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**POTENTIAL DEGRADATION OF HARMFUL SYNTHETIC  
DYES USING ISOLATED AND PURIFIED LACCASES  
EXTRACTED FROM TRIGONELLA FOENUM GRACEUM**

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**Keywords**

Bioremediation,  
synthetic dyes,  
laccase,  
Trigonella foenum-  
graceum.

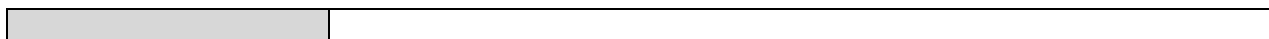
**Abstract**

The increasing use of synthetic dyes in the textile, paper, and plastic industries has led to severe environmental pollution owing to their toxic, carcinogenic, and non-biodegradable nature. Conventional physicochemical treatment methods often fall short because of high cost and secondary pollution. Sustainable alternative enzymatic degradation is a promising strategy for dye removal. Laccases are multicopper oxidases capable of oxidizing a wide range of phenolic and nonphenolic compounds in dyes. In this study, laccase was isolated and purified from Trigonella foenum-graceum (fenugreek seeds). The extracted enzyme was characterized in terms of optimum pH-7, temperature of 35°C, and time of 30 minutes. Enzymatic degradation assays were conducted using synthetic dyes such as thiazine, azo, phthalein, sulfonphthalein and triarylmethane dyes. The results demonstrated a significant decolourization efficiency with degradation rates exceeding 50%. Spectrophotometric analysis confirmed the breakdown of the complex dye molecules into less toxic intermediates. These findings highlight the potential of plant-derived laccases as eco-friendly biocatalysts in wastewater treatment and industrial dye degradation.



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## 1. INTRODUCTION

In recent years, one of the primary concerns has been environmental pollution resulting from human activities, which has garnered significant attention from researchers. Industrialization and urbanization have been pinpointed as the key contributors to environmental pollution [1–4]. Among the various industrial processes that can lead to the release of pollutants, the textile industry is recognized as a major source of dye contamination. Dyes produced by the paper, printing, and textile sectors can significantly pollute rivers and waterways. It is estimated that over 700,000 tons of dyestuffs are produced annually. Other industries contributing to dye pollution include pulp and paper mills, distilleries, dyestuff manufacturers, and tanneries [4–6]. The presence of different dye classes, such as xanthenes, anthraquinone, phthalocyanine, and quinonimine, in environmental matrices is due to their structural characteristics. Azo dyes are the most prevalent in the environment because of their natural colour, easy availability, and cost-effectiveness [10–13]. Removing dyes is crucial due to their potential carcinogenic properties, which can severely harm aquatic life and obstruct light penetration in aquatic environments, affecting plant growth. It has been reported that approximately 9% of the total dye substances produced globally are discharged into wastewater from textile industries [1, 2, 14]. There are numerous categories of dyes, which can be synthetic or natural, while synthetic dyes are more commonly employed in textile fibers, whereas naturally occurring dyes are employed as coloring agents in food [7, 08, 09].

Impact on the ecosystem and the environment at large. The removal of dye therefore becomes necessary. Various methods have been employed for the removal of dyes some of which include physical, chemical, and biological approaches some of the methods have their inherent limitations and challenges. Some of the physical approaches include filtration, flocculation/coagulation, and adsorption, while chemical methods include photocatalysis, electro-Fenton, and ozonation. Microorganisms and enzymes are employed in biological methods of removing dyes.

Dye pollution has been of increasing concern due to its environmental and health impacts. The removal of dyes has been achieved through various conventional and advanced methods. The major concern of scientists in recent times is developing highly efficient approaches to dye removal that are environmentally friendly. The need for sustainability in the dye removal approach is also paramount. Dye waste must be properly treated before being released into the environment in order to reduce environmental pollution by dyes.

Laccase (E.C.1.10.32) is an oxidoreductase enzyme belonging to the blue multi-copper oxidases, capable of degrading synthetic dyes (Shraddha et al., 2011). It is widely found in bacteria, fungi, plants, and insects, with extensive studies conducted in fungi. However, research on laccase in plants is limited (Vinit Kumar et al., 2013). In this study, the laccase enzyme was isolated from the seeds of *Trigonella foenum-graceum* (fenugreek seeds), and dye decolorization experiments were conducted.

