dyes were subjected to different analysis for isolation, purification, identification and quantitative determination of single dye components.

Analysis indicated that there were four compounds which were the main components for the produced dyes. GC indicated the following identification of each compound:

Compound 1:
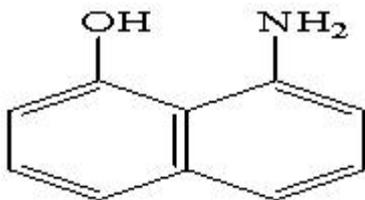Compound 1 was suggested to be 8-aminonaphthol.

Figure 1: 8-aminonaphthol, $C_{10}H_9NO$, Mol. Wt. 159

The mass spectrum (rel. int. %) of compound 1 (Figure 1) showed M^+ at m/z 159 (11%), corresponding to molecular formula $C_{10}H_9NO$. It losses H_2O to give fragment at m/z 141 (M^+ - H_2O, 18). Also losses another

fragment NH_2 to give (M^+ - NH_2) at m/z 127 (7) and other fragments at m/z 121 (10), 117 (16), 109 (10), 98 (16) and 96 (28).

Compound 2:
Compound 2 was suggested to be 4-(1-naphthol-8-amino)-4'-(1'-naphthol-8'-amino-6'-sulfonic acid).
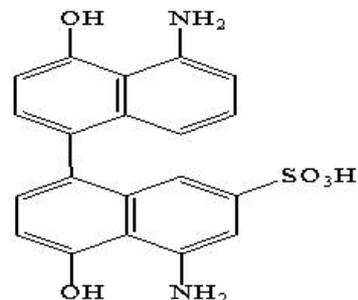

Figure 2: 4-(1-naphthol-8-amino)-4'-(1'-naphthol-8'-amino-6'-sulfonic acid), $C_{20}H_{16}N_2O_5S$, Mol. Wt. 396

The mass spectrum (rel. int. %) of compound 2 (Figure 2) showed M^+ at m/z 396 (1 %), corresponding to molecular formula $C_{20}H_{16}N_2O_5S$. It losses fragment H_2O to give (M^+ - H_2O) at m/z 378 (3). Other fragments were lost at m/z 348 (11), 226 (6), 215 (8), 195 (11), 188 (5) and 185 (8).

Compound 3:
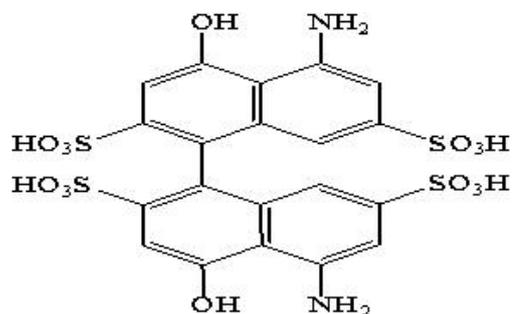Compound 3 was suggested to be 4,4'-bis-(1-naphthol-8-amino-3,6-disulfonic acid).

Figure 3: 4,4'-bis-(1-naphthol-8-amino-3,6-disulfonic acid), $C_{26}H_{16}N_2O_{14}S_4$, Mol. Wt. 636

The mass spectrum (rel. int. %) of compound 3 (Figure 3) suggested that M^+ 636 corresponding to molecular formula $C_{26}H_{16}N_2O_{14}S_4$. It losses 2 SO_3H to give fragment at m/z 478 (3 %). It also losses NH_2 to give fragment at m/z 462 (M^+ - NH_2, 2). Also losses another fragments at m/z 440 (17), 426 (13), 410 (33), 396 (10), 385 (17), 358 (11), 348 (15), 330 (14), 310 (10) and 297 (12).

Compound 4:

Compound 4 was suggested to be 4-(1-naphthol-8-amino)-4'-(1'-naphthol-8'-amino-3',6'-disulfonic acid).
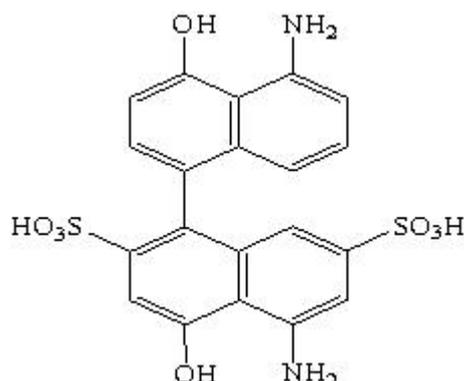

