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Biodegradation of Crystal Violet by Ascomycetous Fungi Isolated from Local Dump Sites: An Approach for Potential Mycoremediation of Textile Dyes

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Abstract

Dyes are integral elements used to impart colours to textile materials. The wastewater effluent generated from textile industries during the processing and treatment of the dye contains inorganic and organic compounds that are hazardous, thereby posing a serious threat to ecosystems. This study focused on the assessment of the decolourization capability of textile dyes using Eleven (11) ascomycetous fungal isolates, obtained from selected local dump site habitats in Sri Lanka's. These fungi were tested against a model compound, Crystal Violet, a synthetic dye that belongs to the chemical group of triphenylmethane. Results revealed that *Fusarium falciforme* (USJCC-0046) has the highest decolourization potential, removing 83% of Crystal Violet within seven days of incubation in a dye solution (50 mg/L, pH 7, at 27±2°C, and 150 rpm). The biosorption of the dye into fungal mycelia was relatively low, less than 20% indicating a predominance of enzymatic degradation over physical adsorption. Moreover, Fourier Transform Infrared Spectroscopy analysis conducted before and after fungal treatment revealed the biodegradation of the chromophore structures of Crystal Violet by fungal enzymatic activity. These findings suggest that *F. falciforme* could be optimized for large-scale textile dye effluent treatment due to its ability to effectively target diverse dye structures.

***Keywords**:* Crystal violet, *Fusarium falciforme,* mycoremediation, soil fungi, textile dyes

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Introduction

The textile industry utilises a considerable amount of water containing dyes and other substances, such as emulsifiers, dispersing agents, levelling agents, and heavy metals (Syed Agha Hassan, 2009). It is estimated that the textile industry produces over 1 million tons of synthetic dyes annually worldwide, employing more than 10,000 dye types, with as much as 50% of these dyes ending up in wastewater due to inefficient dyeing processes (Kalyani *et al*., 2008; Lade *et al*., 2015). Although physicochemical treatment methods like flocculation and ozonation are used, they are costly and generate substantial sludge, posing further environmental and disposal issues (Fu & Viraraghavan, 2001; Surwase *et al*., 2013).

Hence, Biological treatment has become a favoured alternative for managing dye wastewater due to its cost efficiency, minimal sludge generation, and environmental friendliness. However, the high toxicity of dye effluents poses a challenge, with some microorganisms unable to withstand these conditions, highlighting a critical gap for effective and eco-friendly bioremediation methods. The marine microorganisms and white-rot fungi have been the focus of many studies related to textile dye degradation. However, only a few studies have focused on this area, resulting in a scarcity of research on soil-dwelling Ascomycete fungi, which are predominantly terrestrial.

Triphenylmethane (TPM) dyes, such as Crystal Violet (CV), are among the largest and most used synthetic colorants in the textile industry (Chen *et al*., 2018). Therefore, this study aims to evaluate the use of newly isolated soil ascomycete fungi from local dumpsite areas as a biological agent to absorb and biodegrade toxic TPM dye, using CV as the model substrate.

Materials and Methods

Selection of test-cultures from the USJ culture collection

Eleven newly isolated fungal strains from local dumpsites were obtained from the University of Sri Jayewardenepura culture collection for the study. Table 01 provides a comprehensive overview of the isolates selected for the present study.

Table 1: *Fungal isolates selected for this dye decolorization study.*

Identity of the isolate	GenBank Accession	Culture Collection No.
Fusarium falciforme	MW084628	USJCC-0046
Purpureocillium lilacinum	MW084627	USJCC-0044
Talaromyces purpureogenus	MT756246	USJCC-0051
T. purpureogenus	MW084629	USJCC-0047
Aspergillus terreus	MW084632	USJCC-0050
A. flavus	MW084625	USJCC-0042
A. flavus	MW084624	USJCC-0041

A. flavus	MW084630	USJCC-0048
A. japonicus	MW084626	USJCC-0043
A. japonicus	MW084631	USJCC-0049
Penicillium citrinum	MT920323	USJCC-0045

Preliminary screening of dye decolourisation

Preliminary screening of fungal isolates for decolourisation of crystal violet was carried out according to Diwaniyan *et al*., (2010) with slight modifications. Fungal cultures were grown on Malt-Extract Agar (MEA media in triplicates, which was supplemented with synthetic dye at a final concentration of 0.005% (w/v). Then plates were incubated at 30 ^{0}C for 7 days in darkness. The formation of decolorized zones under or around the developing mycelia was monitored visually for dye decolourisation.

