Assessment of the dyeing properties of pigments from *Monascus purpureus*

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Abstract: *Monascus purpureus* C322 was cultivated on well-established production media to yield prevalingly red or orange pigment-rich ethanolic extracts. Once these extracts had been diluted by an overall factor of 50, they were used as such to dye raw wool standard specimens differently premordanted using alum or stannic chloride. Independently of the mordant used, the specimens dyed with the red pigment-rich extracts showed a pale red colour tending to pink, whereas the specimens dyed with the orange pigment-rich extracts exhibited a more definite orange colour. By carrying out a few colourfastness standard tests (manual washing at 40 °C, acid and basic perspiration and hot pressing), stannic chloride-premordanted wool specimens dyed with the red pigment-rich extracts were found to be less resistant to acid and basic perspiration than their orange counterparts. Since the production of the orange pigment-rich ethanolic extracts appeared to be more cost-effective than that of their red counterparts, the former might support the present demand for colorants of natural origin in the textile sector.

Keywords: colourfastness tests; dyeing tests; *Monascus purpureus*; mordants; orange and red pigments; wool specimens

INTRODUCTION

The worldwide demand for colorants of natural origin, especially yellow and red pigments, is rapidly increasing in the food, cosmetic and textile sectors.

As far as the textile sector is concerned, it nowadays makes almost exclusive use of synthetic dyes. Not only their production techniques but also their uses to manufacture coloured fabrics or yarns have a tremendous environmental impact. To process 1 Mg of textile, quite large volumes (230–270 m³) of water are needed and this gives rise to enormous volumes of exhausted dye baths containing up to 90% of the chemicals used in the finishing processes, which have to be disposed of. Moreover, a great deal of concern has been raised by the presumed or actual noxious effects of some synthetic dyes on human health as sources of skin cancer, disorders and allergic reactions.

The general consumer in the USA and EU appears to be quite aware of such problems. For instance, the commercial success of natural-coloured underwear is just one indication of a new positive attitude towards natural colorants in spite of the fact that their synthetic counterparts are generally advantaged by far lower prices.

Several research projects have so far been carried out to evaluate the techno-economic feasibility of today’s alternative dye crops. Among the species examined, common madder (*Rubia tinctorum* L.), woad (*Isatis tinctoria* L.) and weld (*Reseda luteola* L.) proved to be quite interesting sources of red (alizarin), indigo (indigotin) and yellow (luteolin) dyes respectively, either for their agronomic characteristics or for their dyeing properties. In fact, all three dyes were extensively exploited until the commercial success of their synthetic analogues.

The main disadvantage of these natural dyes lies in the order of magnitude of their extraction yield factors (a few grams of pigment per kg of dried raw material). This makes their current market price about US$1/g, thus limiting their application to high-value-added natural-coloured garments only.

To overcome this limitation, it was suggested to exploit the potentiality of other biological sources such as fungi (both moulds and yeasts), bacteria, algae and plant cell cultures, since appropriate selection, mutation or genetic engineering techniques are likely to improve significantly the pigment production yields with respect to wild organisms.

Among the several pigment-producing microorganisms described in the literature, the fungus *Monascus* has been thoroughly studied. It has been traditionally used for manufacturing food colorants (e.g. red rice) and fermented foods and beverages in...
Southern and Far Eastern Asia, the latter being also used in medical therapy to promote blood circulation and proper cholesterol levels, prevent gastric and intestinal disorders, stimulate digestion, etc. The several pigments produced by Monascus are oligoketides and have been subdivided into three groups. Rubropunctatin and monascorubrin are orange pigments, presenting different side chains on the ozolactone ring. Their two azoto analogues are the red pigments rubropunctamine and monascorubramine, whereas their reduced forms are the yellow pigments monascin and ankaflavin. Two other novel yellow pigments, xanthomonascin A and ‘Yellow II’, were also identified.