## 2. MATERIALS AND METHODS:



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Extraction: About 20gm of the seeds of *Trigonella foenum-graceum* was weighed and homogenized in 100ml of 0.1M phosphate-citrate buffer (pH=5). It was centrifuged at 10000 rpm for 15 minutes at 4<sup>0</sup> C. The supernatant was used as crude enzyme sample. (Benjamin, 1997).

➤ **Partial purification of laccase:**

**Ammonium Sulphate Precipitation:** Crude enzyme was filtered through Whatman filter paper No.1 and the total volume measured was 100ml. The crude enzyme was precipitated by 80% saturation. The beaker containing the sample was placed on a magnetic stirrer and ammonium sulphate salt was added little by little with continuous stirring. Once the total salt was added the beaker placed at 4<sup>0</sup> C overnight. It was then centrifuged at 10000rpm for 15 minutes. Supernatant was discarded and the pellet was dissolved in 10mM Tris HCl. (Distasioe et al., 1976)

**Purification:** The sample was further purified using column chromatography via the gravity flow method. A solid, self-indicating silica gel was used as the stationary phase, packed into the column, while the elution buffer served as the mobile phase. The gel was cast, washed, and equilibrated with the elution buffer before loading the sample. Fractions (2 mL each) were collected during elution and analysed for enzyme activity.

**Dye Degradation:** Four synthetic dyes, each representing a different chemical class—thiazine, azo, sulfonphthalein, and triarylmethane—were selected for the study. The dyes used were methylene blue, Congo red, bromocresol purple, and crystal violet, respectively. Stock solutions of each dye were prepared at a concentration of 1 mg/mL by dissolving 0.1 g of the dye in 100 mL of solvent. To achieve a working concentration of 25 ppm, 0.25 mL of the stock solution was diluted with 99.75 mL of distilled water. The prepared dye solutions were then incubated with the enzyme sample to assess decolorization activity.

The maximum absorbance wavelengths ( $\lambda_{max}$ ) of the dyes were as follows: methylene blue at 520 nm, Congo red at 680 nm, bromocresol purple at 420 nm, eosin yellow at 620nm and crystal violet at 420 nm. After incubation, the absorbance of each solution was measured using a UV-Visible spectrophotometer.

$$\% \text{ Decolorization} = \frac{\text{initial decolorization} - \text{final decolorization}}{\text{Final decolorization}} \times 100$$

