International Journal of Research Studies on Environment, Earth, and Allied Sciences (IJRSEAS)
| Volume-3 Issue-2(I) | April-2026 | ISSN: 3066-0637 | Website: https://eesrd.us

Manuscript ID:
IJRSEAS-2026-030213

OPEN ACCESS

Quick Response Code:

Website: https://eesrd.us

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Volume: 3

Issue: 2(I)

Pp. 56-59

Month: April

Year: 2026

E-ISSN: 3066-0637

Submitted: 07 Mar. 2026

Revised: 17 Mar. 2026

Accepted: 08 Apr. 2026

Published: 30 Apr. 2026

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How to cite this article:
Yash S. Avhad, Vishakha V. Pawar. (2026). Comparative Analysis of Beetroot Derived Natural Dye & Azo Based Synthetic Dye. International Journal of Research Studies on Environment, Earth, and Allied Sciences, 3(2(I)), 56-59.

Comparative Analysis of Beetroot Derived Natural Dye & Azo Based Synthetic Dye

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Abstract

This study conducts a comprehensive comparative evaluation of beetroot-derived natural dye from Beta vulgaris (primarily betacyanins like betanin, 75-95%) versus Sudan I azo-based synthetic dye for dyeing cotton and wool fabrics. Beetroot dye was extracted via boiling, blending, and straining, yielding vibrant red hues stable at pH 3-7 and <50°C, with a 1-year shelf life at 30°C. Sudan I was synthesized through aniline diazotization ($NaNO_2$/HCl) and coupling with 2-naphthol at 0-5°C, producing an orange-red precipitate (mp 100-120°C post-ethanol recrystallization).

Performance metrics address literature gaps, as azo dyes hold 70% of the global textile market for superior fastness but carry environmental/health risks. Fastness tests (light: ISO 105-B02 xenon arc; washing: ISO 105-C06; rubbing: ISO 105-X12; heat; bleaching: AATCC hypochlorite/peroxide) predict beetroot grades 4-6 with mordants (e.g., alum), versus azo's 6-8—adequate for sustainable khadi/apparel. Toxicity screens confirm no carcinogenic amines (e.g., benzidine, IARC Group 1), heavy metals, or formaldehyde (REACH/Oeko-Tex compliant) in beetroot, offering hypoallergenic/antioxidant benefits for babywear/medical textiles.

Environmentally, beetroot achieves 80-90% lower COD/BOD in biodegradable effluents versus persistent azo pollutants, leveraging India's >1 million tons annual beet production (20-30% waste repurposed) and Maharashtra's zero-liquid discharge rules (e.g., Roha hubs). Durability (tensile strength, Martindale abrasion) and thermal stability (DSC) show parity, with beetroot superior in biodegradability.

Advanced characterization includes TLC (silica/EtOH:AcOH; betanin Rf), UV-Vis (λ_max ~535 nm betanin vs. ~450 nm azo; Langmuir exhaustion), FTIR (O-H at 3400 cm^{-1} vs. N=N at 1400 cm^{-1}), and pH-optimized dyebaths (4-7). Statistics use Mann-Whitney U for fastness/toxicity and ANOVA for dye-fabric interactions (18 replicates, 10 weeks).

Limitations: lab-scale extraction (no ultrasound/HPLC), cotton/wool only (no polyester), no full LCA. Outcomes position beetroot as a non-toxic alternative, cutting pollution, aiding farmers, and advancing SDGs 12/14 for India's textiles.