Monascus pigment production either by submerged culture using free or immobilised cells or by solid state culture in complex media has been thoroughly studied. The use of Monascus cultures as food additives is not approved either in the EU or in the USA, though it is currently permitted in Japan. All reagents were of analytical grade. All reagents were of analytical grade.

Materials and Methods

Micro-organism

Monascus purpureus C322 was obtained from the culture collection of the Department of Food Science and Technology, University of California, Davis, CA, USA. Stock cultures were maintained on medium MPI (see below) agarised with 20 g dm\(^{-3}\) technical agar at 4 °C and subcultured every month.

Raw material

For all dyeing tests, premordanted raw wool (CIELAB co-ordinates \(L^* = 64.0 \pm 0.1, a^* = -0.6 \pm 0.6, b^* = -0.3 \pm 0.2; \) density 0.0225 g cm\(^{-3}\) ) fabrics were used.

Culture media and operating conditions

The medium MPI developed by Shepherd was used for spore development and vegetative seed culture. Media for the production of orange, red and yellow pigments were MPIIII, MSG and GA respectively. These media contained (g dm\(^{-3}\) deionised water): MPI—glucose, 40.0; K\(_2\)HPO\(_4\), 3.0; yeast extract, 10.0; MPIIII—glucose, 40.0; K\(_2\)HPO\(_4\), 1.0; MgSO\(_4\) \(\times\) 7H\(_2\)O, 0.5; NaCl, 0.5; FeSO\(_4\) \(\times\) 7H\(_2\)O, 0.1; NH\(_4\)Cl, 0.5; MSG—maltose, 50.0; anhydrous sodium glutamate, 12.6; K\(_2\)HPO\(_4\), 2.4; KH\(_2\)PO\(_4\), 2.4; MgSO\(_4\) \(\times\) 7H\(_2\)O, 8.0; KCl, 0.5; FeSO\(_4\) \(\times\) 7H\(_2\)O, 0.01; ZnSO\(_4\) \(\times\) 7H\(_2\)O, 0.01; MnSO\(_4\) \(\times\) 7H\(_2\)O, 0.003; GA—starch, 25.0; glutamic acid, 2.0; peptone, 4.0. All reagents were of analytical grade.

Analytical methods

The pigments produced by M. purpureus were extracted by adding two volumes of 95% ethanol per volume of exhausted culture broth according to the following procedure: (i) after dilution with about 60% of the solvent volume needed, the resulting mixture was kept on the rotary shaker at 180 rev min\(^{-1}\) and 30 °C for 30 min; (ii) the ethanol mixture was centrifuged at 3780 \(\times\) g for 15 min; (iii) once the supernatant had been recovered, the residue was dispersed in the remaining volume of ethanol and centrifuged again at 3780 \(\times\) g for 5 min; (iv) the supernatants collected were filtered through a preweighed Whatman GF/C disc (47 mm) and further diluted with 95% ethanol to a final volumetric dilution factor of 20; (v) their absorption spectrum in the range 350–500 nm was recorded using a Hitachi U-2000 spectrometer (Hitachi Ltd, Tokyo, Japan). According to Johns and Stuart, the optical density (OD) measured at about 400, 470 or 500 nm (these wavelengths representing the absorption maxima for yellow, orange and red pigments respectively) was multiplied by the above dilution factor, thus yielding the so-called yellow, orange or red pigment production, expressed in units of absorbance (UA\(_X\)) at a given wavelength (\(\lambda\)). Finally, the mycelial biomass recovered by centrifugation was dispersed in deionised water, filtered through a preweighed Whatman GF/C disc (47 mm), dried at 105 °C for 12–15 h and weighed to yield the biomass (X) concentration.

Preparation of fabric specimens

Raw wool fabrics were subordinated into standard specimens (10 cm x 4 cm) in accordance with the Italian Association of Textile Colourists.
Mordanting procedures
To assess the most appropriate mordant for Monascus pigments, a series of mordants generally used in natural dyeing procedures (alum, A; tannic acid, TA; copper sulphate, CS; iron sulphate, IS; stannic chloride, SC) was tested. All reagents were of analytical grade. Their doses, expressed in g per 100 g of fabric and extracted from Refs 26 and 27, are listed in Table 1. To mordant 100 g of raw wool specimen, the following general procedure was used.

