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Eco-Friendly Dyeing of Cotton Fabric Mordanted with *Glycine max* Protein Extract Using Natural Pigments from Achiote (*Bixa orellana*) Seeds

Authors Details

Redait Zerihun Negiya[1*]
Department of Textile processing research and development desk, Textile and garment industry research and development center, Addis Ababa, Ethiopia.

Yesuf Seyd Ahmed[2]
Department of Textile processing research and development desk, Textile and garment industry research and development center, Addis Ababa, Ethiopia

Corresponding Author: *Redait Zerihun Negiya*

ABSTRACT: This study investigates an eco-friendly approach to cotton fabric coloration using a natural dye extracted from achiote tree seeds (Bixa orellana) combined with a sustainable, bio-mordant. The primary objective is to extract and characterize plant based biodegradable natural dye and mordant that can be used as alternative option for toxic synthetic colorants and hazardous metallic mordants in dyeing of cotton. Through optimized alkaline extraction at 85°C for 2 hours, a maximum crude dye yield of 25 % was achieved. Subsequent exhaust dyeing trials demonstrated optimum color strength (K/S) of 5.18. The protein-mordanted cotton substrates exhibited reliable fastness characteristics, presenting a sustainable, non-hazardous alternative for eco-conscious textile product development.

Keywords: *Glycine max protein mordant, Bixa orellana seed, color strength, color fastness*

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1. INTRODUCTION

While wet chemical processing significantly enhances the market value of textile materials, it inherently accelerates environmental degradation through the intensive consumption of water and auxiliary chemicals, leading to heavily contaminated effluents [1]–[5]. To achieve uniform coloration, dye application is typically carried out at various processing stages, including fiber, yarn, or fabric forms [6], [7]. Dye is a term used to describe colored substances that are commonly used to color goods in the textile, printing, rubber, cosmetic, plastic, and leather industries [8], [9]. Dyeing is the most popular method for giving fibers, yarns, and fabrics color. Color for the textile industry is primarily derived from pigments and colors, with dyes being applied to textiles as a paste or solution. Color is an important factor in the appeal and marketability of textile products [7]. Textile dyes are divided into natural and synthetic types. Natural dyes, obtained from animals, flowers, fruits, stems, roots, and leaves of plants, are important for textile substrates, organic, and require a binder for application [10]–[13]. Nowadays, the majority of Synthetic colors are employed in industrial textile dyeing. They are created using synthesis using a variety of methods from fossil fuel residues, such as Aromatic derivatives, including aniline [14]–[18]. Up until the nineteenth century's end, the main colorants used on textiles were natural dyes [19]. Natural dyes were less popular following Synthetic dyes were invented in 1856 because they lacked some of its virtues, such as color fastness, high shade reproducibility, brilliance, and ease of application. Allergies may be triggered by synthetic dyes, which are also cancerous and harmful to human well-being. Recent economic developments favor natural colors made from plants over synthetic dyes since they are non-hazardous and biodegradable [20]–[23]. Since natural colors are biodegradable and come from vegetarian and renewable sources, they are typically renewable, in contrast to non-renewable resources fundamental raw materials used to create synthetic colors [24], [25]. Natural dyes can provide unique colors unlike those made by commercial dyes [26], [27]. Although Mordants made of metal are commonly utilized to increase color fastness in natural dyeing, their high toxicity and non-biodegradable nature present severe hazards to both environmental ecosystems and human health [28]–[30]. This is because metal salt mordants typically contain a high concentration of metal ions that remain after coloring [31], [32]. during dyeing, significant amount of the metal ions remains unused in leftover mordant baths, which are subsequently released into wastewater, raising environmental and

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public health concerns [33], [34]. Growing environmental awareness has increased interest in the use of bio-mordants for natural textile dyeing in order to avoid hazardous metallic salts [35]. Therefore, this work assess extraction and application of natural dye from *Bixa orellana* seed and bio-based mordant from *Glycine max* protein in aspects of reducing the negative impact of synthetic dyes and metallic mordants on the environment.