### 3. RESULTS:

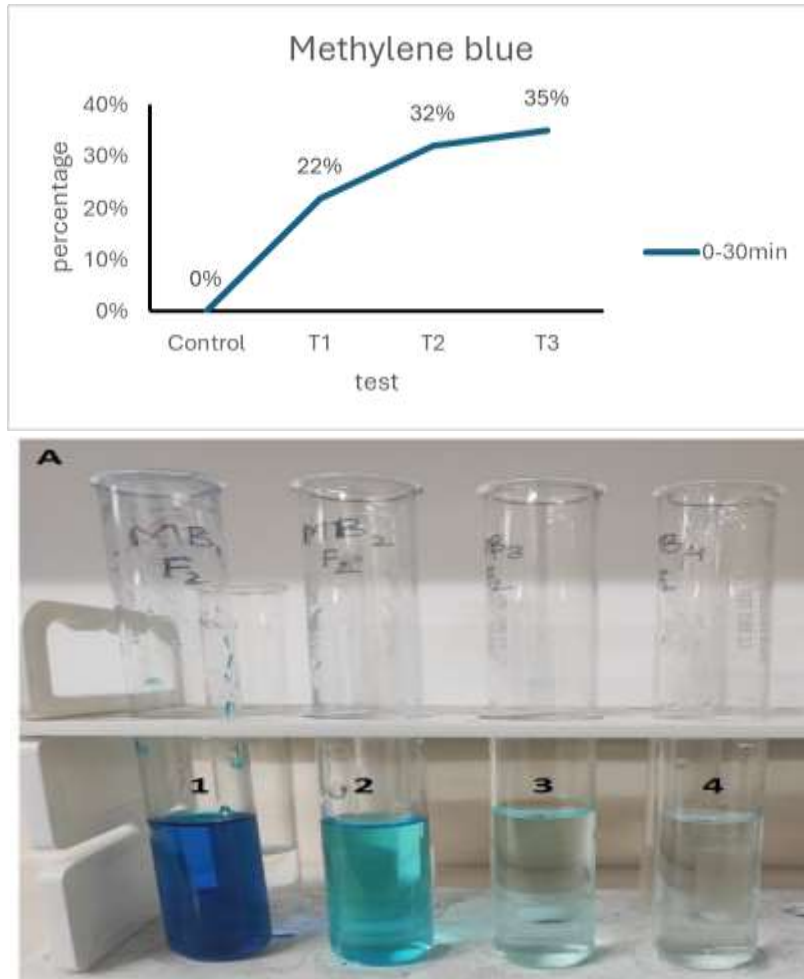
**Dye degradation:**

In case of methylene blue, the dye was degraded with increasing concentration of sample. The degradation percentage was found to be 22, 32 and 35% respectively at 0.1, 0.2 and 0.3ml of treatment.

Methylene blue



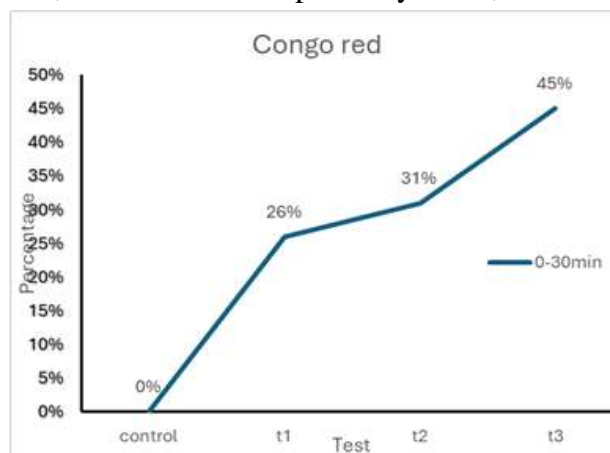
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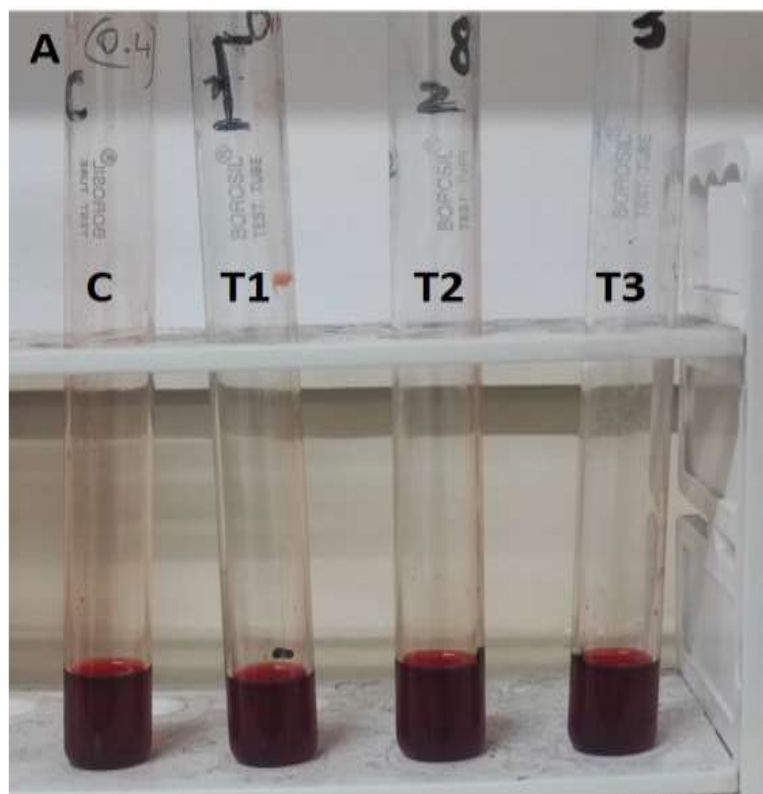
The figure demonstrates the degradation of methylene blue by serial dilution method.

