



Eco dye & Bio cleanser: Innovating Sustainability with Natural Fabric Dyes and Microbial Decolorizers

Shruti P. Salunke^{1*} and Devkirani P. Pawar²

¹Student, ²Assistant professor, Department of biotechnology, Deogiri College, Chhatrapati Sambhajinagar, IN

*Corresponding author email: salunkesp109@gmail.com¹, devikapawar43@gmail.com²

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ABSTRACT

The textile industry is at a turning point with the increasing demand for eco-friendly products. Natural fabric dyes and organic decolorizers are a ray of hope for sustainable clothing. *Bougainvillea*, *Balsamina*, *Clitoria Ternatea*, *Cestrum Nocturnum*, *Mirabilis Jalapa*, and *Red Malabar Spinach* seeds are some of the plant materials used to dye clothes. Cream of tartar is used to enhance dye retention, making clothes more sustainable. Fabrics are immersed directly in extracted pigments for 24 hours after moderation. Microbial decolorizers offer a sustainable solution for removing dyes from fabrics. *Bacillus* species can produce laccases, peroxidases, and azoreductases that break down synthetic and natural dyes, allowing for efficient decolorization without harsh chemicals. These bacteria can effectively decolorize natural dyes through enzymatic reactions. Microorganisms like *Aspergillus Niger* and yeast (*Saccharomyces cerevisiae*) have enzymatic activities that can effectively decolorize natural dyes in fabrics. Activated charcoal is another efficient solution for removing colorants from dye-containing solutions, with great adsorption properties and the ability to be reused. These eco-friendly alternatives offer sustainable solutions for reducing the environmental impact of dyeing processes in the textile industry. We were able to obtain various shades of dyed fabrics by using different plant sources. For instance, Night Jasmine yielded a yellow color, *Balsamina* flowers produced a yellowish-brown hue, *Red Malabar Spinach* created a Magenta color, *Bougainvillea* flowers resulted in a pink shade, *Clitoria Ternatea* produced a blue color for both cotton and polyester fabrics. At the same time, *Curcuma longa* (turmeric) and baking soda pigment gave us an orange color and *Mirabilis Jalapa* was used to dye grey-colored fabric. This paper investigates the decolorization of various dyed fabrics using four different methods: bacteria (*bacillus* species), yeast, fungus (*Aspergillus Niger*), and activated charcoal. The results showed that while all four methods were successful in decolorizing the fabrics, Bacteria took 7 days, yeast took 5 days, and fungus and activated charcoal took 9 days to decolorize fabrics. This study provides useful information on removing dyes from textiles.

Keywords: Natural fabric dye, *Bougainvillea*, *Balsamina*, *Clitoria Ternatea*, *Cestrum Nocturnum*, *Mirabilis Jalapa*, and *Red Malabar Spinach*, bacteria (*bacillus* species), yeast, fungus (*Aspergillus Niger*), and activated charcoal.

INTRODUCTION

The people are moving more towards natural products which has been attributed to several factors including consumer preferences and interests. With ongoing studies and investigations, consumers are

getting more apprehensive of the implicit health threats associated with synthetic or chemical components in colorful products. They're looking for choices perceived as safer and further salutary for their health along with two critical perspectives of ambient and frugality. Natural fabric stains are gaining fashion ability for both environmental and health-related considerations. Because the yield of synthetic pigments is dependent on the petrochemical source and some of the colorants contain carcinogenic amines, the use of natural stains is boosting day by day because of their non-toxicity, non-carcinogenic, non-poisonous, less contaminating, reduced chemical exposure, easy accessibility, and numerous further. As it can be acquired from sustainable and renewable sources, for example, florals, it's now more extensively taken into consideration. Also, synthetic stains don't degrade and accumulate in the ambient making them destructive for the surroundings as well. It has been estimated that nearly. tones of synthetic pigment were used per annum. The synthetic stain may create pollution, skin conditions, and health hazards to humans and other important organisms. Disquisition on natural stains covers a broad diapason of motifs, ranging from the identification of new colorant sources to the enhancement of dyeing processes and the investigation of their usage in colorful industries. However, natural colorants contain antibacterial, and antifungal, If we look at them from different angles besides their coloring properties. India has a rich floral biodiversity, ranked 11th as the biggest biodiversity in the world. It has roughly 490,000 floral species and there's no query that the floral kingdom is a treasure-house of different natural products (Grover and Patani, 2011). Particularly flowers are used as a source of natural stains. Floral pigments delivered colorful hues as well as aroma effects on the fabrics. They're rich in tannin, an important source of yielding different hues of red, pink, brown, unheroic, etc. Natural stains produce profitable occasions for original communities involved in the culture, harvesting, and processing of colorant-yielding florals, contributing to sustainable livelihoods.