2. EXPERIMENTAL

2.1 Materials and Reagents

- **Substrate:** 100% cotton, half-bleached, plain weave fabric (140g/m^2), sourced from MNS Manufacturing Plc. (Legetafo, Ethiopia).

- **Natural Colorant:** Raw seeds of the achiote tree, obtained from the Wondogenet Agricultural Research Center.

- **Mordants & Auxiliaries:** Commercial-grade Copper (II) sulfate was utilized as a metallic mordant. Locally sourced soybeans (*Glycine max*) were processed to obtain a proteinaceous macromolecular extract serving as the bio-mordant. Laboratory Sodium Hydroxide (NaOH) was used as the extraction media. ISO standard soap and sodium carbonate were used for post-dyeing wash-off sequences.

2.2 Methods

2.2.1 Alkali-Assisted Extraction

Dye extraction was performed in an atmospheric washer-dyeing machine at a constant material-to-liquor ratio (MLR) of 1:30. Dried achiote seeds were suspended in an aqueous solution containing sodium hydroxide (NaOH) to establish an alkaline medium. The alkaline environment facilitates the hydrolysis of glycosides embedded within the seed coat, breaking down complex pigment clusters and increasing their solubility in the liquor. The temperature was systematically evaluated at 65°C, 75°C, and 85°C for 1, 1and half, and 2 hours. The resulting solution was separated using a two-stage filtration process: initial filtration through a polyester fabric mesh, followed by secondary purification via Whatman filter paper to extract a pure, particulate-free dye solution and the dye solution was subsequently dried under vacuum using Rota evaporator. To obtain the yield percentage of the dye plant, the following formula was used: -

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$$\text{Extraction Yield (\%)} = \frac{\text{Actual weight}}{\text{Initial weight}} * 100$$

2.2.2 Characterization Techniques

- **UV-Visible Spectroscopy:** The light absorption profile of the extracted dye liquor was evaluated using a Jenway UV-Vis Spectrophotometer across a scan spectrum of 300 to 600 nm to determine the maximum absorption wavelength (λ_{max}).
- **FT-IR Spectroscopy:** Chemical functional group transitions and coordination linkages between the fiber and mordant were analyzed using a Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) system (SHIMADZU FTIR-8400S).

2.2.3 Fabric Mordanting Procedures

1. **Glycine max Protein Mordanting:** Glycine max seeds were soaked in water at a1:5 gravimetric ratios for 12 hours to isolate active proteinaceous macromolecules. Cotton samples were immersed in this extract, padded, and subsequently stored in a controlled dry climate for 1 week to facilitate proper protein curing on the cellulose matrix.
2. **Metallic Mordanting:** For comparison, reference cotton samples were treated with 2% o.w.f. (on weight of fabric) Copper (II) sulfate solution at a controlled boiling temperature for 1 hour using a laboratory hot plate.

2.2.4 Exhaust Dyeing Process

Pre-mordanted and un-mordanted cotton fabric samples were dyed via the exhaust method using an atmospheric water bath dyeing machine at a fixed MLR of 1:30 under alkaline conditions. To optimize performance, three process variables were evaluated across three distinct levels:

- **Concentration:** 10%, 15%, and 20% (o.w.f.)
- **Temperature:** 30°C, 45°C, and 60°C
- **Time:** 30, 45, and 60 minutes

Post-dyeing, all fabrics underwent thorough cold water rinsing to remove loosely bound surface colorants, followed by a soaping process using 2 g/L of non-ionic soap at 60°C for 15 minutes to clear unreacted pigment matrices before final air drying.

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3. Experimental Design and Statistical Optimization

Taguchi and Box-Behnken designs were used to optimize extraction and dyeing. For extraction, a Taguchi L9 orthogonal array evaluated time and temperature across three levels (9 runs) to maximize dye yield. For dyeing, a three-factor Box-Behnken response surface design (15 runs) modeled dye concentration, temperature, and time using a second-order polynomial to predict the optimum parameters for maximum fabric color strength and fastness.