Figure 4: 4-(1-naphthol-8-amino)-4'-(1'-naphthol-8'-amino-3',6'-disulfonic acid), $C_{26}H_{16}N_2O_8S_2$, Mol. Wt. 477

The mass spectrum (rel. int. %) of compound 4 (Figure 4) showed M^+ at m/z 478 (6 %), corresponding to molecular formula $C_{26}H_{16}N_2O_8S_2$. It losses SO_3H_2 to give fragment at m/z 396 (M^+ - SO_3H, 10). Also losses another fragments H_2O and NH_2 to give (M^+ - H_2O and - NH_2) at m/z 362 (6) and losses another fragment SO_3H to give (M^+ - SO_3H) at m/z 282 (5). Other fragments are lost at m/z 252 (16), 232 (6), 223 (6), 160 (15), 147 (13) and 123 (22).

Production model of the dye

Figure 5 illustrate the suggested structures of the dye compounds produced by fungi and their inter-relations, also illustrate the relation of these compounds to the dye precursor (H-acid).

The change in compounds percentages in the fermentation medium (Table 1), deduced from the change of groups due to fungal activity exerted in H-acid during the fermentation process. This was in agreement with what stated by Nagia and EL-Mohamedy (2007) on fungal culture of *F. oxysporum* isolate No. 4, and (Turner and Aldridge, 1983; Yagi *et al.*, 1993) on dermatocyke cinaborina.

Table 1: Quantitative determination of single dye components, mg/L produced by the tested fungi

Fungal strain	Color	Dye	λ max.	2nd Week				3rd Week				K/S values
				C1	C2	C3	C4	C1	C2	C3	C4	
Acrostalagmus (NRC 90)	Brown		485	130	98	76	75	190	113	100	109	10.4
Alternaria alternata (NRC 117)	R. brown		485	76	111	95	87	50	125	111	115	8.0
Alternaria sp (NRC 97)	Brown		482	105	ND	186	79	80	ND	310	108	10.5
Aspergillus niger (NRC 95)	Brown		482	73	143	158	79	40	131	220	106	7.26
Bisporomyces sp. (NRC 63)	Deep brown		480	76	ND	167	97	40	ND	181	160	13.7
Cunninghamella (NRC 188)	F. R. brown		489	43	ND	53	ND	75	ND	72	104	9.51
Penicillium chrysogenum (NRC 74)	Deep brown		478	113	ND	143	88	190	ND	156	120	6.62
Penicillium italicum (NRC E11)	Brown		471	65	123	163	ND	67	130	200	ND	7.9
Penicillium oxalicum (NRC M25)	F. R. brown		478	43	152	132	65	40	176	166	74	14.3
Penicillium regulosum (NRC 50)	Brown		477	65	ND	43	122	92	ND	31	145	8.0
Phymatotrichum sp. (NRC 151)	R. brown		480	130	144	108	165	150	75	124	275	17.6

C1: Compound 1; C2: Compound 2; Compound 3; C4: Compound 4; K/S: Absorbance / Scattering; ND: Not detected

$C_{20}H_{16}N_2O_{14}S_4$
Mol. Wt.: 636

4,4'-bis (1-naphthol-8-amino-3,6-disulfonic acid)

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Dimerization

$C_{10}H_9NO_7S_2$
Mol. Wt.: 319.3

1-naphthol-8-amino-3,6-disulfonic acidl

-2 SO$_3$H

$C_{20}H_{16}N_2O_8S_2$
Mol. Wt.: 477

Fusion

$C_{10}H_9NO$
Mol. Wt.: 159

4- (1-naphthol-8-amino)-4'-(1'-naphthol-8'-amino-3',6'-disulfonic acid)