1. Dissolve the amount of cream of tartar shown in Table 1 in 2.5 dm³ of demineralised warm water.
2. Heat the solution to boiling point and dissolve the amount of mordant shown in Table 1.
3. Add cold water to a final volume of 4 dm³ and regulate the bath temperature at 40 °C.
4. Dip the wool specimen, previously submerged in water for 20 min, in the mordanting solution and heat the suspension to boiling point for 2 h in the case of alum or for 1 h in the case of all other mordants tested; then let the suspension cool to room temperature for at least 3 h.

Dye bath preparation
The dye bath (500 cm³) was prepared by diluting the ethanolic extracts with deionised water by a volumetric factor of 10 or 2.5 and then adding 2 g dm⁻³ NaCl.²⁶

Dyeing procedure
Premordanted raw wool standard specimens (100 g) were dipped in the dye bath (0.5 dm³), which was slowly heated to 22, 50 or 90 °C. The temperature was then kept constant for 1 h, stirring regularly. After letting the specimens cool off in the pot, they were rinsed in tap water repeatedly until the rinsewater looked clear and then hung to dry in the shade.

Colourfastness standard tests
All dyed specimens were submitted to a few colourfastness standard tests, namely manual washing at 40 °C, acid (pH 5.5) and basic (pH 8) perspiration and hot pressing at 150 ± 2 °C for 15 s.²⁵

Colour measurements
The colour of specimens, before and after dyeing or any colourfastness test, was measured using a D25-PC2 portable colour-measuring instrument (Hunterlab, Restow, VA, USA) with a diffuse (0/45°) illumination viewing geometry and a 50 mm diameter specimen aperture. After calibrating the instrument, the specimen was mounted between two glass plates and its colour was assayed in three different (left, central and right) positions by recording the resulting CIELAB co-ordinates (L*, a*, b*).

RESULTS AND DISCUSSION
Pigment production
Table 2 shows the main results of the 7–8 day fermentation trials of Monascus purpureus C322 on the media given above, while Fig 1 shows the absorption spectra of the corresponding clarified culture broths diluted with ethanol by an overall volumetric factor of 40. The extracts obtained from media MPIII, MSG and GA presented absorption maxima at 440, 520 and 380 nm respectively. According to Johns and Stuart,²³ they were labelled as orange, red and yellow pigment-rich extracts respectively.

Maximum production (>20 UA) of pigments was observed when the fungus was cultivated on medium MPIII, a medium that contained ammonium chloride as nitrogen source. This confirmed the importance of this salt for high pigment yield factors.¹⁹,²⁸

Using medium MSG, the red pigments should be largely predominant as a consequence of the presence of sodium glutamate as sole nitrogen source. This confirmed the importance of a cultural pH around neutral.¹⁷,²⁹ In this case, in spite of the fact that there was no statistically significant difference between red and yellow pigment was then kept constant for 1 h, stirring regularly. After letting the specimens cool off in the pot, they were rinsed in tap water repeatedly until the rinsewater looked clear and then hung to dry in the shade.

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Table 1. Doses of mordant and cream of tartar used per 100 g of fabric, as extracted from Refs 26 and 27

<table>
<thead>
<tr>
<th>Mordant</th>
<th>Raw formula</th>
<th>Cream of tartar</th>
<th>Dose of tartar (g per 100 g of fabric)</th>
<th>Cream of tartar (g per 100 g of fabric)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alum (A)</td>
<td>KAl(SO₄)₂·12H₂O</td>
<td>25</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Tannic acid (TA)</td>
<td>C₆H₁₀O₆</td>
<td>12</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Copper sulphate (CS)</td>
<td>CuSO₄</td>
<td>12</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Iron sulphate (IS)</td>
<td>FeSO₄</td>
<td>12</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Stannic chloride (SC)</td>
<td>SnCl₄</td>
<td>3</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Overall results of 7–8 day activity of Monascus purpureus C322 on different culture media: mean values and standard deviations of pH and biomass concentration (X) in culture broth as well as of yellow, orange and red pigment production (UA) at different wavelengths (λ, expressed in nm) and prevailing colour of clarified culture broths diluted with ethanol by an overall volumetric factor of 40