Phase 1-Expermental design for extraction

Utilized a Taguchi experimental design to reduce process variation and optimize cost efficiency. The independent variables were extraction time and temperature across three levels, with dye yield percentage serving as the response variable.

Table1. Factor and level for extraction

Factor	Description	Levels		
A	Temperature(°C)	65°C	75°C	85°C
B	Time (time)	1hr	1.5hr	2hr

Table2. Experimental observation for extraction

Experiment No	Temperature(°C)	Time (hours)	Yield%
1	65	1.0	11.0
2	65	1.5	13.4
3	65	2.0	15.5
4	75	1.0	17.0
5	75	1.5	19.0
6	75	2.0	21.7
7	85	1.0	22.0
8	85	1.5	23.9
9	85	2.0	25.0

Phase-2 Experimental design plan for dyeing

The independent variables were dye concentration, dyeing temperature and dyeing time with three levels while the response variables were color strength and color fastness.

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Table3. Factors and level for dyeing

Factor	Factors Name	Min	Max
A	Dye concentration(o.w.f)	10	20
B	Temperature ((°C)	30	60
C	time (min)	30	60

Table4. Experimental design box and Behenken(BBD) for dyeing

StdOrder	Run Order	PtType	Blocks	Concentration (%)	Temperature (°C)	Time (Min)
10	1	2	1	15	60	30
12	2	2	1	15	60	60
4	3	2	1	20	60	45
1	4	2	1	10	30	45
13	5	0	1	15	45	45
14	6	0	1	15	45	45
8	7	2	1	20	45	60
6	8	2	1	20	45	30
5	9	2	1	10	45	30
11	10	2	1	15	30	60
7	11	2	1	10	45	60
9	12	2	1	15	30	30
15	13	0	1	15	45	45
2	14	2	1	20	30	45
3	15	2	1	10	60	45

4. RESULTS AND DISCUSSION

4.1 Impact of Extraction Variables on Dye Yield

The gravimetric yields obtained from the alkaline aqueous extraction process are summarized in Table 2. The maximum purified pigment yield of 25.0% was recorded at an extraction temperature of 85°C over a 2-hour processing cycle. Thermal acceleration up to 85°C significantly enhanced the solubility and mass transfer of the carotenoid colorants;

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however, increasing the temperature beyond this limit yielded no notable mass gains. Similarly, prolonging the extraction time past 120 minutes had a negligible impact on the overall yield, indicating that the system had reached mass transfer equilibrium.

Table5. Analysis of Variance for Response yield percentage

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	p-value	significance
Temperature	2	161.342	**86.18%**	161.342	80.6711	305.06	0.000	**< 0.05 highly significant**
Time	2	24.816	**13.26%**	24.816	12.4078	46.92	0.002	
Error	4	1.058	**0.57%**	1.058	0.2644			
Total	8	187.216	**100.00%**					

An analysis of variance (ANOVA) was conducted to evaluate the influence of extraction temperature and time on the dye yield percentage (Table5). The results demonstrate that extraction temperature is the highly dominant factor, accounting for 86.18% of the variability in the yield. Extraction time is also statistically significant, contributing 13.26% to the total variance. Notably, the p-values for both factors are less than 0.05 ($P < 0.05$), confirming their strong statistical significance in the extraction process.

4.2 Spectroscopic Identification

4.2.1 FT-IR Spectroscopy Analysis

Fig.1. shows the Fourier-Transform Infrared (FTIR) spectra of the Unmordanted Fabric and Bio-mordanted Fabric. The FTIR of bio-mordanted fabric shows a substantial increase in absorbance at region of 1648 cm^{-1} which is corresponding to **C=O** stretching vibrations of Amide I and the presence of quaternary nitrogen group (- N^+ - 3R) in which the 'R' groups are substituted with amino groups of Glycine max protein [36]. And the newly formed absorption peak at 2140 cm^{-1} represents C≡C or C≡N stretching, indicate the presence of alkyne or nitrile groups Indicates minor secondary metabolites or conjugated carbon triple-bond structures present in crude plant extracts which suggests the successful attachment of *Glycine max* protein extract. Also the FTIR of bio-mordanted fabric exhibits a notable shift and deepened absorption at 1400 cm^{-1} suggesting C-H bending vibrations in-plane O-H bending of phenolic hydroxyl groups and symmetric stretching of carboxylate

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(COO^{-}) groups which subsequently verifies the involvement of polyphenolic or organic acid components from the bio-mordant in cross-linking with the polymeric backbone of the cotton fibre.