Clitoria Ternatea: - substantially belongs to the family Fabaceae. Due to its pharmacological exercise, it's extensively used as an antimicrobial, antipyretic, anti-inflammatory, analgesic, and numerous further. The flower can be used to stain natural threads and is used by traditional societies in Asia to do so. Blue single petal floral size "8 – 16" altitudinous (including pot).

Red Malabar Spinach: It belongs to the family Primulaceae. It's gathered in veritably large quantities due to its 75 ayurvedic medications. It's rich in anthelmintic, carminative, antibacterial, antibiotic, hypoglycemic, and anti-fertility properties. (Syed and Rajasekharan, 2011)

Curcuma Longa (Turmeric) + baking soda: A cremating brew. It belongs to the family Zingiberaceae. Turmeric is rich in anti-inflammatory, antimicrobial, antioxidant, and anti-neoplastic properties. It also has curcumin which may be used medically to treat a variety of dermatologic conditions. Pre-dye study revealed the following Turmeric does not need a caustic to set the stain. It's also what is called a fugitive stain- the chemical nature of fugitive colorants means they are kind of semi-permanent.

Bougainvillea: It belongs to the family Nyctaginaceae. It's rich in anticancer, antihepatotoxic, anti-inflammatory, antihyperlipidemic, antimicrobial, antioxidant, and anti-ulcer properties. Flowers are introduced in axillary clusters of three, each flower with a grandiloquent, red, pink, or orange bract beneath. The calyx is tubular with 5 lobes roughly 2 cm long. The tube is the same color as the bracts, with roughly 3 mm long white lobes and 5- 10 stamens.

Balsamina: It belongs to the family Balsaminaceae. The floral grows up to a height of 20 to 75 cm. They've mug-shaped, various flowers with four to five petals and grow up to one to three altitudes in size. In Southeast Asia, it was also used in making stains. For example, in Nepal, balsam leaves were crushed to color fingernails on the day of Shravan Sankranti. (Balasubramanian, 2023)

Cestrum Nocturnum (Night Jasmine): It belongs to the family Oleaceae. The flowers are ambrosial, with a five- to eight-lobed white corolla with an orange-red center; they're produced in clusters of two to seven together, with individual flowers opening at dusk and finishing at dawn. The leaves have been used in Ayurvedic medication and Homoeopathy. like antioxidant, antibacterial, antifungal, anticancer, antimalarial, antiepileptic, cytotoxic, analgesic, anti-inflammatory, anti-HIV, hepatoprotective, antipyretic, and injury recovery properties. (Singh and Vyas, 2018).

Mirabilis Jalapa: - Also known as the four o'clock flower. It belongs to the family Nyctaginaceae. The

flowers generally open late evening or dusk (between 4 and 8 o'clock). The flowers aren't formed from petals – rather they're a colored difference of the calyx. The flowers are pipe-shaped and penta-lobed, they've no mug (replaced by bracteal leaves) but are made of a corolla. It has a high capability for bioremediation, reduces inflammation, and numerous further. Used in making stains in the fabric diligence. (Annapoorani and Sundarraj, 2014)

Decolorization by Bacteria, Yeast, Fungus (*Aspergillus Niger*), and activated charcoal

Dyes usually have complex aromatic structures, making them more stable and difficult to degrade. There are more than 40,000 dyes and pigments with some 7000 different chemical structures, out of which more than 3500 dyes are in practical use. Synthetic dyes are classified as azo, nitroso, triphenylmethane, xanthene, and anthraquinone dyes. The existing methods that are used in the degradation process are found to be highly expensive and due to the production of chemical sludge, it is very harmful to the flora and fauna including human beings. Microbial degradation and decolorization of dyes are seen as a cost-effective method for removing this pollution from the environment (Ibrahim et al., 1996; Pearce et al., 2003). Several bacterial, fungal, and algal species can adsorb and/or break down azo dyes. (Stolz, 2001). Moreover, bacterial decolorization is normally faster than fungal systems concerning the decolorization and mineralization of azo dyes (Ibrahim et al., 1996). Numerous bacterial species including *Bacillus*, *Pseudomonas*, *Enterobacter*, *Halobacter*, and *Aeromonas* have been decolorized and detoxified by a wide range of azo dyes compared to phenylamine, benzenediazonium chloride or phenol (Telke et al., 2014). Activated carbon is a remarkable material that possesses a structurally homogeneous composition, boasts a high surface area, and demonstrates exceptional radiation stability. With its microporous structure, it has become a go-to solution for adsorption, catalysis, and catalyst support in various industrial processes. The effectiveness of activated carbon's adsorption properties largely depends on its particle size, porosity, ash content, degree of carbonization, and method of activation. The concentration of hydrogen ions, also known as pH, significantly impacts the dyes' ionization and the adsorbents' surface properties. The adsorption of the selected dyes decreases with an increase in pH, except for turmeric (containing 30% baking soda) on

activated charcoal. The reason for this is that activated charcoal forms a positively charged surface. When the pH value is low, the negative charge on the surface of the charcoal decreases, increasing the positive charge on the surface, which, in turn, enhances the adsorption of the negatively charged adsorbate.

