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RESEARCH ARTICLE

# Adventitious root culture dyeing and optimisation of dyeing parameters using roots of *Gynochthodes umbellata* (L.) Razafim and B. Bremer

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## Abstract

This study offers a new source of natural dye. Taguchi's design of experiments was used to statistically and optimize the dyeing parameters of different fabrics. The results varied with different fabrics and the optimum conditions for cotton were found to be ultrasonication for 5 min, a material-to-liquor ratio of 10 mL dye to 100 mL water, a temperature of 60 ºC, and a pH of 8. For linen, the optimum values were ultra-sonication for 15 min, 15 mL dye to 100 mL water, pH of 8, and temperature of 80 ºC. Optimum combinations for silk were a temperature of 80 ºC, the material-to -liquor ratio of 20 mL of dye extract to 100 mL water, pH of 8, and ultrasonication for 15 min. The results indicated the commercial viability of dyeing fabrics using this procedure.

## Keywords

anthraquinones; dye; fabrics; natural dyeing; root culture; Taguchi; textiles

## Introduction

At least 3000 years have passed since the invention of textiles and dyes. For the most part, the high-quality cloth of that era was made using procedures that were empirically determined and relied on the unique expertise and experience of innumerable weavers, spinners, and dyers (1). Since ancient times, natural dyes have been used to colour both synthetic and natural textile materials, including wool, cotton, silk, nylon, fur, and leather. The use of natural colours declined when synthetic dyes were introduced in the middle of the 19$^{th}$ century (2, 3). Synthetic dyes and pigments are widely utilized in the textile industry for their wide spectrum of colours, improved colour fastness qualities, and affordable cost (4). However, due to their toxicity and carcinogenic effects and the fact that they are neither biodegradable nor eco-friendly, many producers nowadays avoid synthetic dyes and pigments (5). During the past few decades, researchers worldwide have been focusing on various natural dye applications (6). Another issue related to this is the interaction between different dyeing parameters during optimisation. Against this backdrop, the present investigation was carried out to identify the limiting parameter in the process using the Taguchi L9 Design of Experiments (DOE). The methodology adopted in this study was novel and was introduced in the dyeing of fabrics. Moreover, this study was the first to use *Gynochthodes umbellata* (L.) Razafim and B. Bremer as a source of natural dye. We started investigating *Gynocthodes* *umbellata* as a source of dye since the presence of anthraquinones in this species had been established before (7). In traditional Indian systems of medicine, the plant parts of *Gynocthodes umbellata* are considered to be useful in treating diseases such as intestinal pain, indigestion, syphilis and gonorrhea (8), it has also been reported that the plant is a rich source of minerals, vitamins and fibres.

## Materials and Methods

This research paper is a combination of 2 different experiments. The first one is the dyeing of cotton fabric alone, using dye obtained from the adventitious root culture of *Gynocthodes umbellata*, with 3 different mordanting methods, i.e., pre-mordanting, simultaneous mordanting, and post-mordanting, all in combinations with each of the 3 different mordants, which are an alum, caustic soda and ferrous sulphate. The second experiment is the optimisation of the dyeing parameters for the dyeing of 3 different fabrics, which are cotton, silk and linen, using the *in-vivo* root of *Gynocthodes umbellata*, using a single natural mordant, i.e., a 10 % solution of the powdered seed of *Terminalia chebula*, by the pre-mordanting method. This method of dyeing is highly eco-friendly, as both the dye and the mordant are extracted from natural sources. The optimisation of the dyeing parameters for this experiment was done with the help of Taguchi L9 orthogonal array design.

### Plant material

*Gynochthodes umbellata* (L.) Razafim and B. Bremer are the plant species used in the current investigation. *Gynochthodes umbellata* (L.) Razafim and B. Bremer (syn: *Morinda umbellata*), is a medicinal plant also known as "Nuna" in Tamil and "Pitadaru" in Sanskrit (7). It is a woody climber. *Gynochthodes umbellata* was collected for the current study from a natural population in the Kariavattom campus of the University of Kerala, Thiruvananthapuram, Kerala (Fig. 1).

**Figure 1**: Habit of *Gynochthodes umbellata*

### Initiation and establishment of adventitious root culture

For the establishment and maintenance of the root culture of *Gynochthodes umbellata*, a nodal explant (4th node from the tip) was used. The medium used was Murashige and Skoog (MS), with 3 % sucrose and 2 $mgL^{-1}$ BA solidified with 0.8 % agar, as standardized earlier in our laboratory (7). Young shoot cuttings of *Gynocthodes umbellata* were taken from field-grown plants, defoliated, thoroughly cleaned with water, and then cleaned using the detergent Labolene (Glaxo India Ltd., Mumbai). Afterward, these explants were washed under running water for 30 min and then rinsed with distilled water. Later 0.1 % (w/v) Mercuric Chloride ($HgCl_2$) was used for surface sterilisation. After treatment with $HgCl_2$ for 6 min and washing with distilled water thrice, the nodal segment (1-1.5 cm) was transferred to the above-mentioned MS medium. After 4 weeks, the *in vitro* shoots generated were sub-cultured and maintained at the same hormone concentration (Fig. 2). The culture conditions were maintained at 25°C under a 12 h photoperiod, with 50 $molm^{-2}s^{-1}$ irradiance of a white fluorescent tube (Philips, India) and 60-65 % relative humidity.

**Figure 2**: *In vitro* shoot multiplication in MS medium containing 3% sucrose and 2 $mgL^{-1}$ BA solidified with 0.8% agar

To initiate adventitious root culture, an internodal segment of 1 cm length from micro-propagated cultures was transferred to a half-strength MS solid medium augmented with different concentrations of NAA (0.1-0.5 $mgL^{-1}$). Internodal explants with 0.3 $mgL^{-1}$ NAA showed the best result and were selected for the initiation of adventitious root culture (Fig. 3). Small roots from the internode were harvested and transferred to a 50 mL half-strength MS liquid medium with 3 % sucrose and 0.3 % $mgL^{-1}$ NAA in a 250 mL Erlenmeyer flask. The cultures were kept in a rotary shaker at 90 rpm. After 90 days of culture, the roots were harvested and subjected to anthraquinone

**Figure 3**: Internode with adventitious roots

extraction (8).