<table>
<thead>
<tr>
<th>Culture medium</th>
<th>pH</th>
<th>X (g dm⁻³)</th>
<th>UA₄₀₀</th>
<th>UA₄₇₀</th>
<th>UA₅₅₀</th>
<th>Extract coloura</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPIII</td>
<td>2.4 ± 0.1</td>
<td>5.2 ± 0.9</td>
<td>21.1 ± 0.6</td>
<td>20.5 ± 0.3</td>
<td>11.6 ± 1</td>
<td>O</td>
</tr>
<tr>
<td>MSG</td>
<td>6.8 ± 0.2</td>
<td>13.2 ± 1.9</td>
<td>10.0 ± 0.3</td>
<td>7.2 ± 0.4</td>
<td>10.2 ± 0.0</td>
<td>R</td>
</tr>
<tr>
<td>GA</td>
<td>2.2 ± 0.8</td>
<td>4.0 ± 0.5</td>
<td>12.4 ± 0.5</td>
<td>3.6 ± 0.9</td>
<td>3.4 ± 0.7</td>
<td>O/R</td>
</tr>
</tbody>
</table>

a O, orange; R, red.
Data refer to three independent fermentation runs.
productions at a confidence level of 0.05 (Table 1), the prevailing colour of both these ethanolic extracts was red (Table 1). Moreover, owing to quite high mycelial growth (13.2 ± 1.9 g dm⁻³), the fermentation trials performed on medium MSG were stopped after 7 days, ie 1 day before the trials performed on media MPIII and GA.

Orange pigments were mainly produced by M purpureus C322 on medium MPIII at an acidic pH (the final value of pH being 2.4), although the favourable pH for accumulating these pigments is typically above 5.29

Generally speaking, the visible colour of any ethanolic extract depends on the relative amounts of the three pigment types.

Owing to the complexity of the pigment mixture produced by M purpureus, the pigment composition determined via absorbance measurements (as shown in Fig 1) was found to differ from that found by HPLC analysis,30–32 probably because of the formation of one or more unknown compounds that interfere with the absorption maxima of pigments from Monascus species.28 Thus the use of ethanolic extracts with the same optical density at the three reference wavelengths mentioned above does not guarantee the same colour at the grid origin (a' = 0, b' = 0) is achromatic (grey). On the horizontal axis a positive value of a' indicates a hue of red/purple and a negative value a hue of bluish-green. On the vertical axis a positive value of b' indicates a hue of yellow and a negative value a hue of blue.34

When alum (A) or stannic chloride (SC) was used to mordant raw wool, its original grey colour was practically unaltered. In fact, by measuring the CIELAB co-ordinates (L*, a*, b*) of several jth premordanted wool specimens in three different (left, central and right) positions, which were averaged as

\[
A : L_A^* = 65.2 ± 0.1, \quad a_A^* = -1.1 ± 0.6, \quad b_A^* = -0.7 ± 0.9
\]

\[
SC : L_{SC}^* = 64.7 ± 0.2, \quad a_{SC}^* = -1.7 ± 2.0, \quad b_{SC}^* = -2.8 ± 0.3
\]

it was not possible to assess any statistically significant colour difference between raw wool and A- or SC-premordanted wool specimens at a confidence level of 0.05, as also shown in Fig 2.
using orange or red pigment-rich extracts diluted with ethanol by the same factor of 50.