2. MATERIAL AND METHODS

Collection of samples:

We collected the samples from various places to get the color pigments from various plant sources. The *Clitoria Ternatea* flowers were collected from Ramakrishna Mission Ashram, Satara Parisar, *Mirabilis Jalapa*, *Red Malabar Spinach*, *Balsamina* were collected from the Shivsena Pramukh Sriman Balasaheb Thakre Botanical Buddi Garden, Chaitanya Housing Society, Cidco Rd, Shivdatta Housing Society, N 8, Yashashri Colony N 8, *Bougainvillea* flower was collected from Prayas Biodiversity HotSpot, Nyay Nagar, *Curcuma Longa* (Turmeric) was collected from K.K Dawasaz, shahganj, *Cestrum nocturnum* (Night Jasmine) flower was collected from the Parijat Botanical Garden, sector N4 Cidco. Other Glassware like Flasks, beakers, test tubes, glass rods, Petri plates, weighing balance, spatula, cream of tartar, burner, mortar, pestle, etc. were collected from the laboratory of the Biotechnology department of Deogiri College. All the flowers were collected from locations situated in Chhatrapati, Sambhajinagar.

Extraction Of sample:

The aqueous extraction method was used for the extraction of dyes from different parts of the flowering plants including seeds. In this method dyes from flowers were extracted by preparing an aqueous solution of flowers. Firstly, the flowers were exposed to the natural sunlight until and unless they become completely dry. Once it dried completely, they were crushed into fine powder manually in mortar and pestle. Then, they were soaked in water to get the pigments for 3-4 hours or sometimes overnight depending upon the flowers. The quantity of the powder to be put in the water was dependent upon the availability of the flower powder (the standard can be taken as 10 gm in 100 ml distilled water). Then they were boiled at (80-85 degrees) until a nice color was obtained and then filtered to remove the non-coloring particles. The Dye colors were then stored in the refrigerator for further use.

Exception: In the case of seeds of Red Malabar Spinach, seeds were crushed into a fine paste using a mortar and pestle. Once the fine paste was obtained it was mixed with water for some time and then filtered using filter paper. For the preparation of orange dye, baking soda was added to the turmeric powder to create a color variation from yellow to orange. The process has been explained in detail in the below diagram of the Bougainvillea flower. The same process

was carried out for the rest of the flowers used in the experiment: *Bougainvillea Mirabilis Jalapa*, *Clitoria Ternatea*, *Cestrum Nocturnum* (Night jasmine), *Balsamina*, *Red Malabar Spinach*.

For the extraction of dye from the seeds of Red Malabar Spinach, firstly the seeds were crushed into fine paste using filter paper. After the preparation of the fine paste, Paste was mixed with water and then filtered out. No boiling is required in the use of seeds



Fig.1: Collection of sample A: Balsamina B: Red Malabar Spinach C: Bougainvillea D: Cestrum Nocturnum (Night Jasmine) E: Clitoria ternatea F: Mirabilis Jalapa