### Congo red

In case of congo red, the dye was degraded with increasing concentration of sample. The degradation percentage was found to be 26%, 31% and 45% respectively at 0.1, 0.2 and 0.3ml of treatment.



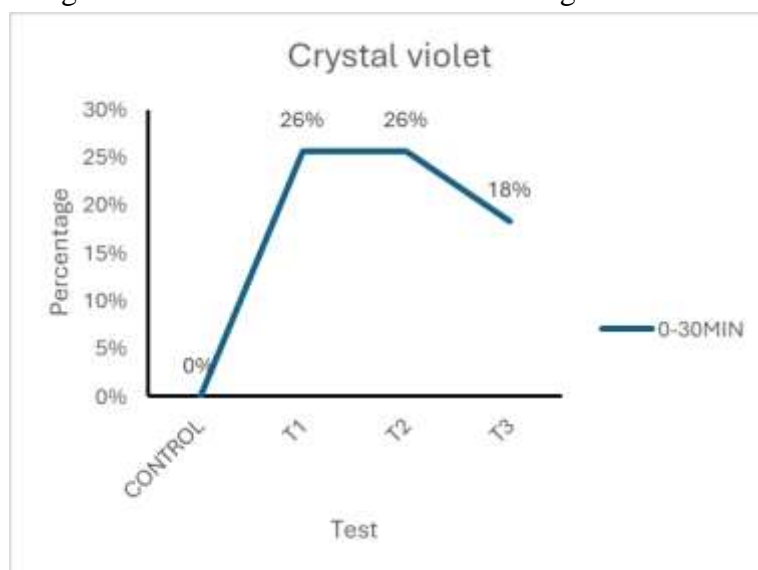
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The figure demonstrates the degradation of Congo red.

### Crystal violet

In case of crystal violet, the dye was degraded with different concentration of sample. The degradation percentage was found to be 26%, 26% and 18% respectively at 0.1, 0.2 and 0.3ml of treatment. The T1 shows highest concentration among the other two test samples, the maximum concentration of sample might be attained in the T1 and further degradation was not found.