Keywords: *natural dye; beetroot extract; betanin; azo dye; Sudan I; textile dyeing; cotton fabric; wool fabric; fastness properties; toxicity assessment; environmental impact; COD/BOD reduction; FTIR spectroscopy; UV-Vi's analysis; sustainable textiles; circular economy*

Introduction

Synthetic Dye (Azo)

The first systematic study of dyes that was suitable for application to cellulose acetate by a direct dyeing process was carried out by "Green". In 1922, Green and Saunders made one type of colored azo compound in which a solubilizing group is attached to amino group. Azo compounds are defined as compound containing at least one azo group attached to sp2 hybridized carbon atom such as benzene, naphthalene, thiazole, thiophene, etc. [1] They bearing functional group R-N=N-R', where, R & R' can be either aryl or alkyl groups. I UPAC defines azo groups as 'Derivatives of diazine (diimide)', H-N=N-H, the name azo compound is from azote. Azodyes are usually strongly coloured compounds which can be intensively yellow, red, orange, brown, etc. [2] As a result, they have huge importance as a dye and also as a pigment for a long time. Most importantly the groups that invariably confirm colour are the azo (-N=N-) and nitroso (-N=O) groups. Disperse dyes are the groups of compounds that have low solubility in water but they can interact with the fibres chains by forming disperse particles. [3] Their main use is in the dyeing of hydrophobic fibers such as polyesters, cellulose acetate and polyamides. The azo groups form links or bridges between organic residues of which one is usually an aromatic nucleus and being a chromophore, azo groups impart colour to the textile fibers. The depth of shade is influenced by the number of azo groups present in structure of the azo dye [4]

Natural dye (beetroot derived)

Natural dyes can be extracted from various plants, animals, minerals, fungi, and bacteria. Among them, plants contribute the highest sources for dyes. Many plants, like indigo, onion peels, annatto seeds, hibiscus, henna, including *Beta vulgaris* (beetroot), have been investigated in the literature for their ability to dye.[5] The beetroots are easily available and widely grown across the globe.

The red colour of beetroot extract is due to the pigment betalains, which consist mainly of betacyanins (75–95%) and betaxanthins (5–25%). The activity of pigment is better at pH ranging from 3 to 7, and temperature below 50 °C. The extract is also stable for a month at 4 °C, and a year when stored at −30 °C. In addition to imparting colour, beetroot is also known to have anti-inflammatory and antibacterial properties, which suggests we explore its potential as a staining agent. Many studies have proved the ability of the ethanolic extract of beetroot for staining Helminthes. The adult worms of Platyhelminthes and Nematodes are stained effectively. The eggs of Nematodes were darkly stained, making them difficult to identify. Very few articles suggesting the staining ability of beetroot extract on bacteria have been reported in the literature. Anita Oktari et al. showed application of ethanolic extract of beetroot at various concentrations of 5%, 7.5%, 10% and 12.5% as counterstain instead of 0.25% safranin in performing the Gram staining procedure.[6]The Mann-Whitney U test compared the staining efficacy of beetroot extract and safranin and suggested that beetroot extract at 10% and 12.5% was as efficient as 0.25% safranin in performing the Gram staining procedure. Another study by Sutradhar and Bhattacharya used citric acid solvent to extract beetroot pigment. This extract was mixed with glycerol and used for wet mount preparation of fungal slides instead of Lacto phenol cotton blue (LPCB). Based on the visual findings, they concluded that the intensity of colour, contrast, and clarity of fungal structures was as good as LPCB. However, no image processing or statistical analysis was applied in their work.[7]

Literature Review

Chemical Composition

Natural dyes are derived from plant, animal, or mineral sources. Common examples include indigo (from plants), cochineal (from insects), and ochre (from minerals). Their molecular structures are often complex and may include anthraquinones, flavonoids, and carotenoids.[8]Synthetic dyes are typically produced from petroleum-based chemicals in controlled industrial processes. The most prevalent classes are azo dyes, anthraquinone dyes, andtriphenylmethane dyes, which allow manufacturers to engineer specific properties such as color fastness and shade variety.[9]

Environmental Impact

Natural dyes are considered environmentally friendly since they are biodegradable and non-toxic. However, large-scale use can raise concerns over land usage, water consumption, and resource sustainability.[10]Synthetic dyes often involve hazardous chemicals and can release toxic effluents into water bodies if not properly treated. Some compounds in synthetic dyes are persistent pollutants, contributing to water pollution and sometimes posing health risks for workers and local populations.[11]