Quantitative screening of decolourisation

Decolourization was tested in a 100 mL Erlenmeyer flask with 50 mL sterile malt extract Broth (MEB) supplemented with CV at a final concentration of 0.005% (w/v) at pH 6.5 in accordance with Yang *et al*., (2016). Flasks were inoculated with two fungal disks (1 cm Diameter) taken from the periphery of the 7-day-old fungal cultures in MEA plates and incubated the flasks at room temperature (26 - 30 ^{0}C) in an orbital shaker with 150 rpm agitation speed for seven days under dark conditions. Three replicates were done for each fungal isolate. The percentage decolourization for a regular interval of 24h for seven days was calculated using the following equation (Shah *et al*., 2023).

$$DP = (1 - C / C_0) \times 100\%$$

Where DP is the decolourization percentage (%), C_0 is the initial concentration of the dye (mg/L), and C is the final concentration of the dye after decolourization (mg/L). Fungal isolates that exhibited the highest decolourization rate after seven days of incubation were selected for further studies.

Enzyme activity assays

The selected fungal species was subjected to determine the extracellular enzyme production during the decolourization process. The enzymatic activity, expressed in international units per millilitre (IU/mL), was calculated based on the concentration of the oxidised substrate. The key enzymes, such as Manganese peroxidase (MnP) (Chen *et al*., 2019) Lignin peroxidase (LiP) (Kalyani et al., 2008), and tyrosinase (Chen et al., 2019), were assayed.

Biosorption assay

Biosorption assay was carried out using the mycelia fraction obtained from the filtration of culture broth incubated for one week as described in Yang *et al*., 2016. Each set of mycelial fractions was triplicated to ensure the significance of the observations.

Fourier Transform Infrared (FT-IR) Spectroscopy analysis

FT-IR spectra of the CV dye before and after fungal degradation were obtained using the KBr pellet method at the Instrument Centre, Faculty of Applied Sciences, University of Sri Jayewardenepura.

Results

Preliminary Decolourization Screening

According to Figure 1, out of eleven (11) isolates, *A. terreus* (USJCC-0050), *F. falciforme* (USJCC-0046), *P. citrinum* (USJCC-0045) and *T. purpureogenus* (USJCC-0047) showed relatively strong dye decolorising activity for crystal violet dye. Isolates: *T. purpureogenus* (USJCC-0051) and *A. flavus* (USJCC-0041, USJCC-0048 and USJCC-0042) showed moderate dye decolorising activity, and isolates: *P. lilacinum* (USJCC-0044), *A. japonicus* (USJCC-0049 and USJCC-0043) did not show decolorising activity when compared with the negative control.