4

8-aminonaphthol

1

-SO$_3$H

$C_{20}H_{16}N_2O_5S$
Mol. Wt.: 396

4- (1-naphthol-8-amino)-4'-(1'-naphthol-8'-amino-6'-sulfonic acid)
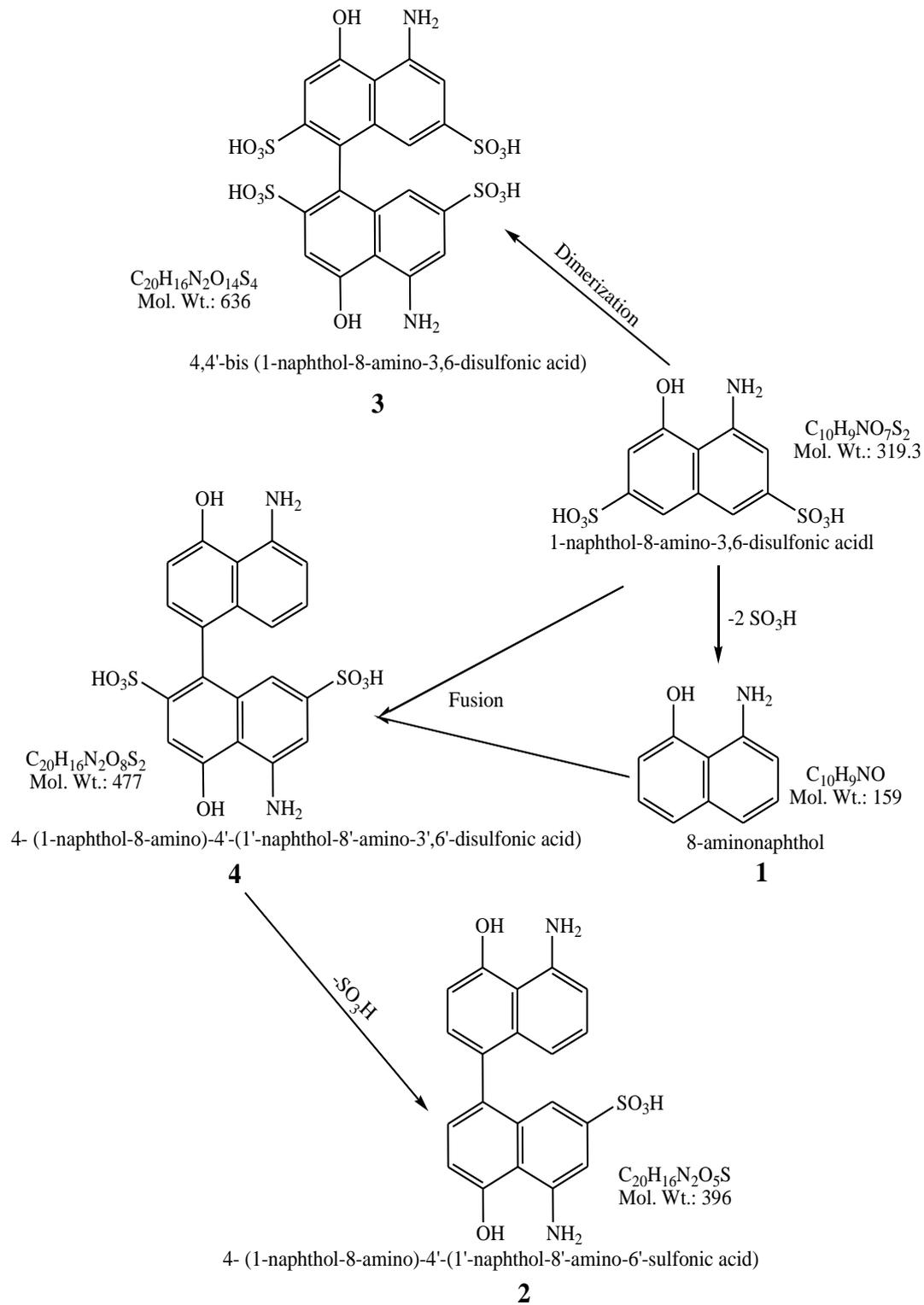
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Figure 5: Suggested structures of compounds produced by fungi and their inter-relations

Production of dyes by fungi

Acrostalagmus sp. (NRC 90)

Acrostalagmus sp. (NRC 90) produced brown dye during the fermentation process. The dye that has the best fastness criteria and good reflectance (485 nm) was produced at the end of the fermentation period (21 days). Table 1 indicated the quantitative amounts (mg/L) of the dye components. The produced dye color is composed of dye components ratio (C1) 37%, (C2) 22%, (C3) 20% and (C4) 21%. The dyed wool fabrics exhibited very good ratings to wash fastness, good to very good ratings to perspiration fastness.

The K/S of dyed samples showed high value (10.4) (Table 1). There was a significant variation in the K/S values and the dye exhaustion, which is proportional to the amount of dye in the fiber. This led us to assume that when the dye absorption on the fiber is faster and exhaustion tends to be completed, the dye is transferred from the endocuticle to the sulphur-rich exocuticle rather than into the cortex (Musnickas, 2005).

Dyed wool samples also exhibited very good ratings to light fastness (Table 2). Nearly all natural dyes have a light-fastness below blue scale grade 5. Most have a fastness below 4. Nearly all natural dyes will fade badly during an exposure to 50 million lux hours of artificial light, or to a much smaller dose of daylight (Tim and Sheila, 1966).