### Adventitious root culture

The maximum shoot induction was noticed in the 4th node in MS medium fortified with 2 $mgL^{-1}$ BA, 0.8 % agar and 3 % sucrose. The sub-cultured *in vitro* shoots were used for the initiation of adventitious root culture. Medium fortified with 0.3 $mgL^{-1}$ NAA, was used for root culture and the internodal segment was selected as the explant. The initiated roots were transferred to a liquid medium with the same hormonal combination and without agar. After 90 days of growth, roots were harvested and the dye was extracted. The extracted anthraquinones were used for the dyeing of fabrics.

### Mordanting and dyeing of adventitious root culture

Mordanting is a crucial step in the process of dyeing cotton. Cotton dyeing is thought to be a more challenging art than dyeing silk and wool. Due to its non-porous nature, cotton cannot retain dyes without undergoing more involved procedures for diffusing the dye into the fibre or creating an affinity between the dye and the fibre. The mordanting makes the dye more soluble in the fibre and creates more affinity between the dye and the fibre. The cotton samples were treated with different metallic salts known as mordants by following three methods.

### Pre-mordanting

7 cm x 7 cm uniform-sized strips of cotton fabric were immersed in three different mordant solutions (alum, caustic soda, and ferrous sulphate) for 20 minutes each. The fabrics were then left to air dry for roughly 2 h. The mordanted cotton fabrics were submerged for 20 min in a specific dye bath. The materials were allowed to dry in the air for around 2 hours after dyeing. To assess the level of color variation, pictures of all the treated cotton fabric strips were taken.

### Post-mordanting

As mentioned above, the cotton fabric strips were subjected to a dye bath for 20 min, after which they were left to air dry for around 2 hours. The dyed fabrics were then immersed in a special mordant bath for 20 min, followed by approximately 2 hours of air drying.

### Simultaneous mordanting

The above-mentioned cotton fabrics were simultaneously mordanted by dipping in equal amounts of dye bath and mordant bath for 20 min, followed by 2 h of air drying.

### Determination of optical density of washout of cotton fabrics

The dried cotton fabrics obtained after pre-mordanting, post-mordanting, or simultaneous mordanting and dyeing were cut into equal halves and the second half was washed in 10 mL water and stored in small bottles. The effluent was analyzed using a UV/visible spectrophotometer. The absorption wavelength was recorded based on the maximum absorption peak exhibited by effluent using a UV/visible spectrophotometer after finding the absorption wavelength, the absorbance of each sample was measured. The optical density of the effluent was recorded. The most suitable type of mordant was characterized by the least Optical density (OD) value as the minimum release of dyestuffs to the effluent.

### Measure of color strength

Color characteristics of all the dyed fabrics were assessed in terms of CIE L*, a*, b*, reflectance, and color strength (K/S) values at 400 nm. In 1976, the International Commission on Illumination (CIE) defined the CIE LAB color space, also known as the L*a*b*, as a system that expresses color as 3 values: L* for perceptual lightness and a* and b* for the 4 unique colors of human vision; red, green, blue and yellow. The spectral reflectances of dyed samples were measured using a USB200-Reflectance spectrophotometer MARC SPEC (Ocean Optics). The color strength (K/S) values were calculated by the

Kubelka -Munk equation. $K/S=(1-R)^2/2R$, (Eqn. 1)

where R is the reflectance of the dyed sample, K is the absorption coefficient and S is the scattering coefficient.

### Optimisation of dyeing parameters using *in vivo* root and *Terminalia chebula*

We used 3 different fabrics for the experiments, which are cotton, silk, and linen. 7 cm × 7 cm strips of each fabric were taken and all of them underwent pre-mordanting using a 10 % solution of the powdered seed of *Terminalia chebula*. The mordant was employed at a material-to-liquor ratio of 1:10 and mordanting was carried out overnight at room temperature. The dye was made by adding 15 g of the dried and powdered root of *Gynocthodes umbellata* to 100 mL water and then boiling the solution until it reduces to 30 mL of dye extract, which takes about 20 min. Using Taguchi L9 orthogonal array design, we obtained 9 potential experiment runs for each of the 3 fabrics. To optimize dyeing we set the response as reflectance, i.e., the experiment run with the lowest reflectance will have the optimum dye absorption. As an example, we can take an experiment run on cotton. The 7 cm x 7 cm cotton fabric that had undergone pre-mordanting is immersed in a dye solution of a material-to-liquor ratio of 1:10 and the pH of the solution is adjusted to 6. Then the solution is heated to 40 °C. Finally, the whole solution is subjected to ultrasonication for 5 min, all the while maintaining the temperature.

### Analysis of Taguchi Orthogonal Array Design

The Taguchi method was used to optimize the process parameters for the reflectance. The Taguchi method is one of the best experimental methodologies that can be used to find the minimum number of experiments to be performed within the permissible limit of factors and levels. To find the best combination of conditions, we have to look for the one that gives the least S/N ratio. The smaller the S/N ratio, the better.

Taguchi experiments often use a 2-step optimisation process. In the first step, the signal-to-noise ratio is used to identify those control factors that reduce variability. The second step identifies the control factors that move the mean to target and have a small or no effect on the signal-to-noise ratio. Box Behnken's design of experiments was used to achieve the optimisation of enzymatic and ultrasonic bio-scouring of the cellulosic linen fibre. Sonication (LAB MAN Ultrasonic cleaner) time was in the range of 20-60 min (10).

### Optimisation of dyeing parameters

MINITAB statistical software (version 21.1.1) was used to produce the Taguchi Design of Experiments for the different dyeing parameters and their 3 different levels. The optimisation was performed based on the reflectance of the fabric at 400 nm by different combinations of 4 parameters and their different levels, as presented (Tables 1-3). Analysis of mean (ANOM) and Analysis of variance (ANOVA) were used for the analysis of the treatments. ANOVA determines whether the means of the treatment vary from one another. ANOM is used to determine if the means of the treatment deviate from the general mean.

### Interaction plots

To comprehend the relationships between process parameters, an interaction graph was used. There are 2 approaches to defining interaction behavior: parallel and non-parallel lines. A weak connection can be characterised if the lines are parallel and non-parallel lines confirm strong interactions.