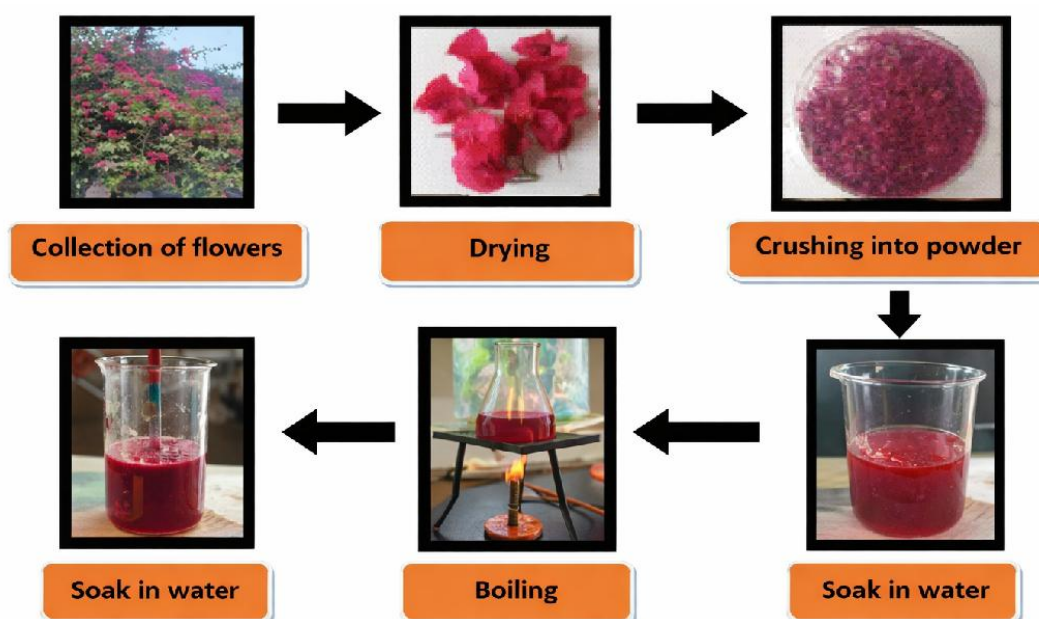


Fig. 2 Extraction process of dye from Bougainvillea flower



Fig. 3 Extractions of flowers

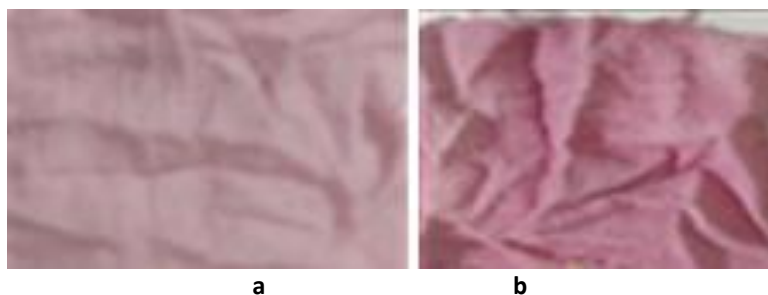


Fig. 4 Mordanting process:
a. Mordanting by salt b. Mordanting by cream of tartar

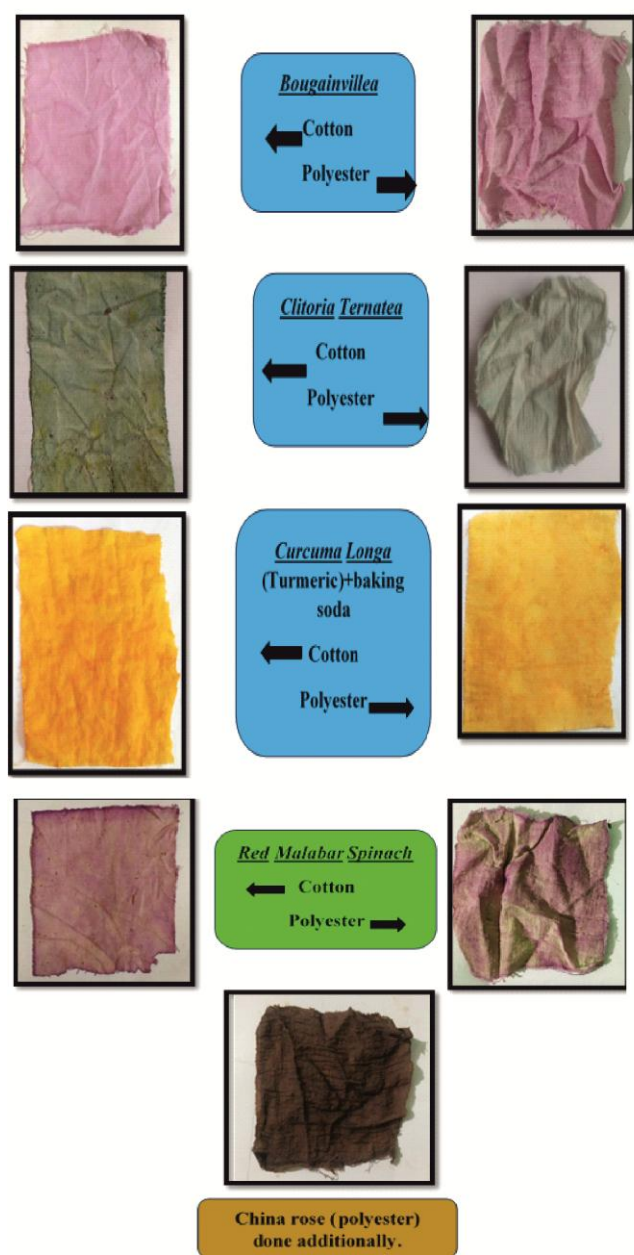


Fig. 5. All dyed fabrics



Fig. 6. Mordanting process

3 Mordanting of the Fabric:

Mordants: are chemicals in the form of metallic salts that are generally used to create an affinity between the fiber and the pigment. The mordant which was used for the mordanting was Cream of Tartar.

Cream of tartar: Also known as tartaric acid, it is a byproduct left after the fermentation of grapes into wine. It is used as 6% fiber weight for 100 g of fiber. The process of mordanting is carried out in two stages:

Pre mordanting and Meta mordanting: Before that, the flask was filled with tap water and made it lukewarm. Add the standard amount of cream of tartar to the flask and allow it to dissolve completely.