The colour of any generic \( j \)th dyed specimen was measured by recording its CIELAB co-ordinates \( (L^*_j, a^*_j, b^*_j) \) in three different (left, central and right) positions, which were averaged as shown in Table 3.

For any set of dyed specimens the differences between any measured CIELAB co-ordinate in any of the three positions tested and its corresponding average value listed in Table 3 were found to be statistically insignificant at the 95\% confidence level, thus involving no colour change not only within the same \( j \)th dyed specimen but also within each set of specimens tested. Moreover, by calculating the colour difference \( (\Delta E'_j) \) between each \( j \)th wool specimen differently premordanted and dyed and the original raw one via eqn (1), it was possible to assess that both mordanting and dyeing procedures gave rise to a uniform difference in colour between dyed and raw specimens (Fig 3). In particular, when using alum or tin chloride as mordant, \( \Delta E'_j \) ranged from 40 to 45 with an average value of 42\pm2 in the case of orange pigments and from 22 to 25 with an average value of 23\pm1 in the case of red pigments. Such a colour difference is mainly due to the fact that the average lightness coefficient \( (L^*_j) \) of red pigment-dyed specimens was about two-thirds greater than that pertaining to the orange pigment-dyed ones (Table 3). By plotting the hue sequence and hue angle orientation for any set of specimens in the \((a^*, b^*)\) plane (Fig 2), it is possible to collect further information on specimen colour.

In all tests, independently of the \( j \)th mordant used, the characteristic colour points of red (R) and orange

Moreover, to better quantify the differences in metric lightness and chromaticity for the different sets of wool specimens, the colour difference \( (\Delta E'_j) \) between each \( j \)th premordanted wool specimen and the original raw one (the CIELAB co-ordinates of which are reported under ‘Raw material’) was calculated as the square root of the squares of the respective differences of \( L^*, a^* \) and \( b^* \):

\[
\Delta E'_j = \sqrt{(L^*_j - L^*o_j)^2 + (a^*_j - a^*o_j)^2 + (b^*_j - b^*o_j)^2} \tag{1}
\]

thus resulting in an insignificant colour difference \( \Delta E'_j \) of 1.6 or 3.3 when using A or TS as mordant respectively.

Following previous work on pigment production by free or immobilised cells of \( M. \) purpureus C322,\(^{22}\) a series of preliminary dyeing tests was carried out to assess the effects of the overall dilution factor \( (D) \) of the ethanolic extracts mentioned above and the dye bath temperature \( (T_{DB}) \) in the ranges 50–200 and 22–90\degreeC\, respectively.

For instance, when using a 200-fold diluted orange pigment-rich extract at 90\degreeC, the average colour difference \( (\Delta E'_j) \) between each \( j \)th alum-premordanted and dyed specimen and the original raw one was less than 30. When using a 50-fold diluted red pigment-rich extract at 22 or 50\degreeC, the average colour difference \( (\Delta E'_j) \) between each \( j \)th alum-premordanted and dyed specimen and the original raw one was of the order of 12 or 26 respectively. By accounting for the sensitivity to heat of red Monascus pigments,\(^{20}\) \( T_{DB} \) was set to 50\degreeC.

To assess the most appropriate mordant, four sets of 10 raw wool standard specimens were differently mordanted using A or SC and then dyed at 50\degreeC.
Table 3. Colour characteristics of 10 wool standard specimens premordanted using alumin or stannic chloride and dyed at 50 °C using orange or red pigment-rich extracts diluted with ethanol by an overall factor of 50: average values and standard deviations of CIELAB co-ordinates (L\(^*\), a\(^*\), b\(^*\)) measured in three different (left, central and right) positions