**Table 2**: Experimental layout for reflectance in coded form

| Batch | MLR | TMP | pH | US |
|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 |
| 2 | 1 | 2 | 2 | 2 |
| 3 | 1 | 3 | 3 | 3 |
| 4 | 2 | 1 | 2 | 3 |
| 5 | 2 | 2 | 3 | 1 |
| 6 | 2 | 3 | 1 | 2 |
| 7 | 3 | 1 | 3 | 2 |
| 8 | 3 | 2 | 1 | 3 |
| 9 | 3 | 3 | 2 | 1 |

**Table 3:** Dyeing process variables for Reflectance

| Batch runs | MLR | TMP | pH | US |
|---|---|---|---|---|
| 1 | 10 | 40 | 6 | 5 |
| 2 | 10 | 60 | 7 | 15 |
| 3 | 10 | 80 | 8 | 25 |
| 4 | 15 | 40 | 7 | 25 |
| 5 | 15 | 60 | 8 | 5 |
| 6 | 15 | 80 | 6 | 15 |
| 7 | 20 | 40 | 8 | 15 |
| 8 | 20 | 60 | 6 | 25 |
| 9 | 20 | 80 | 7 | 5 |

**Table 1:** Selected dyeing process variables and respective levels in the experimental design.

| | Process variables | Units | Level 1(L1) | Level 2(L2) | Level 3(L3) |
|---|---|---|---|---|---|
| A | Material to liquor ratio | mL | 10 | 15 | 20 |
| B | Temperature | degree Celsius | 40 | 60 | 80 |
| C | pH | - | 6 | 7 | 8 |
| D | Ultrasonication | minutes | 5 | 15 | 25 |

## Results and Discussion

### Adventitious root culture and dyeing

### Dyeing

Three different types of dyeing methods were tested for dyeing using the adventitious root extract. The dyeing of cotton using adventitious root produced different shades of color (Fig. 4). The comparative effectiveness of the three mordants showed that alum had the highest efficiency in dyeing with an OD value of 0.01. Among the 3 mordanting methods tried, simultaneous mordanting showed promising results with the lowest OD value (0.01). Mordants such as caustic soda and alum gave better color


**Figure 4**: Adventitious root dyeing using different mordants and mordanting methods

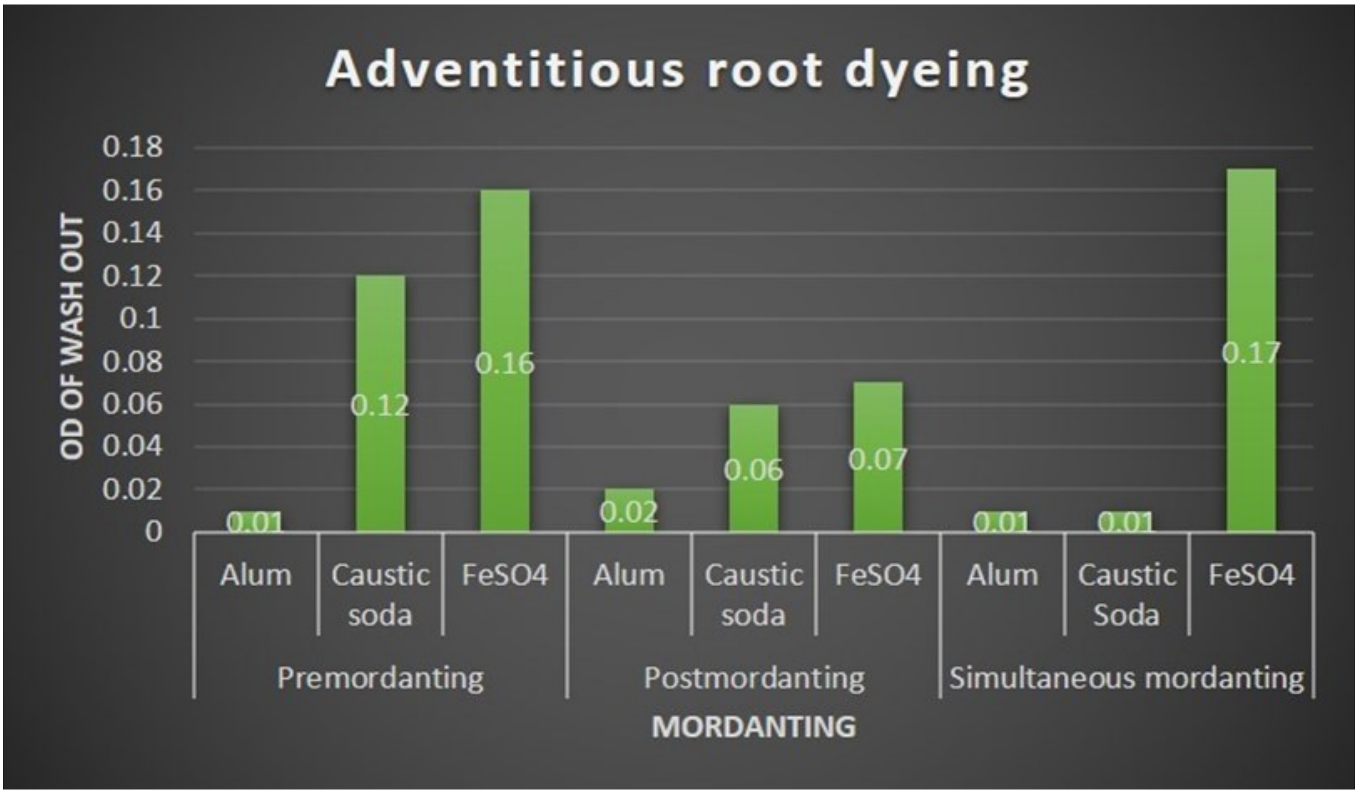


**Figure 5**: Effect of mordants in dyeing cotton fabrics with adventitious root extract on OD of washout

fastness response concerning the OD of effluents (Fig. 5).