Pre-Mordanting: Firstly, the fabrics were treated with mordanting. It was treated for about 40 minutes to 1 hour at 30- 35 degrees.

Meta-mordanting: Fabrics were immersed in mordant and the dye extract for 40 mins at a lukewarm temperature.

Process of Mordanting

Before pre- and meta-mordating, fill the flask with tap water and make it lukewarm with the help of a burner. Add the standard amount of cream of tartar to the flask and allow it to dissolve completely. Then add the fabric to the solution for about half an hour. Make sure the fabric is covered completely in the solution. After half an hour, let the fabric cool completely and wash it under tap water. After that, the dye was added to the mordanting cloth and left for a few hours according to the dyeing fastness.

Decolorization by the Bacteria, Yeast, Fungus and Activated Charcoal

Three methods are tested for the decolorization of fabric dyes such as activated charcoal, bacillus sp., yeast, and fungus (*Aspergillus Niger*).

Preparation of the nutrient broth containing a bacterial culture

Firstly, the nutrient agar was prepared. The volume of nutrient Agar required was calculated as per the standard volume mentioned on the bottle (28gm in 1000 ml distilled water.) and 1% of Agar agar powder was added to it. Thus, the media was prepared. After the preparation of the media, it was autoclaved at 121

degrees for 15 minutes. Media was poured into the Petri plate in the Laminar Air Flow under Sterile conditions and cooled down. After the media solidified, the loopful sample from 10^{-6} test tubes of soil serial dilution was taken and 4 quadrant streaking was done and incubated at 37 °C for 24 hours. After the incubation, the loopful of bacterial colonies were taken from a cultured plate and inoculated in the nutrient broth under Laminar Air Flow at sterile conditions and incubated overnight. After the incubation, an equal amount of volume was distributed in each test tube containing small pieces of each dyed fabric. Then the test tubes were left for several days until the decolorization was observed in each test tube and the results were recorded.

Preparation of the yeast culture

Firstly, the dry yeast was taken from the laboratory and put in the glucose solution of standard concentration (1g in 100 ml). Incubate the solution overnight at room temperature. After that, an equal amount of volume was distributed in each test tube containing small pieces of dyed fabric.

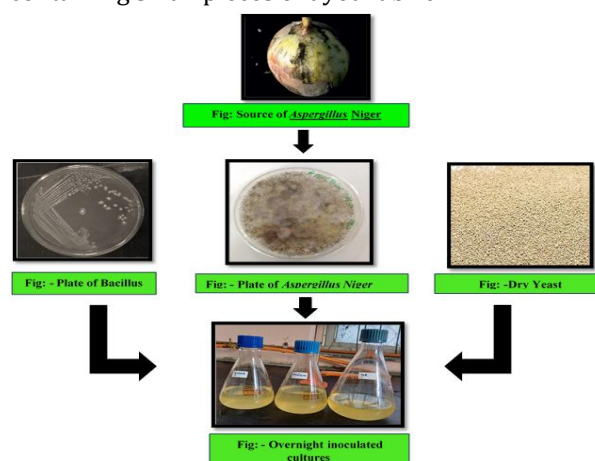


Fig.7 Inoculated culture of Bacillus, Fungus and Yeast

Preparation of the Czepedox Broth containing *Aspergillus Niger*

Firstly, the Czepedox broth was prepared. The volume of czepedox broth required was calculated as per the standard volume mentioned on the bottle (35.01g in 1000 ml distilled water.) and 1% of Agar agar powder was added according to it. After the preparation of the Czepedox media, it was autoclaved at 121 degrees for 15 minutes. After the media preparation, the loopful of black mold was picked from the onion and spotted on the czepedox plate. Incubated the plate for 5 days to get the *Aspergillus Niger*. After the incubation, the loopful colony was inoculated in nutrient broth

(prepared by the standard volume of 25g in 1000 ml of distilled water) and again incubated overnight. After overnight incubation, broth was distributed 20 ml in each test tube containing small pieces of dyed fabric. Then it was left for several days until the decolorization was observed and the results were recorded.

Preparation of Activated Charcoal Decolorizer: 5 grams of activated charcoal was dissolved in 100 ml of distilled water mixed well and then filtered out filter paper to get water like clear solution of activated charcoal. Then colored cloth strips were dipped into it.