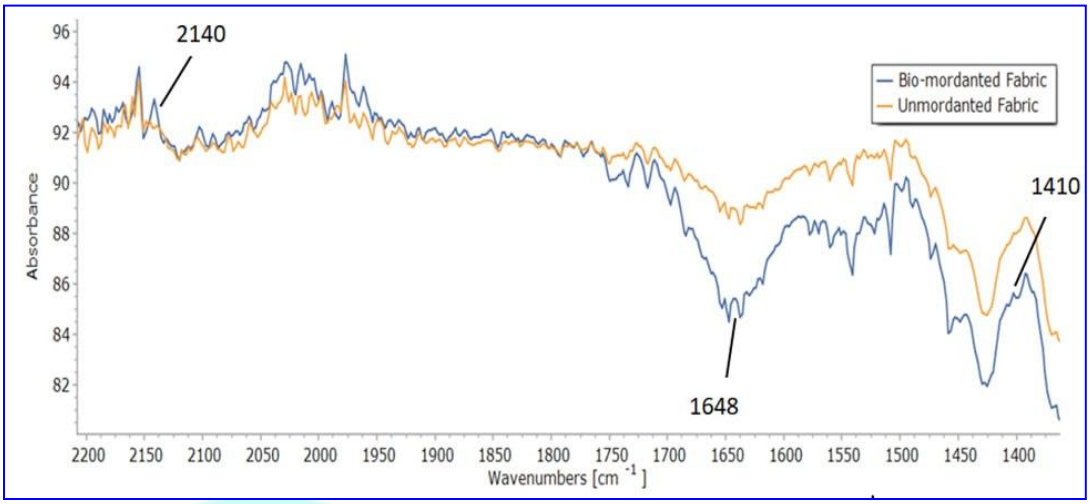

Figure1. FT-IR analysis of Bio-Mordanted and unmordanted fabric

4.2.2 UV-Vis Spectroscopy

The spectra of absorption of the extracted pigment were assessed in both sodium hydroxide and ammonia media. The maximum absorbance The wavelength (λ max) was detected at 420 nm in the sodium hydroxide solvent (pH 11.0)