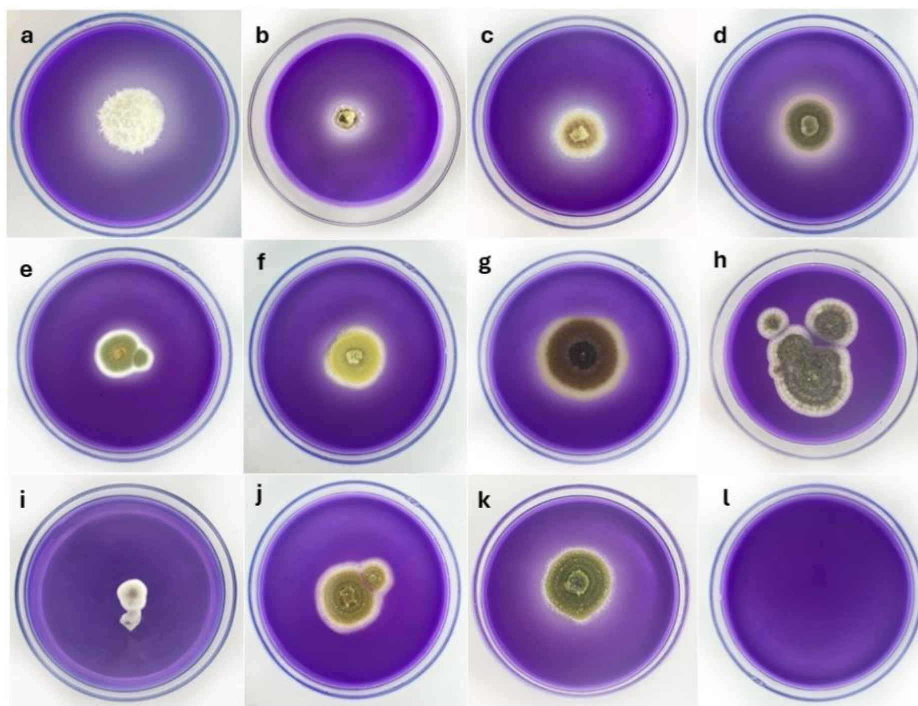

Figure 1: *Decolourization of crystal violet dye by Eleven (11) fungal isolates after seven (7) days incubation at 30°C in darkness. Colourless zones indicating decolourization activities of fungal isolates; a: F. falciforme(USJCC-0046), b: P. citrinum(USJCC-0045), c: A. terrus(USJCC-0050), d: T. purpureogenus (USJCC-0047), e: A. flavus (USJCC-0041), f: T. purpureogenus (USJCC-0051), g: A. flavus (USJCC-0048), h: A. japonicus (USJCC 0049), i: A. japonicus (USJCC-0043), j: P. lilacinum (USJCC-0044), k: A. flavus (USJCC-0042), l: un-inoculated plate (Negative control).*

Quantitative Decolourization Screening

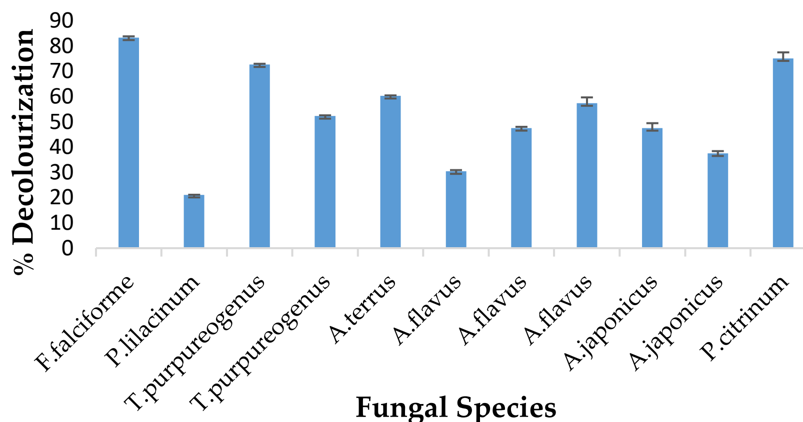

Figure 2: *Percentage decolorization of crystal violet by Eleven (11) fungal isolates on the final day of the incubation period (Day Seven (07)). Error bars represent the standard deviations of the mean values.*

Enzymatic activity and Biosorption assay

Table 2: *Enzyme production and biosorption of the CV by the selected fungal species*

Type of dye	Culture codes	Fungal species	Enzyme activity (IU/mL)			Percentage Biosorption (%)
			LiP	MnP	Tyrosinase	
Crystal violet	USJCC- 0046	*F. falciforme*	6.52±.0.03	0.26±0.002	0.56±0.002	19.61 ± 1.79

According to the results obtained from these assays, comparatively high amounts of Lip were produced by the selected isolate for CV degradation. During the degradation of Crystal Violet, *F. falciforme* (USJCC-0046) was found to produce the highest amounts of all three enzymes, with Lip reaching a peak activity of 6.52 IU/mL. Amount of dye absorbed into the mycelia was comparatively lower than dye decolourized by extracellular enzymatic activity. This value is nearly 20%, while more than 80% of the CV has been removed by enzymatic degradation.