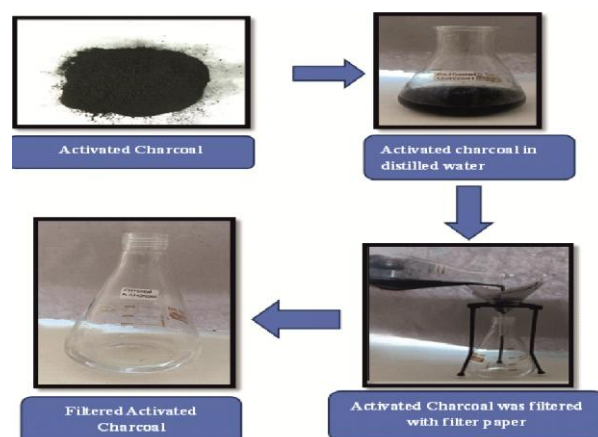


Fig 8: Activated charcoal decolorizer production process

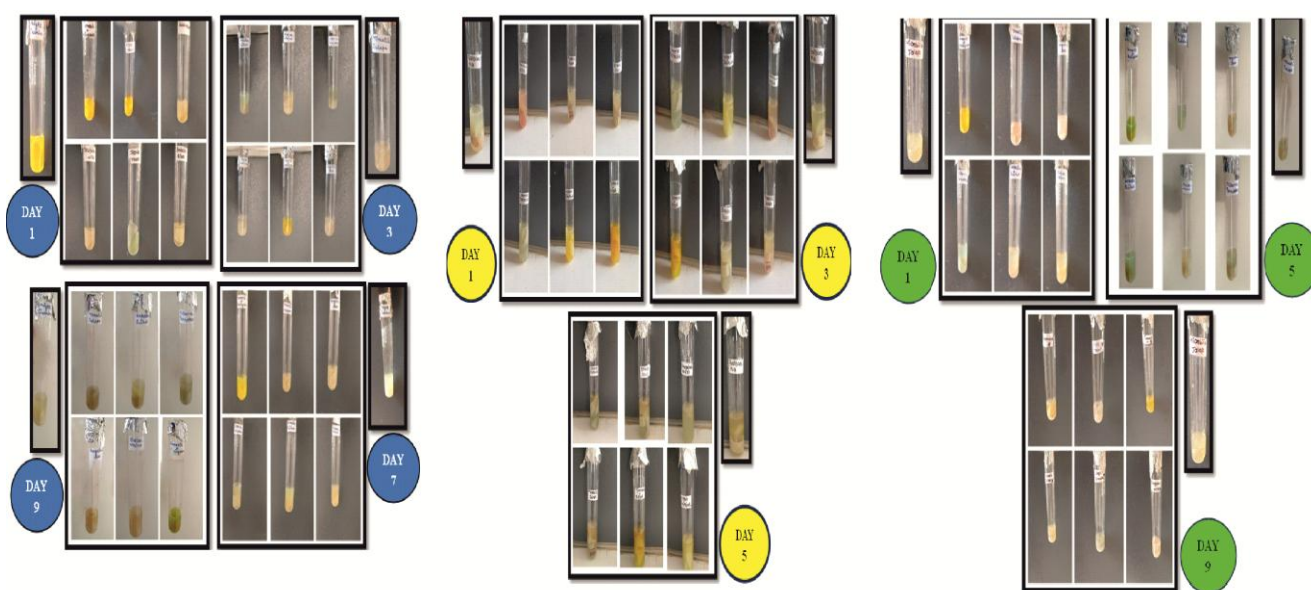


Fig.9. Bacterial, Yeast, Fungi Decolorization

RESULT

These are strips of dyes that have been decolorized using various methods. Different time intervals were observed for different methods used. For instance, clothes that were dyed using the bacterial decolorization method took 7 days to show results, whereas those dyed using the yeast decolorization method took 5 days. Clothes dyed using the fungus decolorization method showed results after 9 days and those dyed using activated charcoal took 9 days as well.