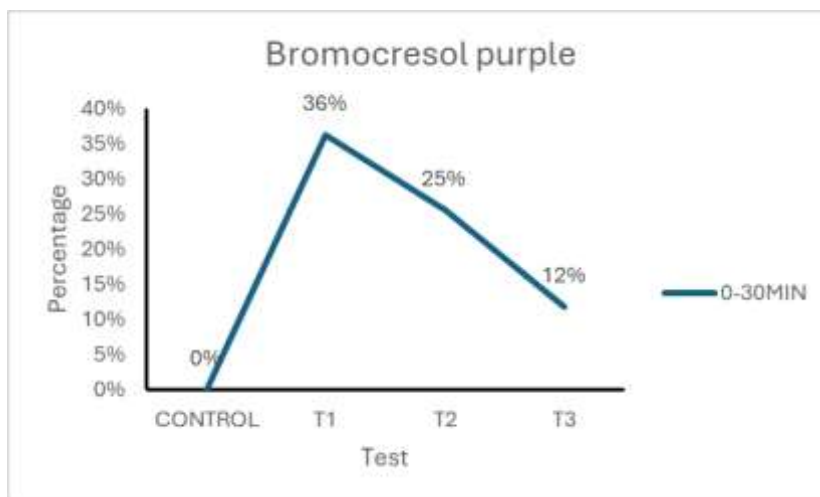
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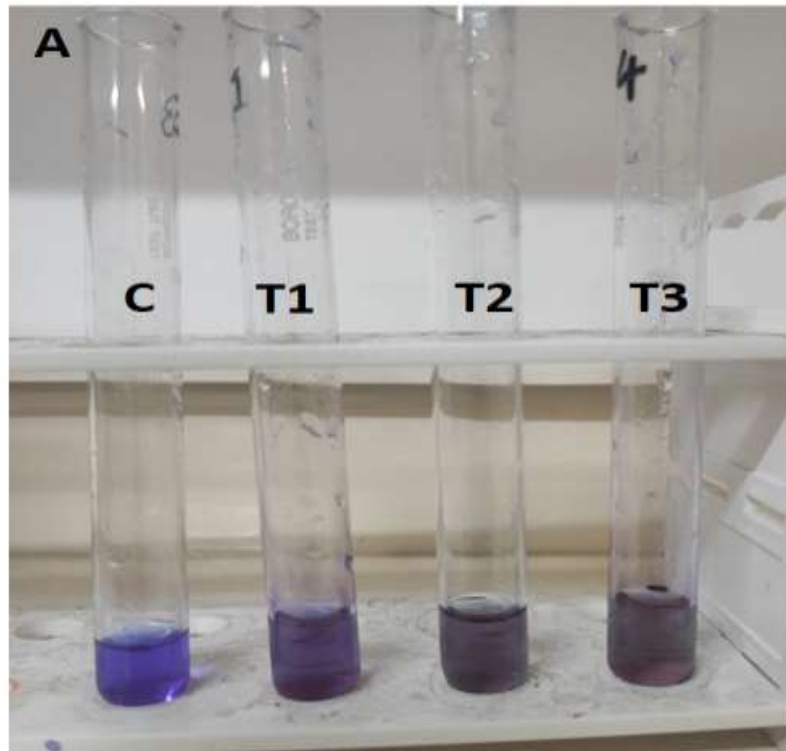
The figure demonstrates the degradation of Crystal violet.

### Bromocresol purple

In case of Bromocresol purple, the dye was degraded with different concentration of sample. The degradation percentage was found to be 36%, 25% and 12% respectively at 0.1, 0.2 and 0.3ml of treatment. The T1 shows highest concentration among the other two test samples, the maximum concentration of sample might be attained in the T1 and further degradation was not found.



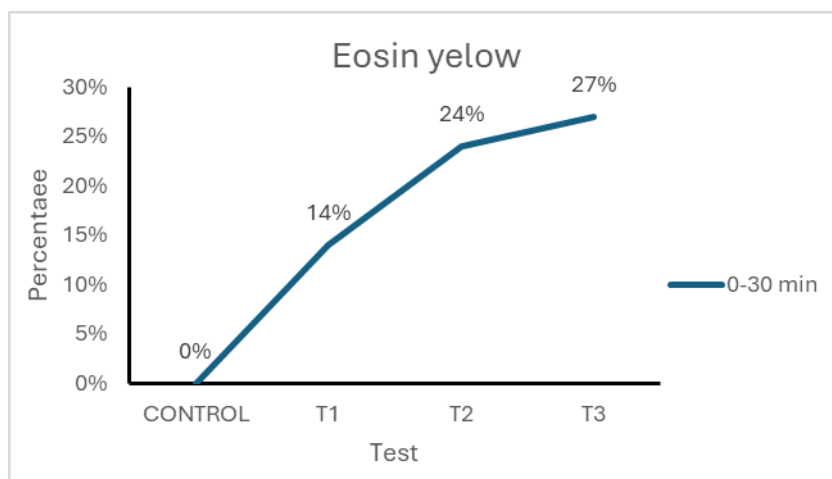
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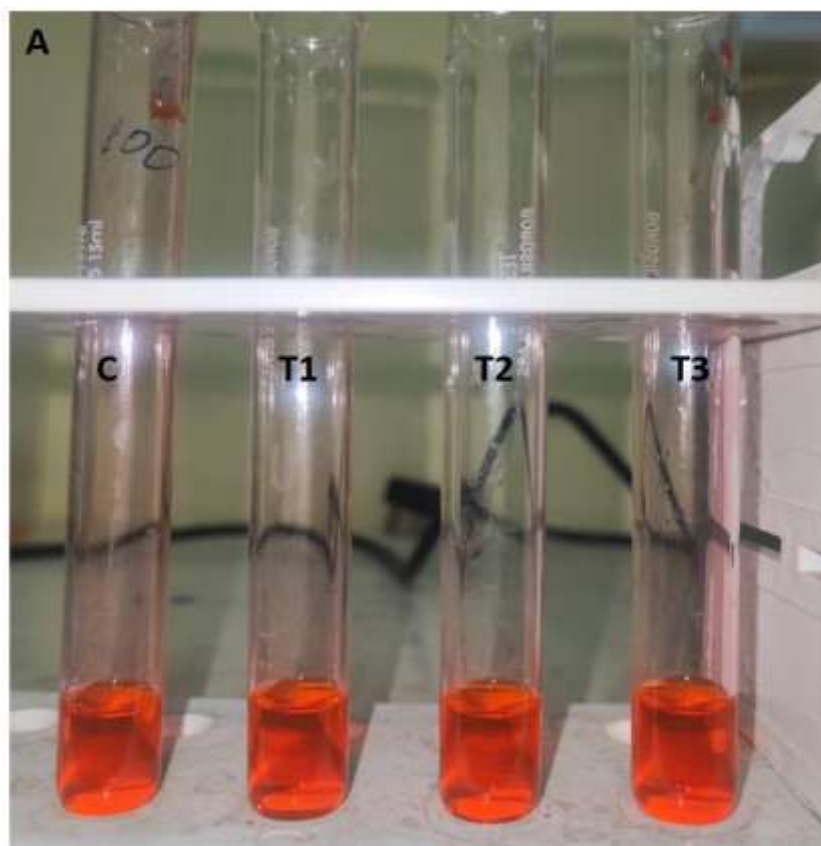
The figure demonstrates the degradation of Bromocresol purple.