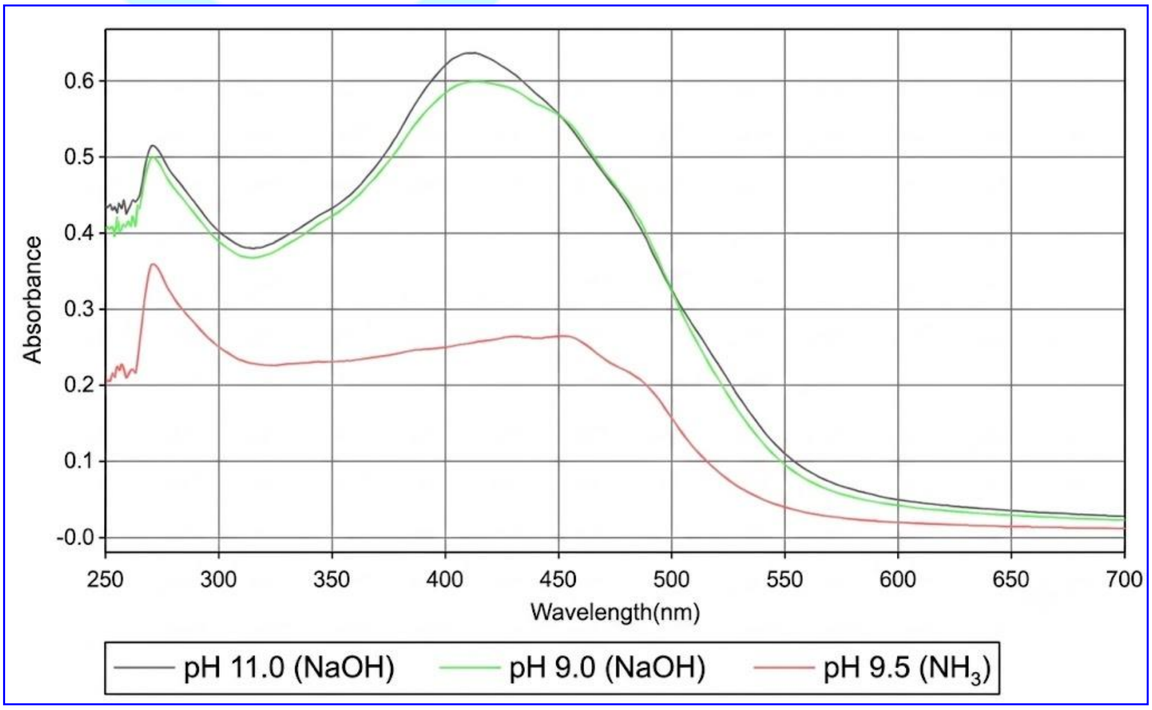

Fig. 2. UV-Vis Spectroscopy analysis of extracted seed of achiote tree dye

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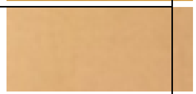
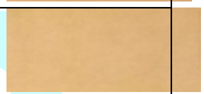
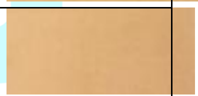
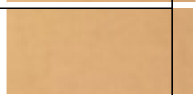
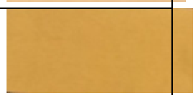
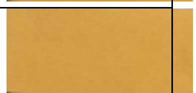
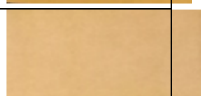
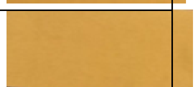
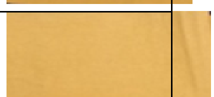
4.3 Analytical Assessment of Dyeing Parameters on Color Strength (K/S)

Color matching coordinates (*L*^*, *a*^*, *b*^*) and resultant Color Strength (K/S) profiles across variables are compiled below:

Table6. Summary table for color strength and color indices test result

Temperature (°C)	Time (minute)	Concentration (%)	Mordant type	K/S	Color indices			Shades
					L	*a**	*b**	
30	30	15	Unmordanted	4.13	74.55	4.08	5.98	
			Glycine max	2.44	75.09	3.66	5.95	
			CuSO4	2.38	74.79	2.91	5.20	
	45	10	Unmordanted	2.55	74.96	3.65	5.37	
			Glycine max	2.43	75.15	3.49	5.88	
			CuSO4	1.55	75.14	2.41	4.49	
	45	20	Unmordanted	4.93	74.13	4.40	5.81	
			Glycine max	5.00	74.38	4.32	6.28	
			CuSO4	2.33	74.80	3.08	5.26	
	60	15	Unmordanted	4.23	74.33	4.13	5.87	
			Glycine max	4.31	74.53	4.08	6.29	
			CuSO4	2.70	74.55	3.24	5.30	
	30	20	Unmordanted	3.95	74.46	4.29	5.84	