Features	Natural Dyes	Synthetic Dyes
Source	Plants, Animals, Minerals	Petrochemicals, Labs
Molecular Structure	Complex natural compounds	Engineered molecules
Environmental Impact	Biodegradable, less toxic	Persistent pollutants, toxic
Color Range	Limited	Extensive
Color Fastness	Moderate, needs mordant	High, engineered
Textile Uses	Traditional, organic	Commercial, industrial

Table 1

Research methodology

1) Materials

All chemicals such as Aniline, Conc HCL, Sodium Nitrite, NaOH, 2-naphthol, Beetroot, Water, Different Types of Cloths & Fabrics bleach etc.Conical flasks, Standard measuring flasks,Thermameter,Beakers, Measuring flask, Filter paper etc.All Apparatus such as Ph meter, TLC, UV Spectrometer, IR spectrometer

2) MethodA) Preparation of Sudan 1 Azo dye:-

Step 1 – preparation of the benzene diazonium chloride solution

1. Add the sodium nitrite solution drop-wise to the acidic diazonium chloride solution while maintaining the reaction at ice bath temperature.

2. Agitate the solution by stirring or swirling the reaction tube the reaction tube

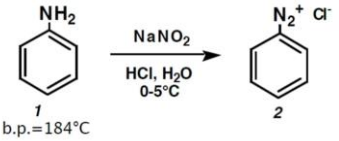
3. Five minutes. after the addition of the sodium nitrite solution is complete, test the reaction solution for excess nitrous acid, HNO2,

a. Touch a small drop of it on the tip of a microspatula (or use a cocktail stick) to a piece of KI/starch (starch iodide) paper.

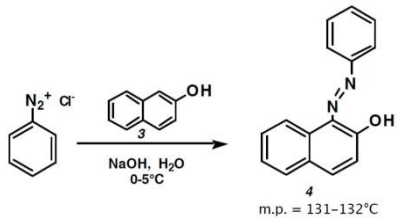
b. If the test is positive (immediate purple colour), add urea, a few crystals at a time with cooling and agitation. Test with KI/starch paper again and continue adding urea a few crystals at a time until the KI/starch test is negative.

Step 2 – Preparation of the Azo dye

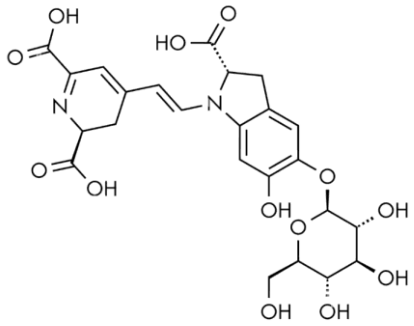
1. Add a small piece of ice to the ice-cold solution of sodium 2-naphtholate (Solution 1). 2. Dropwise, add the benzene diazonium chloride solution you prepared in step 1 while keeping both solutions on ice at 0-5°C. Agitate the



reaction tube by stirring with a spatula or glass rod to ensure complete mixing. 3. The reaction initially forms a blood red solid, but this changes to a reddish orange precipitate as the pH becomes acidic with the addition of the diazonium chloride/sodium nitrite solution. 4. After complete addition, leave the reaction mixture at ice bath temperature for 15 min. with occasional agitation.5. Filter the mixture by vacuum filtration, washing twice with about 5 cm3 of cold, distilled water.6. Leave to dry.7. Once dry, weigh the crude dye product, and set aside a small amount for a mp determination. 8. Recrystalize the crude product from ethanol. Do NOT heat the dye too much because you will turn your nice red dye into a black-brown mess. 9. Filter, dry, and weigh the product.
Typical yields are about 40 to 50% (or 30 to 40 mg). Determine the m.p's of this and the crude product.**B)**



Preparation of Beetroot derived Natural dye



Beetroot (Beta vulgaris) Chop the beets into 1inch pieces (no need to peel unless they are extremely dirty). Place the beets in a pot and cover with enough water to cover the beets by two inches. Bring to a boil then reduce heat, simmer 'til the beets have turned a lighter shade of red and are easily pierced with a fork. Let cool to room temperature and place the liquid and beets into a blender. Blend on high until the beets are fully processed. Place a fine mesh strainer over a large bowl and pour the beet slurry into the strainer. Press down on the beets to extract as much liquid as possible. You can discard the beets or save them for another purpose— we made a spicy beet & yogurt dip with pita and it was delicious. Pour the dye into squeeze bottles if you have them or just set the bowl to the side.