<table>
<thead>
<tr>
<th>Dyed specimen no</th>
<th>Alum</th>
<th>Stannic chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L(^*)</td>
<td>a(^*)</td>
</tr>
<tr>
<td>Orange pigments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>29.0 ± 0.6</td>
<td>20.5 ± 1.5</td>
</tr>
<tr>
<td>2</td>
<td>29.4 ± 1.4</td>
<td>19.3 ± 1.5</td>
</tr>
<tr>
<td>3</td>
<td>29.3 ± 0.7</td>
<td>20.3 ± 0.4</td>
</tr>
<tr>
<td>4</td>
<td>28.3 ± 1.9</td>
<td>19.1 ± 0.7</td>
</tr>
<tr>
<td>5</td>
<td>29.3 ± 1.0</td>
<td>21.4 ± 2.1</td>
</tr>
<tr>
<td>6</td>
<td>28.0 ± 1.0</td>
<td>22.1 ± 1.4</td>
</tr>
<tr>
<td>7</td>
<td>26.7 ± 0.6</td>
<td>22.5 ± 1.0</td>
</tr>
<tr>
<td>8</td>
<td>31.0 ± 1.4</td>
<td>19.3 ± 1.5</td>
</tr>
<tr>
<td>9</td>
<td>29.5 ± 0.2</td>
<td>19.0 ± 0.5</td>
</tr>
<tr>
<td>10</td>
<td>29.6 ± 0.9</td>
<td>19.1 ± 0.9</td>
</tr>
<tr>
<td>Ave</td>
<td>29 ± 1</td>
<td>20 ± 2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Red pigments</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47.6 ± 0.8</td>
<td>13.1 ± 1.4</td>
<td>8.6 ± 0.3</td>
<td>49.8 ± 0.9</td>
<td>13.0 ± 1.6</td>
<td>14.2 ± 0.4</td>
</tr>
<tr>
<td>2</td>
<td>47.3 ± 0.5</td>
<td>12.7 ± 0.6</td>
<td>8.9 ± 0.2</td>
<td>50.9 ± 0.2</td>
<td>9.9 ± 0.4</td>
<td>14.3 ± 0.5</td>
</tr>
<tr>
<td>3</td>
<td>48.3 ± 0.4</td>
<td>12.8 ± 0.6</td>
<td>8.5 ± 0.4</td>
<td>52.1 ± 0.5</td>
<td>10.2 ± 1.3</td>
<td>14.2 ± 0.5</td>
</tr>
<tr>
<td>4</td>
<td>46.9 ± 0.6</td>
<td>14.2 ± 1.2</td>
<td>8.5 ± 0.1</td>
<td>49.8 ± 0.8</td>
<td>12.7 ± 1.2</td>
<td>14.6 ± 0.2</td>
</tr>
<tr>
<td>5</td>
<td>49.0 ± 0.3</td>
<td>12.7 ± 0.6</td>
<td>8.9 ± 0.2</td>
<td>50.0 ± 0.8</td>
<td>10.9 ± 1.1</td>
<td>14.2 ± 0.4</td>
</tr>
<tr>
<td>6</td>
<td>48.1 ± 0.1</td>
<td>13.6 ± 0.4</td>
<td>8.7 ± 0.0</td>
<td>49.6 ± 0.6</td>
<td>11.2 ± 0.9</td>
<td>14.3 ± 0.5</td>
</tr>
<tr>
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<td>13.9 ± 1.4</td>
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<td>49.7 ± 0.8</td>
<td>12.8 ± 1.1</td>
<td>13.8 ± 0.1</td>
</tr>
<tr>
<td>8</td>
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<td>13.9 ± 1.0</td>
<td>8.3 ± 0.2</td>
<td>50.0 ± 1.0</td>
<td>11.5 ± 1.7</td>
<td>13.7 ± 0.4</td>
</tr>
<tr>
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<tr>
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<td>11.2 ± 0.6</td>
<td>13.9 ± 0.3</td>
</tr>
<tr>
<td>Ave</td>
<td>48 ± 1</td>
<td>13 ± 1</td>
<td>9 ± 1</td>
<td>50 ± 1</td>
<td>12 ± 1</td>
<td>14 ± 1</td>
</tr>
</tbody>
</table>