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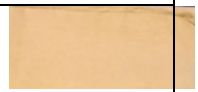
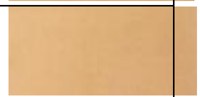
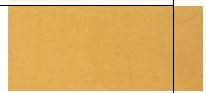
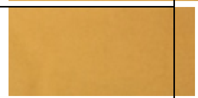
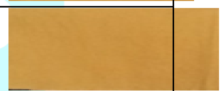
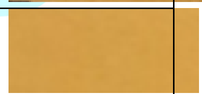
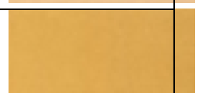
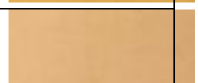
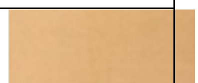
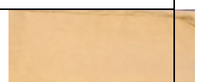
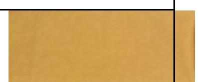
45			*Glycine max*	4.23	74.54	4.27	6.34	
			CuSO4	3.32	74.48	3.28	5.43	
	30	10	Unmordanted	1.91	75.30	3.42	4.94	
			Glycine max	2.36	75.23	3.53	5.80	
			CuSO4	2.10	75.03	2.67	4.95	
	45	15	Unmordanted	3.55	74.62	3.83	5.74	
			Glycine max	3.86	74.71	3.95	6.17	
			CuSO4	2.74	74.70	3.13	5.30	
	60	20	Unmordanted	3.85	74.43	4.30	5.39	
			Glycine max	5.18	74.26	4.45	6.36	
			CuSO4	4.06	74.07	3.30	5.23	
	60	10	Unmordanted	2.09	75.04	3.59	4.94	
			Glycine max	3.36	74.93	3.97	6.22	
			CuSO4	2.50	74.73	2.80	4.91	
60	30	15	Unmordanted	2.62	74.86	3.74	5.37	
			Glycine max	2.05	75.13	3.45	5.50	
			CuSO4	1.64	75.01	2.62	4.55	
	45	20	Unmordanted	3.03	74.59	4.14	5.14	
			Glycine max	4.28	74.44	4.14	6.16	
			CuSO4	2.78	74.44	3.38	5.14	

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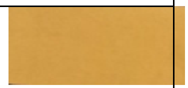
	45	10	Unmordanted	1.82	75.15	3.64	4.36	
			Glycine max	2.47	75	3.56	5.73	
			CuSO4	1.81	74.97	2.54	4.61	
	60	15	Unmordanted	3.38	74.66	4.03	5.81	
			Glycine max	3.72	74.46	4.07	6.03	
			CuSO4	1.78	74.83	2.97	4.71	

4.3.1 Influence of dyeing parameters on dyeing performance

1. **Dye Concentration:** Increasing shade depths from 10% to 20% significantly improved the K/S value across all samples. Higher concentrations expand color density per volume unit, intensifying color depth on the cellulosic boundaries.
2. **Dyeing Temperature:** the highest K/S exhibited at lower to mid-range temperature targets. Thermal migration up to 60°C initiated an apparent drop in color strength. This suggests that excessive thermal action may destabilizes structural molecular chains within natural carotenoid groups.
3. **Dyeing Time:** Color strength progressively optimized from 30 minutes up to 60 minutes, where maximum exhaust equilibrium was reached (K/S = 5.18).
4. **Mordant Performance:** The *Glycine max* protein-based mordant recorded the highest overall color intensity. Interestingly, un-mordanted cotton outperformed the $CuSO_4$ chemical mordant variants suggesting that Achiote extract exhibits an inherent direct affinity to cellulose, whereas metallic copper mordanting generates a surface precipitate film that suppresses overall color expression. At low temperatures (30°C) and short intervals (30 min), the cured macromolecular protein layer on the cotton creates a temporary physical barrier that restricts initial pigment diffusion. Thermal acceleration to 45°C–60°C is required to provide the kinetic energy necessary for the dye molecules to penetrate and chelate with the protein matrix.