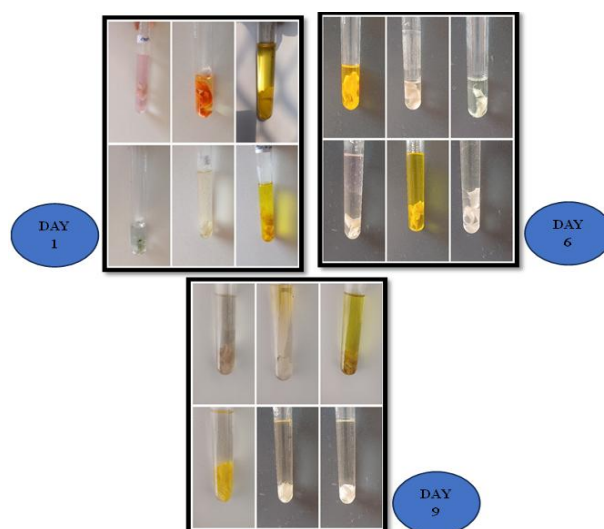


Fig.10 Activated Charcoal Decolourization

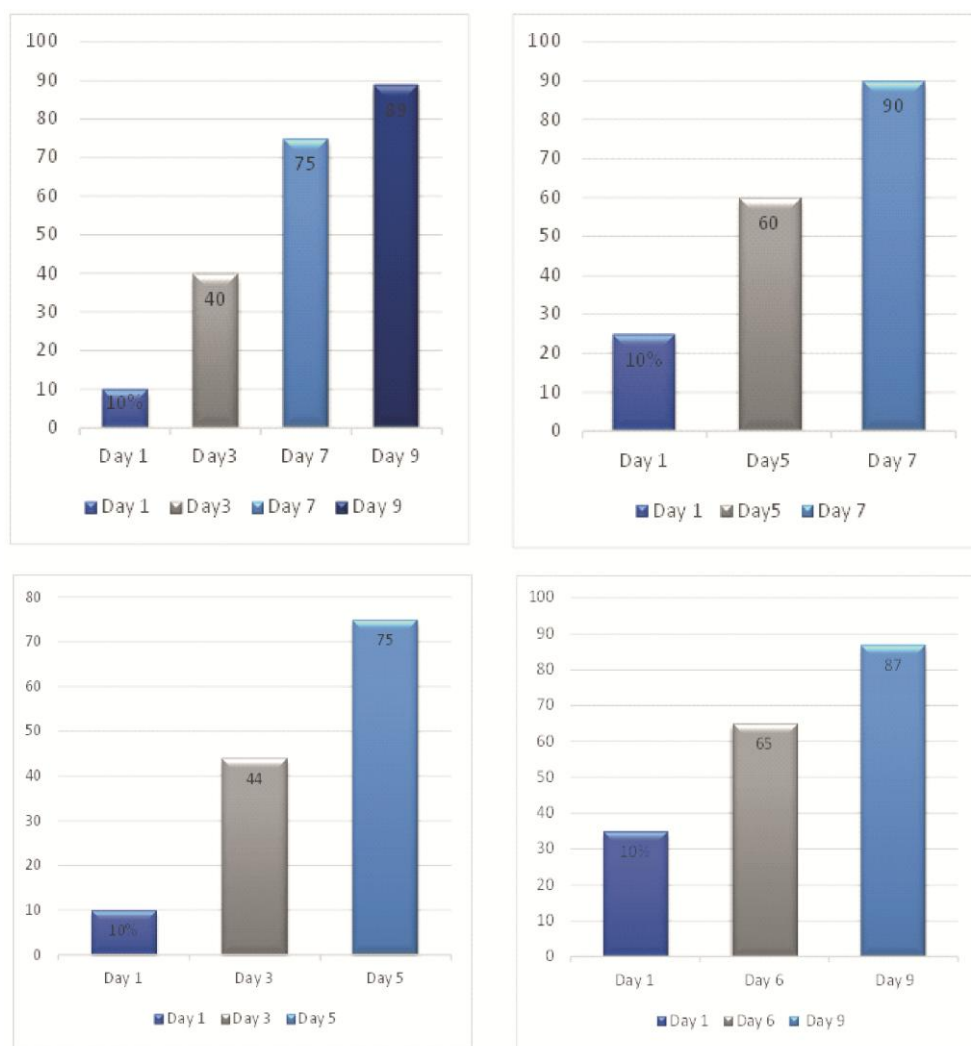


Fig.11. Percentage of Bacterial, Yeast, *Aspergillus niger*, activated charcoal decolorization of dyes for different interval of days

The figures show the percentage of decolorized clothes over time for different decolorization methods. Figure 11. Show the approximate percentage of discolored clothes over specific time intervals in various methods of decolorization such as bacterial, yeast, fungus, and activated charcoal decolorization

The table 1 depicts the types of pigments that are present in flowers and seeds, which are utilized for dyeing purposes. The table 2 shows how the use of salt and cream of tartar as a mordant affects the intensity of fabric dye absorption.

Table 1: Observation Table for Dyes

Sr. no	Scientific name	family	Plant Part used	color	Extracted coloring pigment
1.	<i>Clitoria Ternatea</i>	Solanaceae	Flower	blue	Anthocyanin
2.	<i>Red Malabar Spinach</i>	Primulaceae	seeds	Dark pink	Anthocyanin
3.	<i>Bougainvillea</i>	Nyctaginaceae	flower	Light pink	Anthocyanin
4.	<i>Impatiens Balsamina</i>	Balsaminaceae	flower	Buff brown	Xanthophyll
5.	<i>Mirabilis Jalapa</i>	Nyctaginaceae	flower	Yellowish brown	Anthocyanin
6.	<i>Turmeric+ baking soda</i>	Zingiberaceae	roots	Orange	Beta - Carotene
7.	<i>Cestrum Nocturnum</i>	Solanaceae	flower	Dark yellow	Xanthophyll

Table 2: Indicates Color Intensity

Fabric	Color intensity with mordant		Washing fastness	Rubbing fastness	
	Salt	Cream of Tartar		wet	dry
Cotton	Negative	Positive	Positive	Positive	Positive
Polyester	Negative	Positive	Positive	Positive	Positive

**Fig.12 Chromatography Technique (Paper, Thin layer chromatography)**

Cestrum Nocturnum shows the highest absorbance in water and *Clitoria Ternetea* shows the highest absorbance in acetone as shown in table 4.