## Optimisation of dyeing parameters for cotton

The results of the analyses (Table 4) show that the minimum reflectance was 29.149 for sample RCC 7, which showed the maximum dye absorption and had a maximum color strength (K/S) of 0.86. For cotton fabric, ultrasonication dyeing was identified to be the most significant (0.000) and important factor (Fig. 6 and 7). The optimum conditions were ultrasonication for 5 min, the material-to-liquor ratio of 10 mL dye solution to 100 mL water, pH of 8, and temperature of 60 °C (Table 5). These results were similar to earlier reports (11), wherein ultra-sonicator dyeing of cotton with *Rubia cordifolia* showed an improved dye uptake. It has been shown that pomegranate rind, a natural coloring agent could be used for the dyeing of cotton fabrics (12). The pH-mediated dyeing of cotton for the production of nano micelles improved antibacterial activity as well (13). Thus, it

**Table 4:** Optimisation of dyeing parameters, L*, a*, b*, r, k/s values for dyed fabrics using *in vivo* roots and natural mordant *Terminalia chebula*

| Sample | L* | a* | b* | x | y | z | r | k/s |
|---|---|---|---|---|---|---|---|---|
| **Cotton** | | | | | | | | |
| RCC 1 | 98.5 | 4.2 | 30.6 | 0.4812 | 0.4270 | 0.0917 | 48.990 | 0.28 |
| RCC 2 | 81.8 | 3.7 | 19.3 | 0.4748 | 0.4212 | 0.1040 | 29.7130 | 0.86 |
| RCC 3 | 94.0 | 2.5 | 30.7 | 0.4800 | 0.4300 | 0.0900 | 43.834 | 0.37 |
| RCC 4 | 94.3 | 3.7 | 31.2 | 0.4821 | 0.4288 | 0.0891 | 39.566 | 0.47 |
| RCC 5 | 94.3 | 5.5 | 31.9 | 0.4855 | 0.4270 | 0.0875 | 44.262 | 0.35 |
| RCC 6 | 91.0 | 5.1 | 31.7 | 0.4856 | 0.4278 | 0.0865 | 29.392 | 0.86 |
| RCC 7 | 79.4 | 10.1 | 43.9 | 0.5090 | 0.4307 | 0.0603 | 29.149 | 0.86 |
| RCC 8 | 90.5 | 4.1 | 35.4 | 0.4873 | 0.4319 | 0.0808 | 40.568 | 0.45 |
| RCC 9 | 95.9 | 3.3 | 39.1 | 0.4872 | 0.4344 | 0.0783 | 41.327 | 0.41 |
| **Linen** | | | | | | | | |
| RCL 1 | 76.9 | 6.5 | 33.8 | 0.4953 | 0.4296 | 0.0759 | 43.337 | 0.37 |
| RCL 2 | 69.4 | 5.3 | 24.5 | 0.4875 | 0.4251 | 0.0875 | 48.2683 | 0.28 |
| RCL 3 | 88.5 | 8.6 | 32.5 | 0.4930 | 0.4240 | 0.0830 | 60.8697 | 0.13 |
| RCL 4 | 67.9 | 5.7 | 22.0 | 0.4861 | 0.4224 | 0.0916 | 47.201 | 0.29 |
| RCL 5 | 85.9 | 7.9 | 34.4 | 0.4943 | 0.4267 | 0.0790 | 50.785 | 0.25 |
| RCL 6 | 85.9 | 7.9 | 34.4 | 0.4943 | 0.4267 | 0.0790 | 58.756 | 0.14 |
| RCL 7 | 76.6 | 8.8 | 36.8 | 0.5022 | 0.4283 | 0.0695 | 43.2683 | 0.37 |
| RCL 8 | 72.1 | 7.8 | 33.6 | 0.4999 | 0.4282 | 0.0719 | 40.2683 | 0.45 |
| RCL 9 | 81.8 | 9.0 | 39.4 | 0.5021 | 0.4291 | 0.0687 | 55.8697 | 0.18 |
| **Silk** | | | | | | | | |
| RCS 1 | 47.9 | 4.5 | 20.3 | 0.4915 | 0.4262 | 0.0823 | 6.730 | 7.33 |
| RCS 2 | 66.4 | 8.9 | 29.2 | 0.5010 | 0.4235 | 0.0755 | 9.8133 | 4.55 |
| RCS 3 | 58.3 | 4.6 | 17.5 | 0.4826 | 0.4210 | 0.0964 | 10.6500 | 4.05 |
| RCS 4 | 44.0 | 10.9 | 23.8 | 0.5166 | 0.4157 | 0.0676 | 6.9017 | 7.33 |
| RCS 5 | 56.7 | 3.3 | 15.7 | 0.4779 | 0.4217 | 0.1004 | 10.8133 | 4.05 |
| RCS 6 | 76.8 | 7.7 | 27.9 | 0.4921 | 0.4231 | 0.0847 | 11.6500 | 3.6 |
| RCS 7 | 59.2 | 15.9 | 41.6 | 0.5330 | 0.4201 | 0.0469 | 8.395 | 5.29 |
| RCS 8 | 72.8 | 8.7 | 27.3 | 0.4953 | 0.4215 | 0.0832 | 10.8133 | 4.05 |
| RCS 9 | 76.6 | 12.4 | 41.0 | 0.5123 | 0.4255 | 0.0621 | 11.6500 | 3.6 |