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4.4 Color Strength ANOVA Analysis

Table7. Analysis of Variance for Response color strength

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	p-value	VIF
Model	9	13.6161	97.24%	13.6161	1.51290	19.56	0.002	
Linear	3	12.2526	87.50%	12.2526	4.08419	52.80	0.000	
Concentration (%)	1	8.1406	58.14%	8.1406	8.14061	105.24	0.000	1.00
Temperature (°C)	1	0.3444	2.46%	0.3444	0.34445	4.45	0.089	1.00
Time	1	3.7675	26.91%	3.7675	3.76751	48.70	0.001	1.00
Square	3	1.2085	8.63%	1.2085	0.40284	5.21	0.054	
Concentration (%)*Concentration (%)	1	0.1822	1.30%	0.1051	0.10514	1.36	0.296	1.01
Temperature(°C)*Temperature(°C)	1	0.8025	5.73%	0.8641	0.86405	11.17	0.021	1.01
Time*Time	1	0.2239	1.60%	0.2239	0.22390	2.89	0.150	1.01
2-Way Interaction	3	0.1550	1.11%	0.1550	0.05168	0.67	0.607	
Concentration(%) *Temperature(°C)	1	0.1444	1.03%	0.1444	0.14440	1.87	0.230	1.00
Concentration(%) *Time	1	0.0006	0.00%	0.0006	0.00063	0.01	0.932	1.00
Temperature(°C)* Time	1	0.0100	0.07%	0.0100	0.01000	0.13	0.734	1.00
Error	5	0.3868	2.76%	0.3868	0.07736			
Lack-Of-Fit	3	0.3868	2.76%	0.3868	0.12893	*	*	
Pure Error	2	0.0000	0.00%	0.0000	0.00000			
Total	14	14.0029	100.00%					

The ANOVA results demonstrate an exceptionally strong model fit, where the total model explains 97.24% of the variation in the response variable (representing an excellent R^2 equivalent value) and leaves a negligible 2.76% to error. This high explanatory power is backed by strong statistical significance, as evidenced by the overall model *p*-value of

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0.002, which indicates a highly reliable relationship between the variables. When evaluating individual predictors, Concentration (%) and Time emerge as the highly significant drivers of the process, boasting individual *p*-values of 0.000 and 0.001 respectively. Conversely, the linear effect of Temperature (°C) is not statistically significant with a p-value of 0.089, though temperature does exhibit a Significant quadratic interaction ($p = 0.021$), confirming a parabolic relationship where color strength increases with temperature up to an optimal threshold, beyond which thermal degradation of the natural carotenoid pigment occurs.

4.5 Fastness Evaluation

The color fastness ratings of the dyed cotton samples are detailed across key benchmarking categories:

- **Washing Fastness:** *Glycine max* protein bio-mordanted fabric samples achieved **Moderate to Good (3 to 4)** color change resistance ratings. Conversely, copper-sulfate-treated items displayed satisfactory to poor limits, and staining on adjacent fiber types (e.g., polyester, acrylic, wool) remained negligible (5) across most runs.
- **Rubbing Fastness:** Both bio-mordanted and metallic-mordanted cotton surfaces exhibited **Excellent (4 to 5)** wet and dry crocking numbers, indicating superior mechanical resistance to abrasive color transfer.
- **Light Fastness:** Copper (II) sulfate samples showed good to moderate light stability (4 to 5). Protein-treated substrates achieved fair to poor light fastness scores (3 to 4), while un-mordanted cotton exhibited poor light stability (3), indicating that carotenoid pigments undergo relatively rapid photo-oxidation when exposed to light without chemical stabilization.

5. CONCLUSION

This study successfully demonstrates an environmentally conscious strategy to cotton fabric coloration using natural dye extracted from achiote tree seeds and a sustainable, plant-derived bio-based mordant. Under optimized alkaline extraction conditions (85°C for 2 hours), a maximum purified dye yield of 25% was achieved. Dyeing trials optimized via a Box-Behnken design showed that higher dye concentrations and extended durations (up

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to 60 minutes) significantly enhance color strength (K/S), whereas excessive temperatures degrade the dye.

The plant-based *Glycine max* protein extract outperformed traditional heavy-metal Copper (II) sulfate mordants in color intensity, achieving a maximum K/S value of 5.18 while delivering reliable washing and rubbing fastness properties. This research work highlights the viable role of achiote seeds and bio-mordants in advancing green chemistry and sustainable manufacturing in the global textile sector through eliminating toxic metallic salts and synthetic colorants which allows the textile industry to significantly reduce its ecological footprint and effluent toxicity.

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