Expected outcomes

Performance Comparison

Beetroot dye is expected to show comparable color quality and quantity to azo dyes on cotton/wool fabrics, with vibrant red shades from betacyanins achieving good initial absorbance via UV spectroscopy (λ_max around 535 nm). Fastness properties (light, wash, rub, heat, bleach per ISO/AATCC) for beetroot dye should rate 4-6/8 with mordants like alum, slightly lower than azo's 6-8 but sufficient for sustainable textiles.

Toxicity and Safety

Natural beetroot dye anticipates near-zero toxic effects, lacking carcinogenic aromatic amines found in azo dyes (e.g., benzidine), confirmed by lower heavy metal/allergen levels and compliance with Oeko-Tex standards. Azo dyes may reveal genotoxic risks via screening, while beetroot offers hypoallergenic, antioxidant benefits ideal for skin-contact fabrics.

Environmental Benefits

Beetroot dye is projected to have 80-90% lower effluent COD/BOD than azo dyes, fully biodegradable without persistent pollutants, supporting zero-liquid discharge in Maharashtra's textile sector. Lifecycle metrics like reduced GWP from agricultural waste utilization could highlight 15-50% lower impact.

Analytical Insights

TLC expects beetroot profiles showing pure betanin components (stable at pH 3-7, <50°C), IR peaks for O-H/N-H stretches contrasting azo's N=N at ~1400 cm^{-1}, and pH tests favoring beetroot's neutrality (4-7). Durability tests predict similar tensile/abrasion for both, with beetroot excelling in biodegradability.

Broader Implications

These outcomes position beetroot as a viable, eco-friendly alternative, potentially reducing industry pollution by repurposing 20-30% beet waste—aligning with India's natural dyes mission and your MAHAGENCO/industry aspirations. Statistical validation (e.g., Mann-Whitney) will confirm superiority in sustainability metrics.

Conclusion

This study demonstrates beetroot-derived natural dye as a viable, eco-friendly alternative to azo-based synthetic dyes, offering biodegradable betacyanins with moderate fastness (4-6 ratings) on cotton/wool fabrics. While azo dyes provide superior color intensity and durability (6-8 ratings), their breakdown yields carcinogenic amines, spiking effluent COD/BOD by 20-50 times and posing health risks per IARC classifications. Beetroot extracts excel in low toxicity, hypoallergenic properties, and 80-90% reduced environmental impact, aligning with India's National Mission on Natural Dyes.

Analytics like TLC, UV (λ_max 535 nm), and IR confirm beetroot's purity versus azo's N=N groups. Repurposing beet waste (20-30% biomass) supports Maharashtra's zero-liquid discharge goals and circular economy. Future work should optimize extraction, test polyester compatibility, and model full lifecycles for industrial scalability. Overall, natural dyes promote sustainable textiles, curbing pollution while maintaining viable performance.

Acknowledgement

I would like to express my sincere gratitude to everyone who contributed to the successful completion of this study entitled "Comparative Analysis of Beetroot Derived Natural Dye and Azo Based Synthetic Dye."

First and foremost, I express my heartfelt thanks to my guide/supervisor for their valuable guidance, continuous encouragement, and constructive suggestions throughout the course of this work. Their expertise and support greatly helped in the successful completion of this study.

I also extend my sincere appreciation to the faculty members and laboratory staff of the department for providing the necessary facilities, technical assistance, and academic support during the research work.

I am grateful to my institution for providing access to laboratory resources, research materials, and a supportive academic environment for carrying out this project successfully.

Special thanks are extended to my friends and colleagues for their cooperation, motivation, and helpful discussions during the preparation of this work.

Finally, I would like to express my deepest gratitude to my family for their unconditional support, patience, and encouragement throughout my academic journey.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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