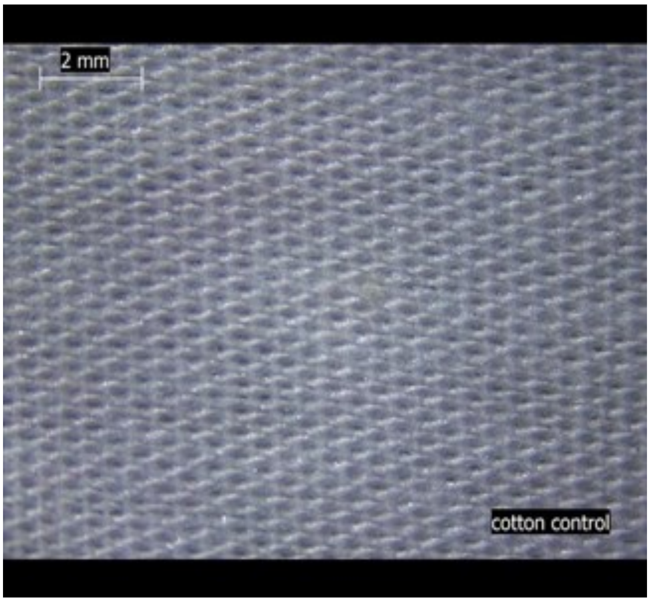


**Figure 6:** Control cotton

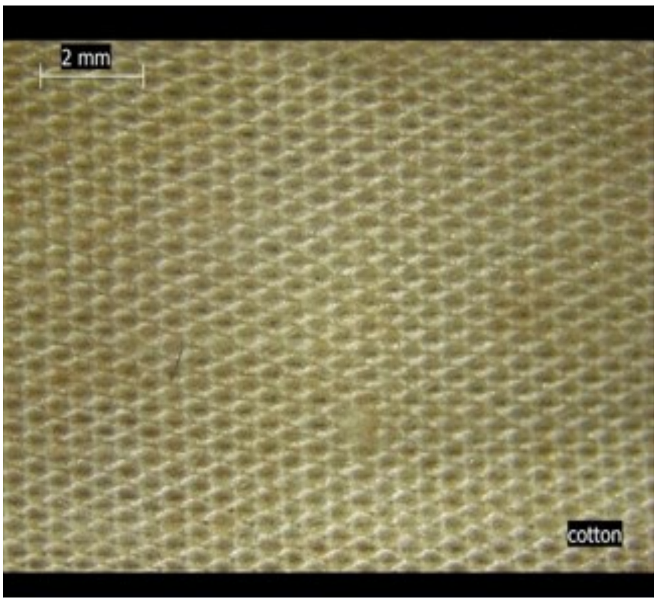


**Figure 7**: Dyed cotton

**Table 5:** Analysis of Mean (ANOM) of Variables in dyeing different fabrics with root dye and *Terminalia chebula* mordant

| Level | MLR A | TMP B | pH C | US D |
|---|---|---|---|---|
| **Cotton** | | | | |
| 1 | 38.18 | 36.57 | 36.98 | 42.19 |
| 2 | 37.74 | 38.18 | 36.87 | 29.42 |
| 3 | 37.01 | 38.18 | 39.08 | 41.32 |
| Delta | 1.17 | 1.62 | 2.21 | 12.78 |
| Rank | 4 | 3 | 2 | 1 |
| Optimum | A1 | B2 | C3 | D1 |
| | 10 | 60 | 8 | 5 |
| **Linen** | | | | |
| 1 | 50.82 | 44.78 | 47.45 | 50.00 |
| 2 | 52.25 | 46.44 | 50.45 | 50.28 |
| 3 | 46.65 | 58.50 | 51.82 | 49.45 |
| Delta | 5.60 | 13.72 | 4.37 | 0.83 |
| Rank | 2 | 1 | 3 | 4 |
| Optimum | A2 | B2 | C3 | D2 |
| | 15 | 80 | 8 | 15 |
| **Silk** | | | | |
| 1 | 9.064 | 7.342 | 9.731 | 9.731 |
| 2 | 9.788 | 10.480 | 9.455 | 9.953 |
| 3 | 10.286 | 11.317 | 9.953 | 9.455 |
| Delta | 1.222 | 3.974 | 0.498 | 0.498 |
| Rank | 2 | 1 | 3.5 | 3.5 |
| Optimum | A3 | B3 | C3 | D2 |
| | 20 | 80 | 8 | 15 |

appears that the pH value of fabric dye is an important parameter (14). pH-responsive cotton was made using madder dye (15).

## Optimisation of dyeing parameters for linen

Linen is a cellulosic fibre from *Linum usitatissimum* (flax plant), which is known to be more comfortable than cotton. Table 4 shows that the linen fabric sample RCL8 possessed the lowest reflectance of 40.2683 and a maximum color strength (K/S) of 0.45. In the case of linen, temperature was an important factor with a significance value of 0.007 (Fig. 8 and 9). Based on Taguchi optimisation the optimum values were ultrasonication of 15 min., 15 mL dye to 100 mL water, at a pH of 8 and a temperature of 80 °C (Table 5). Linen that was dyed naturally showed efficient protection against ultraviolet light, free radicals, and bacteria (16). Natural dye from the fruit shell extract of *Sterculia foetida* has potential application for linen dyeing and it adds excellent ultraviolet protection properties to the linen fabric. The optimal dyeing temperature was 90 °C (17). The linen fabrics dyed with Kapok flower provided efficient UV protection as well as radical scavenging activities (18). An increase in the whiteness index indicated that ultrasonic