#### Eosin yellow

In case of Eosin yellow, the dye was degraded with increasing concentration of sample. The degradation percentage was found to be 14%, 24% and 27% respectively at 0.1, 0.2 and 0.3ml of treatment.



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#### 4. DISCUSSION:

In the case of **Methylene Blue (MB)**, it was observed that dye degradation increased with rising concentrations of the sample. The degradation percentages were recorded as 22%, 32%, and 35% at treatment volumes of 0.1 ml, 0.2 ml, and 0.3 ml, respectively. The amounts of laccase immobilized on carbonate and epoxy groups were 24.6 mg/g and 9.7 mg/g, respectively. Notably, in the presence of a mediator compound such as **acetosyringone**, nearly complete degradation (almost 100%) of both MB and **Carbaryl pesticide (CP)** was achieved using immobilized laccases. In contrast, degradation efficiencies in the absence of a mediator were lower—63% for MB and 71% for CP. These findings are consistent with those reported in a study on the biodegradation of MB and CP using *Trametes versicolor* laccase in the presence of a mediator.

For **Congo Red**, dye degradation also showed a positive correlation with sample concentration. The observed degradation rates were 26%, 31%, and 45% for 0.1 ml, 0.2 ml, and 0.3 ml treatments, respectively. Most fungal laccases are known to have molecular weights ranging from 60 to 70 kDa (Giardina et al., 2010); however, smaller laccases have also been reported, such as a 32 kDa laccase from *Botrytis cinerea* (Pezet, 1998) and a 50 kDa laccase from *Cladosporium cladosporioides* (Aslam et al., 2012).



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In the case of **Crystal Violet**, the degradation percentages were 26% at both 0.1 ml and 0.2 ml treatments, but decreased to 18% at 0.3 ml. The highest degradation was observed in the T1 group, suggesting that the optimal concentration may have been achieved at this treatment level, with no further degradation occurring beyond that point. Optimization of experimental parameters such as enzyme and dye concentrations, pH, and incubation time led to a maximum degradation efficiency of 97.05%. The identification of intermediates like **Leucocrystal Violet**, **Olivetol dimethyl ether** (1,3-Dimethoxy-5-pentylbenzene), and **1,3,5-trimethoxybenzene** via GC–MS analysis confirmed the complete mineralization of Crystal Violet. These results were aligned with a study on *Trichoderma asperellum* laccase-mediated degradation under optimized conditions.