FT-IR Analysis of decolourised products

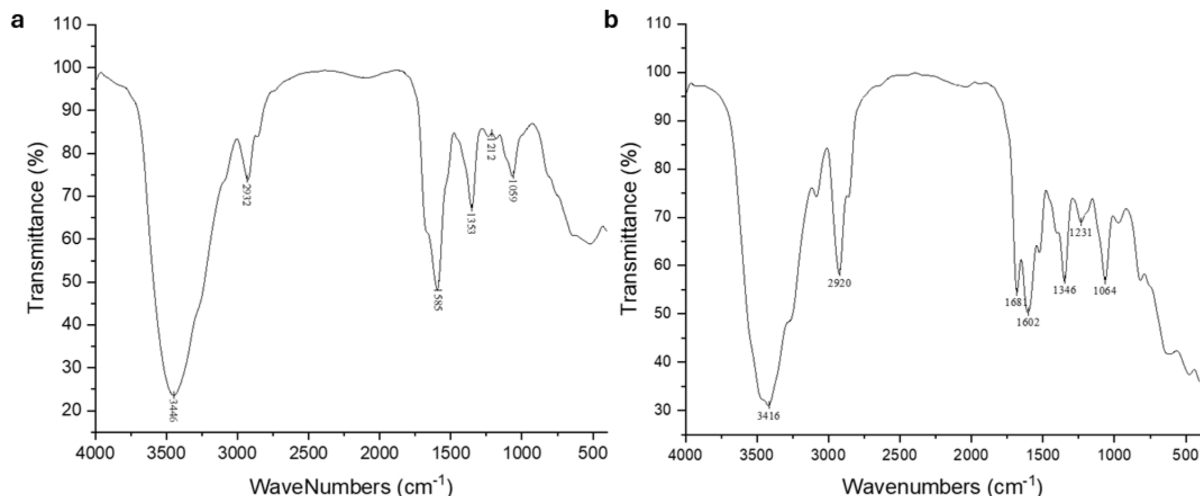

Figure 3: *FTIR spectrum for original Crystal violet dye (a) and decolorized Crystal violet dye (b) by F. falciforme.*

After fungal treatment (b), notable shifts and new peaks were observed: the O-H stretch remained at 3416 cm^{-1}, while a new peak at 2920 cm^{-1} suggested alkyl chain formation or exposure. Additionally, the appearance of new peaks at 1681 cm^{-1} and changes around 1602 cm^{-1} indicated alterations in the aromatic ring structures, possibly due to enzymatic ring opening or attachment of new functional groups such as carbonyls. Furthermore, shifts to 1346 cm^{-1} and 1231 cm^{-1} in the C-N and C-O stretching modes, respectively, along with the emergence of a peak at 1064 cm^{-1} for C-O stretching, suggest the transformation of amine groups and the formation of new bonds, such as ethers or esters.

Discussion

This study evaluated the decolourization potential of 11 ascomycete fungi isolated from local waste dumping sites, against a model compound, crystal violet, a triphenylmethane dye commonly used in the textile industry. Physiological differences among eleven (11) cultures may account for difference in their decolourization abilities on the same dye. Decolourization of a dye requires the destruction of the chromophore (Ali *et al*., 2016), the ease of which depends on the chemical structure of the dye, the relative position of substituents on the aromatic ring, and their resulting interactions with the main bonds of dye compounds (Abadulla *et al*., 2000; Moturi and Charya, 2009; He *et al*., 2018). Among the isolates, *F. falciforme* (USJCC-0046) exhibited the highest decolourization efficiency for crystal violet. Variations in performance were attributed to species-specific enzymatic and metabolic profiles. In the decolourization of crystal violete, *F. falciforme* (USJCC-0046) exhibited the highest decolourization rates in both liquid and plate assays, where similar results were obtained by other researchers (Moturi and Charya, 2009). Although biosorption contributed to less than 20% of the dye removal, it is assumed that the primary mechanism for crystal violet degradation is biodegradation, facilitated by enzymes secreted by the fungus. Lignin peroxidase (LiP) emerged as the main enzyme responsible, facilitating oxidative breakdown of aromatic structures via hydrogen peroxide-dependent free radical generation (Herath *et al*., 2023). Tyrosinase was also significant, despite less clarity on its role in triphenylmethane dye degradation. It likely contributed oxidising phenolics into quinones, which then reacted with LiP byproducts to disrupt the oxidation of phenolics into quinones, thereby altering the dye structure (Vasniwal *et al*., 2021). FTIR analysis confirmed degradation through spectral shifts in the C–N and C–O regions, as well as new bond formations,

indicating structural alterations such as methyl group removal and the formation of ethers or esters. These alterations indicate modifications to the dye's chromophore structure, contributing to its degradation.
This study introduces a novel approach to the biological treatment of textile effluents, highlighting the previously underexplored potential of *F. falciforme* (USJCC-0046) in dye remediation. It highlights their capability to decolourize selected dyes, thereby advancing knowledge of fungal bioremediation. Moreover, the relationship between the *in vitro* and *in situ* degradation of xenobiotic dyes by fungi and their role in natural environmental processes remains to be fully explained.

Conclusion

This study has demonstrated *Fusarium falciforme* (USJCC- 0046), which showed the highest decolourization activity for Crystal Violet, attributed to its production of lignin peroxidase enzymes. FTIR analysis revealed that fungal treatment caused significant structural changes in the chromophore structure of Crystal Violet, including the formation of altered aromatic rings and the introduction of new carbonyl groups, suggesting extensive degradation. This study reveals novel applications of *F. falciforme* (USJCC-0046) in the decolourization of textile dyes, marking the first investigation into these fungal species for their use in mycoremediation of wastewater effluents.

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