Table 2: Fastness properties of the produced dyes

| Fungal strain | Wash fastness | | | Perspiration fastness | | | | | | Light fastness |
| | | | | Acid | | | Alkali | | | |
	Alt.	SC	SW	Alt.	SC	SW	Alt.	SC	SW	
Acrostalagmus (NRC 90)	4	4	4	4-5	3	4	4	3	4	5/8
Alternaria alternate (NRC 117)	4-5	4	3-4	4-5	3-4	4	4	3	3-4	5/8
Alternaria sp. (NRC 97)	4	4-5	4-5	4	4	4	4	3-4	4	5-6/8
Aspergillus niger (NRC 95)	4	4-5	4	4	3-4	4	4	3	4	3/8
Bisporomyces sp. (NRC 63)	4-5	4	4-5	4	3	4	4	2-3	4	6/8
Cunninghamella (NRC 188)	4	4	4	4-5	3-4	4	4-5	3	4	5-6/8
Penicillium chrysogenum (NRC 74)	4-5	4-5	4-5	4	4	4	4	4-5	4-5	3/8
Penicillium italicum (NRC E11)	4	4-5	4-5	4-5	3-4	4-5	4-5	3	4	5-6/8
Penicillium oxalicum (NRC M25)	4-5	4	4	4-5	3	4	4	3	3-4	5-6/8
Penicillium regulosum (NRC 50)	4-5	4	4-5	4	3-4	4	4-5	2-3	4	6/8
Phymatotrichum sp. (NRC 151)	4-5	4-5	4-5	4	4	4	4-5	4-5	4-5	5-6/8

Alt: Alteration of the dye yield of dyed wool in comparison with non treated one; SC: Staining on non-dyed cotton; SW: Staining on non-dyed wool

Alternaria alternata (NRC 117)

Alternaria alternata (NRC 117) produced reddish brown dye at the end of the fermentation process. The dye color is composed of dye component ratio (C1) 12%, (C2) 31%, (C3) 28% and (C4) 29%. The dyed wool fabrics had a very good reflectance (485 nm) and good K/S values (8.0) (Table 1), which indicates the dye-fiber affinity. Also this value was assumed to be in direct proportional to the increase in production of compounds (1) and (4).

Dyed wool samples expressed good to very good fastness properties to washing, perspiration and light (Table 2). Fastness properties of the dyed fabrics with natural anthraquinones produced by *Fusarium oxysporum*

indicate good fastness properties of the dyed samples (Nagia and El-Mohamedy, 2007).

Alternaria sp. (NRC 97)

Alternaria sp. (NRC 97) produced brown dye at the end of the incubation period. The dye color is composed of dye components ratio (C1) 16%, (C3) 62% and (C4) 22%. The dyed wool samples had a very good reflectance (482 nm) and K/S values (10.54) which indicate the dye-fiber affinity. Dyed wool samples expressed good to very good fastness properties to washing, perspiration and light.

Aspergillus niger (NRC 95)

Aspergillus niger (NRC 95) produced brown dye at the end of the incubation period. The dye color is composed of dye component ratio (C1)8%, (C2) %, (C3) 44% and (C4) 21%**.** Production of dye components 1 and 2 were decreased during the third week of the fermentation process, while production of components 3 and 4 increased by time till reached their maxima at the end of incubation (Table 1). It was suggested that compound 1 was transformed by fusion with H-acid to compound 4, while compound 2 was decomposed by the fungus and being utilized for the formation of compound 3 as indicated in Figure 5. The produced dye showed a very good reflectance (482 nm) and good K/S values (7.26).

Dyed wool samples exhibited good to very good ratings to washing, perspiration. They also exhibited good fastness criteria to light (Table 2).

Aspergillus fonsecaeus produce yellow pigments in the vesicles and primary sterigmata, this material is a possible precursor of the brown and black pigments. This result agreed with what produced by *A. niger* (NRC 95), (Oscar *et al.,* 1962).

Bisporomyces sp. (NRC 63)

Bisporomyces sp*.* (NRC 63) produced deep brown dye at the end of the incubation period with good to very good fastness properties to washing, perspiration and UV light (Table 2). As well as a very good reflectance (480 nm) and K/S value (13.7). The dye color was composed of dye component ratio (C1) 10%, (C3) 48% and (C4) 42%. Production of compound 1 decreased till reached the minimum at the end of the fermentation process, while production of compound 3 and 4 increased by time till reached their maxima at the end of incubation period (Table 1).