For **Bromocresol Purple**, the degradation percentages were 36%, 25%, and 12% for 0.1 ml, 0.2 ml, and 0.3 ml of treatment, respectively. Again, the T1 group showed the highest degradation, indicating that a threshold concentration may have been reached, after which no significant degradation occurred. A related study using laccase isolated from *Brassica oleracea* demonstrated efficient degradation of **Bromophenol Blue** (up to 64%) and **Bromocresol Green** (44%), supporting the efficacy of plant-based laccases in dye removal.

Finally, for **Eosin Yellow**, the degradation efficiency increased with higher sample concentrations, showing values of 14%, 24%, and 27% for 0.1 ml, 0.2 ml, and 0.3 ml treatments, respectively. Laccases have been shown to effectively degrade **acid** and **direct azo dyes**, while **reactive azo dyes** tend to be more resistant to enzymatic breakdown. These findings are corroborated by research on the degradation of azo dyes using laccase and ultrasound-assisted treatment (Tauber, Guebitz, & Rehorek).

## 5. CONCLUSION:

This study effectively demonstrated the potential of laccase enzymes isolated and purified from *Trigonella foenum-graecum* (fenugreek) in the degradation of harmful synthetic dyes. The results underscore the enzyme's role as an environmentally friendly biocatalyst with significant application in pollution control. The purified laccases exhibited notable enzymatic activity, leading to considerable decolorization of several synthetic dyes, thereby highlighting their robust oxidative properties and broad substrate specificity. These outcomes validate the feasibility of employing plant-derived enzymes—particularly from *T. foenum-graecum*—as sustainable alternatives to traditional chemical treatment methods, which often generate toxic byproducts and cause secondary environmental contamination.

The extraction and purification protocols used in the study proved to be both effective and efficient, further supporting the potential of *T. foenum-graecum* as a renewable source of laccase. In addition, the utilization of this plant-based enzyme is aligned with the principles of green chemistry, offering a safer, more economical solution for industrial wastewater treatment. Nonetheless, further research is necessary to enhance enzyme yield, improve stability under industrial conditions, and assess performance in real and complex effluent systems. Overall, laccases derived from *T. foenum-*



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*graecum* present a promising and sustainable advancement in biotechnological strategies aimed at mitigating pollution from synthetic dyes.

Laccase-mediated dye degradation functions through a distinct mechanism involving single-electron transfer, which breaks down dye chromophores, often leading to complete mineralization or conversion into non-toxic, colorless compounds. The efficiency of this process can be significantly improved with the addition of redox mediators, which broaden the range of dye substrates and boost degradation rates. Enzyme performance is influenced by various factors, including pH, temperature, dye concentration, and the presence of mediators—all of which can be optimized to achieve high degradation efficiencies within practical durations.

Moreover, plant-based laccases offer several advantages such as easy extraction, lack of pathogenicity, and renewable cultivation potential. These attributes make them particularly suitable for use in decentralized or rural wastewater treatment systems where cost and safety are critical considerations.

The research highlights that *Trigonella foenum-graecum*, a leguminous plant traditionally recognized for its medicinal and nutritional value, is capable of producing laccase-like enzymes with promising oxidative potential against synthetic dyes—especially those in the azo, anthraquinone, and triphenylmethane categories. The findings confirm that laccase from fenugreek seeds represents a viable, plant-based method for dye degradation. Despite the positive results, further studies are necessary to refine enzyme extraction techniques, enhance stability, and explore commercial-scale applications. The eco-friendly nature and effectiveness of fenugreek-derived laccase support its potential role in sustainable wastewater treatment, offering a practical solution for managing dye-laden effluents from the textile and related industries.

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#### **7. AUTHOR(S) CONTRIBUTION**

The writers affirm that they have no connections to, or engagement with, any group or body that provides financial or non-financial assistance for the topics or resources covered in this Manuscript.

#### **8. CONFLICTS OF INTEREST**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

#### **9. PLAGIARISM POLICY**

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