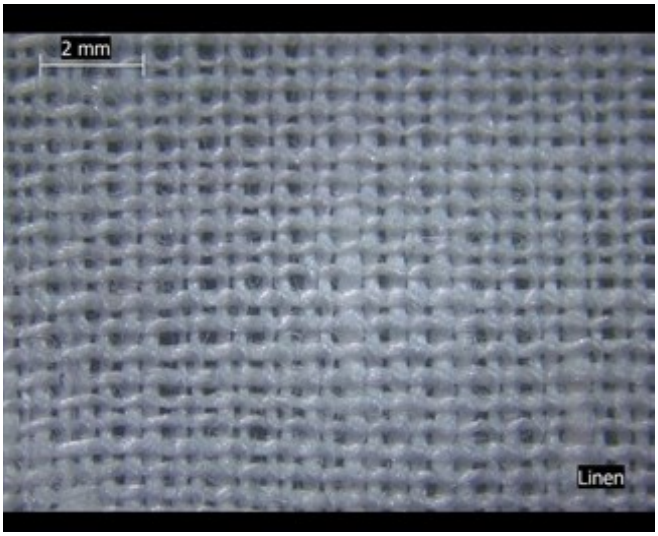


**Figure 8:** Control linen

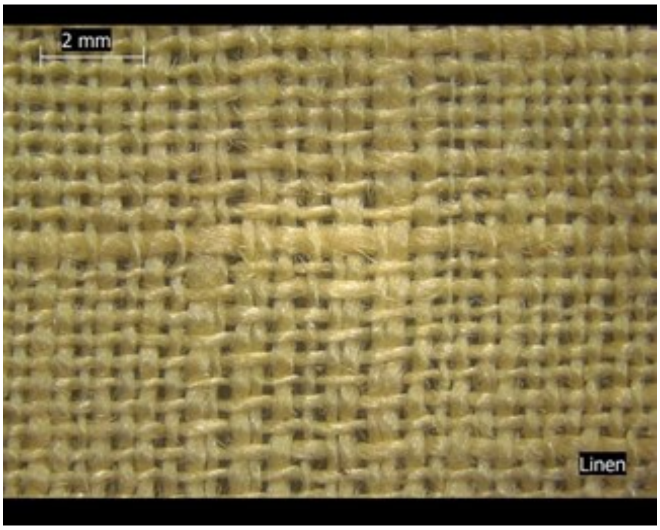


**Figure 9:** Dyed linen

pre-treatment was better at low temperatures for all the treatments. The color strength of the dyed fabric was enhanced by the ultrasonic treatment (19).

## Optimisation of dyeing parameters for silk

In the case of silk, the minimum reflectance value for the sample RCS1 was 6.730 and its maximum K/S ratio was 7.33 (Table 4). Temperature was an important factor for silk fabric and its P value was 0.001 (Fig. 10 and 11). As shown in Table 6, the Taguchi orthogonal array identified the optimisation of silk fabric, the optimum combination being a temperature of 80 °C, a material-to-liquor ratio of 20 mL of dye extract to 100 mL water, at a pH of 8, and ultrasonication for 15 min. Earlier, the efficiency of ultrasonic energy was tested in silk fabric using *Sticta coronata* (20).

## Signal to Noise ratio

The signal-to-noise (S/N) ratio is used in the Taguchi design of experiments to estimate the value of significance in variable selection. The best variables were identified by ranking the delta value in ascending order of significance. A greater delta value denoted a stronger impact of the variable on the overall experiment. Nominal is better, smaller is better, and larger is better were the 3 categories of S/N. In this analysis, the smaller is better principle was


**Figure 10:** Control silk


**Figure 11:** Dyed silk

**Table 6**: Analysis of Variance for means of the percentage contribution of variables in dyeing different fabrics with root dye and *Terminalia chebula*

| Source | DF | AdjSS | AdjMS | F-value | P- value | % Contribution | Rank |
|---|---|---|---|---|---|---|---|
| **Cotton** | | | | | | | |
| US | 2 | 305.62 | 152.810 | 55.19 | 0.00 | 94.84 | 1 |
| pH | 2 | 9.324 | 4.662 | 0.09 | 0.916 | 2.89 | 2 |
| TMP | 2 | 5.204 | 2.602 | 0.05 | 0.952 | 1.61 | 3 |
| MLR | 2 | 2.085 | 1.042 | 0.02 | 0.0981 | 0.64 | 4 |
| **Linen** | | | | | | | |
| TMP | 2 | 336.32 | 168.16 | 12.33 | 0.007 | 80.43 | 1 |
| MLR | 2 | 50.83 | 25.41 | 0.42 | 0.678 | 12.15 | 2 |
| pH | 2 | 29.91 | 14.95 | 0.23 | 0.800 | 7.15 | 3 |
| US | 2 | 1.071 | 0.5353 | 0.01 | 0.992 | 0.992 | 4 |
| **Silk** | | | | | | | |
| TMP | 2 | 26.342 | 13.1708 | 26.25 | 0.001 | 89.74 | 1 |
| MLR | 2 | 2.264 | 1.132 | 0.25 | 0.786 | 7.713 | 2 |
| pH | 2 | 0.3731 | 0.1866 | 0.04 | 0.962 | 0.032 | 3 |
| US | 2 | 0.3731 | 0.1866 | 0.04 | 0.962 | 0.032 | 4 |

applied to provide the optimal mixing ratio and highest compressive strength. Therefore, the better the desired result, the larger the S/N and delta values will be.

The Taguchi technique uses the signal-to-noise ratio to calculate the ideal values of each parameter and to analyze parameter changes. To reduce the effects of uncontrollable factors, Taguchi designs a robust metric to discover uncontrollable factors and to reduce variability in a product or process (noise factors). Controllable design and process parameters are referred to as control factors. Noise issues can be managed during experimentation but not during product use or manufacture. In a Taguchi-designed experiment, we can determine the best control elements to make the process or product robust or resistant to noise factors. Higher signal-to-noise ratio (S/N) values indicate control factor settings that lessen the impact of the noise factor (21). According to Taguchi, the variability of the yield should be minimized concerning noise components and maximized for signal factors. Fig. 12, 13 and 14 show the main effect plots of fabrics to process variables. S/N ratio analysis revealed that the optimum conditions for cotton were A1, B2, C3, and D3. It was A2, B3, C3, and D2 for linen and A3, B3, C3, and D3 was the order for silk. The results show that the factor that influenced the dyeing of cotton the most was ultrasonication. Temperature was the factor that influenced the dyeing of silk and linen the most. To achieve the best dyeing conditions concerning exhaustion %, an orthogonal array design (L9) was used. From the previous reports, the dye bath temperature was the most crucial element for reaching the ideal dye exhaustion, but dyeing time also had a significant impact (22). The extraction of Argy wormwood aerial parts was improved using the Taguchi method to minimize the fluctuation in color intensity caused by the change in phytochemical contents due to seasonal change and other variables. The