Cunninghamella (NRC 188)

Cunninghamella (NRC 188) produced faint reddish brown dye at the end of the incubation period which is composed of dye component ratio (C1) 30%, (C3) 29% and (C4) 41%. Production of the three compounds increased by time till reached their maxima at the end of the fermentation process; this was reflected on the dye color. The produced dye had good reflectance (480 nm) and K/S value (9.51) (Table 1).

Results presented in Table 2 illustrate fastness properties of dyed wool samples to washing, perspiration (acidic and alkaline) where they exhibited good to very good fastness properties. Dyed wool fabrics expressed very good fastness properties upon exposure to UV light.

Penicillium chrysogenum (NRC 74)

Results presented in Table 1 indicated that *Penicillium chrysogenum* (NRC 74) produced deep brown dye at the end of the incubation period. The dye color is composed of dye components percentage as follow, (C1) 41%, (C3)

33% and (C4) 26% and production of these compounds increased by time till reached their maxima at the end of the fermentation process. The dyed wool fabric sample had good reflectance (478 nm) and K/S value (6.62).

Dyed wool samples exhibited good to very good fastness properties to washing and perspiration, while expressed moderate fastness to light Table 2.

Penicillium italicum (NRC E11)

Penicillium italicum (NRC E11) produced brown dye at the end of the incubation period. The dye color is composed of dye components ratio (C1) 17%, (C2) 33% and (C3) 50%. The compound 3 increased during the incubation period till reached the maximum at the end of the experiment, Table 1. The dyed wool fabrics had good reflectance (471 nm) and K/S value (7.9).

Results presented in Table 2 illustrate fastness properties of dyed wool samples to washing, perspiration where they exhibited good to very good fastness properties. Dyed wool fabric expressed very good fastness criteria upon exposure to UV light.

Penicillium oxalicum (NRC M25)

Penicillium oxalicum (NRC M25) produced faint reddish brown dye at the end of the incubation period with good reflectance (478 nm) and very good K/S value (14.38). The dye color is composed of dye component ratio (C1) 9%, (C2) 39%, (C3) 36% and (C4) 16%. Production of compound (1) decreased by time till reached the minimum at the end of the fermentation process. In contrary, production of compounds 2, 3 and 4 increased to reach their maxima at the end of incubation period (Table 1). Dyed wool samples exhibited good to very good fastness properties to washing, perspiration and light (Table 2).

Penicillium regulosum (NRC 50)

Penicillium regulosum (NRC 50) produced brown dye at the end of the incubation period which is composed of dye component ratio (C1) 34%, (C3) 12% and (C4) 54%. Production of compound (3) decreased by time till reached its minimum at the end of the fermentation process. In contrary, production of compound 1 and 4 increased to reach their maxima at the end of incubation period. The dyed wool sample had good reflectance (477 nm) and K/S value (8.0) (Table 1).

Dyed wool samples exhibited good to very good fastness properties to washing, perspiration and light (Table 2).

Phymatotrichum sp. (NRC 151)

Phymatotrichum sp. (NRC 151) produced reddish brown dye at the end of incubation period. The color of the dye is composed of dye components ratio (C1) 24%, (C2) 12%, (C3) 20% and (C4) 44%. Production of compound 2 decreased by time till reached the minimum at the end of the fermentation process. In contrary, production of

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compounds 1, 3 and 4 increased to reach their maxima at the end of incubation period.

Increase in the production of dye component 4 was assumed to affect the color of the produced dye, reflectance (480 nm), as well as the K/S value (17.67). Dyed wool samples exhibited very good fastness properties to washing, perspiration and light (Table 2).

CONCLUSION

In absence of compound 2 the produced dye color became deep brown as in *Bisporomyces* sp. (NRC 63) and *Penicillium chrysogenum* (NRC 74) dye, while absence of compound 2 and 4, the produced dye became faint reddish brown (*Cuningahamella,* 188). These results confirm the importance of the four compounds for the appearance of the reddish brown dye. Absence or presence of compound 4 in a low ratio in the produced dye, the emerged dye became brown.

The dye produced by *Phymatotrichum* sp. (NRC 151) had the best fastness criteria to washing, perspiration and light. So it became clear that the best producer fungal strain for the reddish brown dye is *Phymatotrichum* sp*.* (NRC 151).

It became evident that the dye material have the characteristics of a typical disperse dye like henna, madder and juglone, ie. they are based on a quinine, anthraquinone or naphthoquinone molecules (Deepti, 2000).

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