Among the numerous colourfastness standard tests (such as laundering at different washing temperatures, perspiration, dry cleaning, light, water, chlorine, bleach spotting, chlorinated water, sea-water, hot spotting, dry heat, acid or alkaline spotting, organic solvents, perborate, saliva, etc) available to test, inspect and certificate procedures for textiles and garments (www.textilecomo.com/Tessile_di_como/1_07_eng.html), the above four sets of wool specimens were submitted to the most significant colourfastness standard tests for underwear, namely manual washing at 40 °C, acid (pH 5.5) and basic (pH 8) perspiration and hot pressing at 150 ± 2 °C for 15 s.23

After any colourfastness test the CIELAB coordinates of the generic rth dyed specimen differed neither within the same specimen nor among the different specimens tested at the 95% confidence level. Therefore the results of all colourfastness tests were compared in terms of their mean colour difference and related standard deviation.

As a general consequence of these tests, the initial colour of dyed specimens tended to fade, thus attenuating their colour difference (ΔE) with respect to the raw wool specimens (Fig 4). In particular, when using alum as mordant, the initial value of ΔE for the specimens dyed with red (23 ± 1) or orange (42 ± 1) pigments from *M purpureus* was not affected by hot pressing but was reduced by 10–20% by manual washing and perspiration tests. When using stannic chloride as mordant, the initial value of ΔE (43 ± 1) for the orange pigment-dyed specimens was slightly (less than 2.3%) increased or reduced by manual
Figure 4. Average values of colour difference ($\Delta E$) between raw wool specimens and those premordanted with alum (A) or stannic chloride (SC) and dyed at 50 $^\circ$C using orange (O) or red (R) pigment-rich extracts diluted with ethanol 95% (v/v) by an overall factor of 50 after dyeing (T) or different colourfastness tests (manual washing at 40 $^\circ$C, MW; hot pressing, HP; acid, AP, and basic, BP, perspiration).

Washing and hot pressing or acid and basic perspiration tests respectively. On the contrary, the initial value of $\Delta E$ (23 ± 1) for the SC-mordanted specimens dyed with the red pigments was reduced by as little as 3% by manual washing and hot pressing, 13% by acid perspiration and 7% by basic perspiration tests.

As shown in Fig 4, premordanting with SC increased the resistance to fading of all wool specimens, though the orange pigment-dyed specimens exhibited a generally greater resistance to acid perspiration than the red ones.

CONCLUSIONS
A series of preliminary dyeing tests on differently premordanted raw wool standard specimens using 50-fold diluted ethanolic extracts containing prevalingly red or orange pigments from \textit{M purpureus} C322, as determined spectrophotometrically, gave rise to dyed specimens with different hues depending on the mordant used. Independently of the microbial pigments used, the dyed wool specimens presented a prevailing red or orange shade when alum or stannic chloride was used as mordant respectively. Owing to their greater lightness coefficient ($L^*$), the specimens dyed with the red pigments showed a pale red colour tending to pink, whereas the specimens dyed with the orange pigments exhibited a more definite orange colour.

As a result of the more significant colourfastness standard tests for underclothing (manual washing at 40 $^\circ$C, acid and basic perspiration and hot pressing), the resistance to fading of the red pigment-dyed wool specimens was generally lower than that of the counterparts dyed with the orange pigments, especially when the latter were used to dye stannic chloride-premordanted wool specimens.

Since the formulation of production medium MSG (containing maltose, glutamate and numerous salts) was more expensive than that of medium MPIII (consisting of glucose and fewer salts), the production of orange pigment-rich ethanolic extracts appeared to be more cost-effective than that of the corresponding red items, and thus more suitable to support the growing demand for colorants of natural origin in the textile (underwear and garments) sector. Of course, such preliminary findings have to be further checked by optimising the kinetic and stoichiometric yield parameters of this fungal fermentation process, as well as the pigment-to-fibre and dilution ratios to be used in the dye bath, this being a prerequisite to evaluate the techno-economic feasibility of this novel application for the orange pigments from \textit{M purpureus}.

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