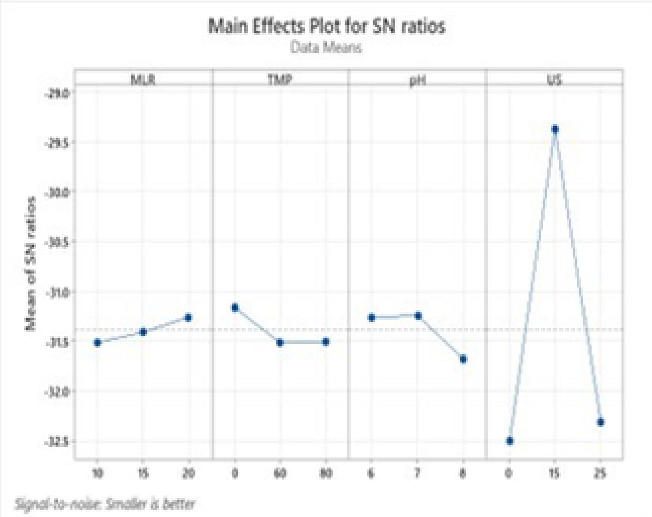


**Figure 12:** Main effects plot for SN ratios for cotton fabric

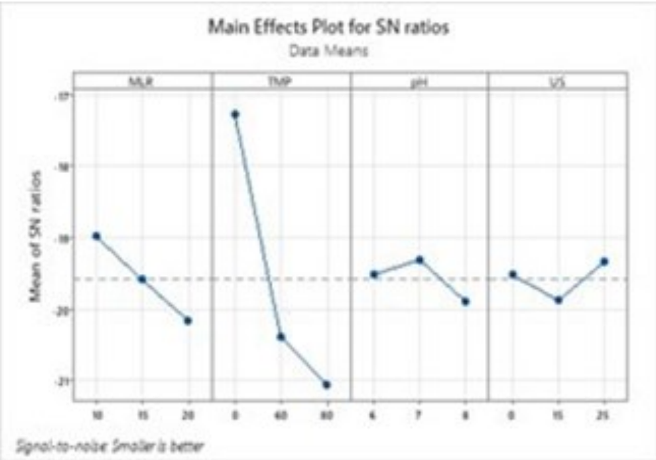


**Figure 14**: Main effects plot for SN ratios for silk fabric

total amount of phytochemicals (phenols, flavonoids, and tannins) present in the extracted solutions served as the basis for optimisation (23).

## Interaction plots

The lines of all four components were not parallel. The interaction map thus shows that the chosen elements considerably impacted the dying process (Fig. 15, 16 and 17).

## Analysis of mean (ANOM)

Table 5 shows a response for the analysis of mean (ANOM) and it demonstrates the equality of sample means. The primary focus of ANOM is to test the effects of a designed experiment in which all the variables were fixed. The ANOM in Table 5 was estimated using an equation. In Table 5, A, B, C, and D are the independent variables, represented in a column of 3 step level, as per range, and were used in setting the dyeing process. For cotton fabric, the optimum combination was ultrasonication of 5 min, material-to-liquor ratio of 10 mL to 100 mL water, temperature of 60 °C, and pH of 8. These optimum values were coded as A1, B2, C3 and D3. For linen, the optimum values were ultrasonication of 15 min, 15 mL dye to 100 mL water, at 8 pH and at 80 °C which is coded as A2, B3, C3,