In the table 5, it is shown that the *Balsamina* flower has the highest absorption in water with 3.60 nm while the *Bougainvillea* flower has the highest absorption in isopropanol with 3.90 nm.

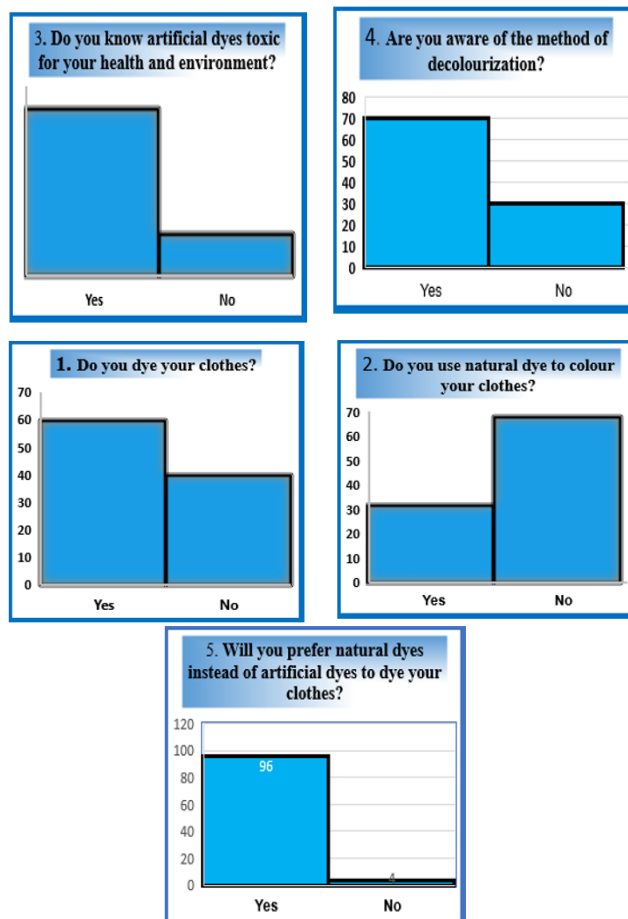
Table 4: For Thin-Layer Chromatography

Sr. no	Flower name	Water	Acetone
1.	Balsamina	0.32	0.20
2.	Bougainvillea	0.34	0.22
3.	<i>Cestrum Nocturnum</i>	0.36	0.22
4.	<i>Clitoria Ternetea</i>	0.34	0.24

Table 5: For Spectroscopy

Sr. no	Flower name	Water OD (360 nm)	Isopropanol OD (360 nm)
1.	Bougainvillea	1.73	3.95
2.	Night jasmine	3.37	0.76
4.	Balsamina	3.60	3.70
5.	Turmeric + soda	3	0.98
6.	Mirabilis Jalapa	3.1	3.32

We surveyed Natural Dye and 50 people answered our questions which are as follows:



We surveyed to determine people's awareness of the harmful effects of artificial dyes. If people are aware of how hazardous these artificial dyes can be, they may opt for natural dyes made from plant-based pigments instead.

Eco dye uses natural fabric dyes from plants, reducing chemical usage. Microbial decolorizes can enhance color fixation.

CONCLUSION

Utilizing natural dyes and colorants is a crucial substitute for synthetic dyes. Because natural dyes have no allergic reactions, are non-toxic, cause less environmental damage, and have fewer negative

effects, they have become a hot topic in the textile dyeing industry. Native American traditional knowledge of plants that generate dye is crucial for the advancement of bioprospecting in the future, so long as appropriate precautions are taken to ensure its sustainability. Some significant and practical sources of flower dye were explored based on this investigation. The data indicates that cotton has a higher absorbance efficiency than polyester. When it comes to decolorization, the usage of bacteria, fungi, and viruses has a faster rate of occurrence than the synthetic decolorizer employed in industries, which can be somewhat hazardous to the environment. When mordants were added to the dye, the result was a variety of colors that could be created from a single plant. The dye is mixed immediately with the mordants, cream of tartar, and alum. When the fabric is heated, the mordant adheres to it well once more, preventing color fading and maintaining its saturation up to the final limit specified.

Conflict of interest

The authors declare no competing interests.

Data Availability Statement: Not applicable.

Correspondence and requests for materials should be addressed to **Shruti P. Salunke**

Umakant Shinde (0000-0001-5145-1897) - ORCID

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