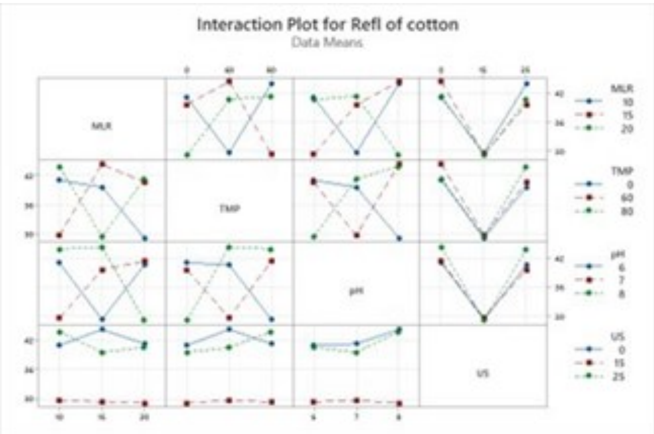


**Figure 15:** Interaction plot of cotton

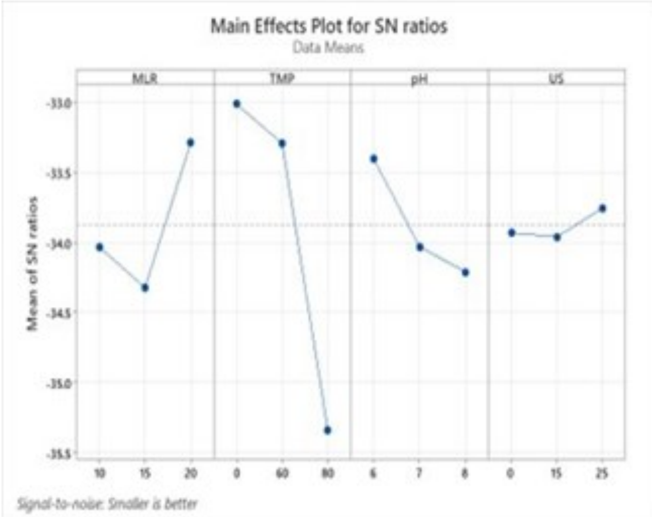


**Figure 13**: Main effects plot for SN ratios for linen fabric

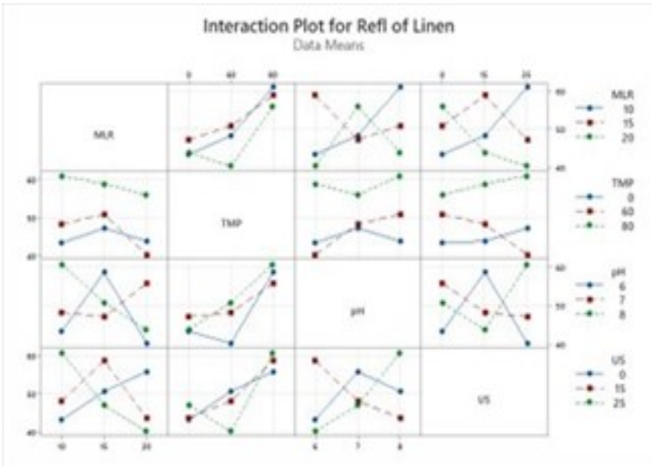


**Figure 16:** Interaction plot of linen

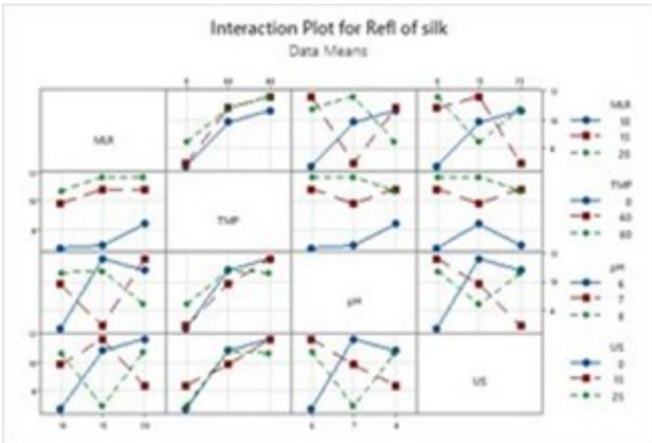


**Figure 17:** Interaction plot of silk

and D2. Optimum combinations for silk were a temperature of 80 ºC, the material-to-liquor ratio of 20 mL of dye extract to 100 mL water, at a pH of 8, and ultrasonication for 15 min and coded as A3, B3, C3, and D2.

## Analysis of variance (ANOVA)

The analysis of variance is a crucial step since it enables the assessment of the significance of the investigated components and their interactions with the outcome based on the p-value of the coefficient. In Table 6, the % contribution of each dyeing variable on reflectance in cotton was shown in this order: Ultrasonication, pH, temperature, and material-to-liquor ratio. For linen, the order was temperature, material-to-liquor ratio, pH, and ultrasonication. In the case of silk, the % contribution was in the order of temperature, material-to-liquor ratio, pH, and ultrasonication (Fig. 18, 19 and 20).

% contribution was calculated as

**%** contribution of parameters used in optimisation = sum of squares of a variable/total sum of squares

## Performance of anthraquinone dye

The optimum conditions established for cotton fabric from the dyeing experiments using the Taguchi design of mean analysis were ultrasonication for 25 min, temperature of 60 ºC, pH of 8, and material-to-liquor ratio of 1:10. In the case of linen, the optimum conditions were ultrasonication for 15 min, temperature of 80 ºC, pH of 8 and a material-to-liquor ratio of 15 mL dye to 100 mL water. For silk, it was ultrasonication of 15 min,

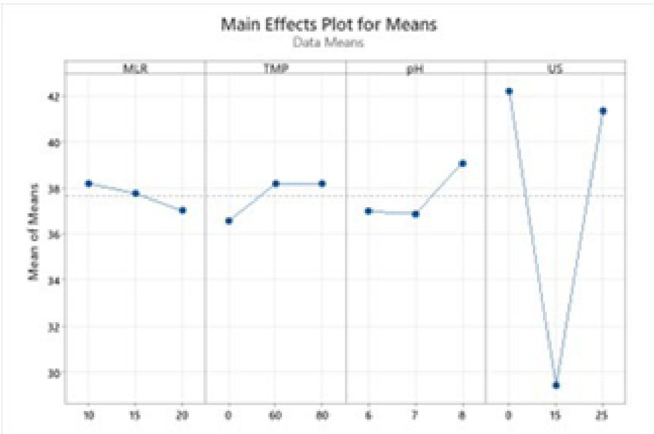


**Figure 18:** Main effects plot for means of cotton fabric


**Figure 19:** Main effects plot for means of linen fabric

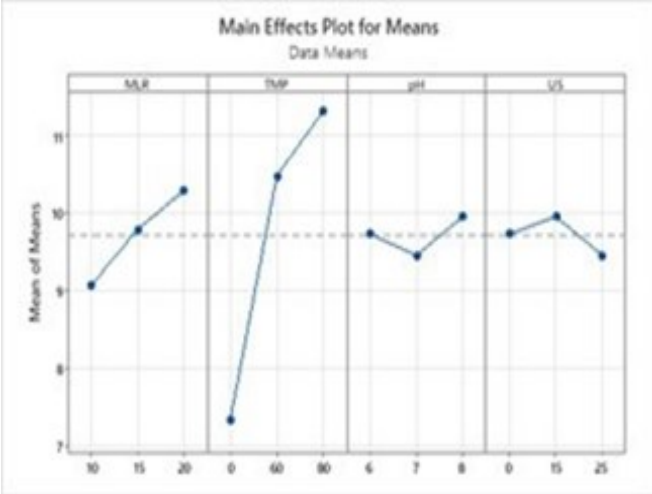


**Figure 20:** Main effects plot for means of silk fabric

temperature of 80 ºC, pH of 8, and a material-to-liquor ratio of 20 mL dye to 100 mL water. Even though the anthraquinone extract used to dye all 3 fabrics was the same, dye absorption and the optimum conditions varied with each fabric. The pH was common for 3 fabrics. An experiment that was performed using the Taguchi design of experiments was the extraction of juglone. Variance analysis (VA) showed that the largest amount of juglone extracted was 37.26 mg, which was produced under the optimal conditions of 150 bar, 35 ºC, and 375 m. Pressure had a 56.46 % contribution and temperature had a 6.88 % contribution to the amount of juglone that can be extracted (24). The response that was chosen for the current experiment was different from this example.

## Conclusion

Anthraquinone dyeing using an adventitious root culture is eco-friendly. The process parameters investigated in this study were ultrasonication, pH, material-to-liquor ratio, and temperature. The DOE based upon L9 orthogonal arrays by Taguchi was used and Analysis of Variance (ANOVA) was employed to analyze the effect of these parameters on the optimisation of dyeing parameters. We demonstrated chemical-free and eco-friendly dyeing of different fabrics such as cotton, silk, and linen with a natural dye from *G. umbellata* and a natural mordant from *Terminalia chebula*. A series of experiments were performed following the Taguchi experimental design and we concluded that the optimisation of the dyeing parameters changes with each fabric. Based on Taguchi's ranking, ultrasonication is the most significant factor for cotton. Ultrasonicator dyeing is a novel approach to anthraquinone dyeing of fabrics. For linen and silk, temperature was the most significant factor. This is a novel process that can be applied to other natural dye-based techniques.

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## Authors' contributions

VAS carried out the experiments and drafted the manuscript. SVR and AGP incorporated the corrections to the manuscript. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None.

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