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ISOLATION, CHARACTERIZATION AND SCREENING OF COMPETENT FUNGAL ISOLATES FROM TEXTILE SLUDGE FOR DECOLORIZATION OF SELECTED SYNTHETIC DYES

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ABSTRACT

Textile industry is predominant in employing dyes and chemicals, cause for environmental degradation and human maladies. Synthetic dyes have an adverse effect on the aquatic ecosystem and their toxic substance has to be removed from the effluent before their exoneration. This research paper aimed, to isolate and screen the competent fungi from textile sludge capable of decolorizing broad spectrum of dyes. Twelve fungal isolates from sludge were preliminary screened by using Czapek Dox agar with 100 mg/ml of dyes. Six fungal isolates have shown decolorization of all three synthetic dyes. Among fungal isolates, *Aspergillus flavus* showed maximum growth (7.33±1.30) and in plate assay, *Aspergillus flavus* showed maximum decolorization zone (6.20 ±1.58) in Reactive violet-2RL followed by Acidic Blue-G and Direct Green-B, *Aspergillus fumigatus* showed 5.33±0.42 and 4.73±0.23. This research work reports that fungi offer an easy, cost effective method and could be used in development of an effective biotreatment method for the removal of dyes from textile effluent.

KEYWORDS: Competent fungal strains, Sludge, Dye agar plate assay, Decolorization and Linear regression.

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INTRODUCTION

Textile industries are economic, sustainable and consume large volumes of water, and employ diverse classes of dyes and chemicals like organic and inorganic complexes[1]. Among the industrial wastewaters, textile effluent is one of the most stringent to treat and comprised of intricate mixture of carbon-based pollutants including dye. Synthetic dyes are classified as azo, heterocyclic, polymeric or triphenylmethane based on the chemical structure of chromogenic groups[2]. In india, synthetic dyes accounts for around 5% of the global production. They are increasingly been used in the textile and dyeing industries because of their ease and cost effectiveness in synthesis, firmness, high stability to light, temperature, detergent and microbial attack and variety in color compared with natural dyes[3]. Discharge of textile effluent containing synthetic dyes especially poly-aromatics and pose threat to health issues like respiratory (coughing, wheezing, itchy or blocked noses and sneezing) and skin sensitization (itching, watery and sore eyes)[4-6]. The textile industry accounts for the largest consumption of dyestuffs. Reactive, Vat and Azo dyes are mainly required for dyeing and printing of cotton fibers. Disperse dyes constitute the largest market with about 21% share followed by direct dyes and reactive dyes with 16% and 11% respectively. Such dyes are recalcitrant to biodegradation, thereby creating an aesthetic problem in receiving waters. Effluents from textile industries, treated before discharged into the environment. Many trials made to develop effective technique to decolorize these effluents.

Treatment methodologies for dye containing wastewater are categorized into three types: physical, chemical and biological. Most of the physical methods, however, simply accumulate and concentrate dyes and create sludge and the problem of disposal exists. Chemical oxidation with either peroxide or ozone can destroy dyestuffs but this approach is costly[7]. Owing to their lower cost and eco-friendliness, biological methods have proved superior over various physico-chemical methods. Various microorganisms like bacteria, fungi, yeasts and algae have been used to remove dyes[8-12]. General mechanisms for the removal of dyes by microorganisms are biosorption, bioaccumulation and biodegradation. Biosorption involves the entrapment of dyes in the matrix of the biomass by processes that do not involve metabolic energy or transport, although such processes may also occur simultaneously where live biomass is used. The process of biosorption can occur in either living or dead biomass[13, 14] whereas bioaccumulation is the accumulation of pollutants by actively growing cells through metabolism and temperature-independent and metabolism-dependent mechanisms. In biodegradation, the dye is fragmented into its metabolites [15-18].

The objectives of this study were:

a) To isolate fungi from textile effluent,

b) To identify the fungal isolates by LCB staining,

c) To investigate the role of fungal isolates for decolorization efficiencies of three chemically different textile dyes namely Reactive Violet-2RL, Acidic Blue-G, Direct Green-B by Dye-agar plate assay.

MATERIALS AND METHODS

(i) Source of sludge

Sludge sample was collected (Mid off - April 2010) from a textile-dyeing mill in perundurai, Erode district, Tamil Nadu, India, in aseptic airtight plastic containers and filtered to remove large suspended particles and stored at 4±1°C in refrigerator until use.

(ii) Isolation and enrichment of fungi

Fungal strains were isolated from textile sludge by dilution plate method (10^{-1} - 10^{-15}) and the plates were observed for the growth of fungi. The fungal strains were sub-cultured by point inoculation method on Czapek Dox agar plates. Pure fungal isolates were maintained on agar slants, stored in 4^{o}C and served as stock

cultures. Sub-cultures were consistently made every 10 days. The fungal isolates were selected for further screening.

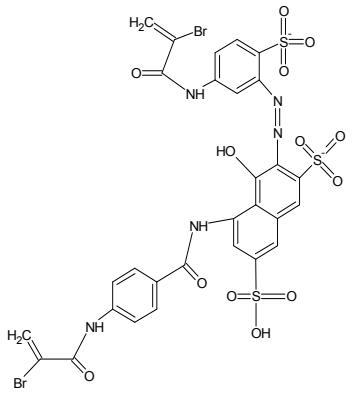
(iii)Dyes

A broad spectrum of dyes namely Reactive Violet-2RL, Direct Green-B and Acidic Blue-G listed in Table 1 and their structure is depicted in Figure 1. Further, to achieve high decolorization ability of the fungal isolates was screened and treated in the laboratory-scale dye-agar plate assay with structurally different dyes. The dyes were procured from dye suppliers, Coimbatore, and were of analytical grade and used further without purification. The dye stock solutions were, prepared accurately by weighed 1gm/l of dyes in double distilled water.

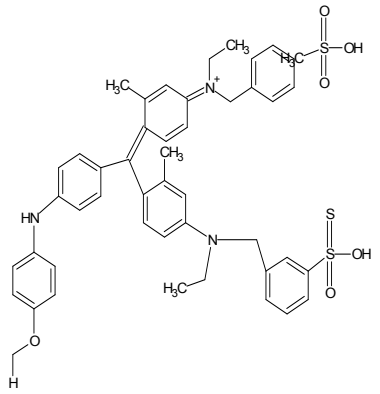
Table 1
Synthetic Dyes used in the Study

DYE [CAS No.]	SYNONYMS	MOLECULAR WEIGHT (KDa)	λ_{max} (nm)
Reactive Violet-2RL [12218-07-4]	Reactive Red 83; Drimarene Violet X-2RL.	969.45	568nm
Acidic Blue-G [6104-58-1]	CBB G-250; Cyanine G; Blue G 250; Acid Blue 90; Serva Blue G; Polar Blue G; Page Blue G90; Brilliant Blue.	854.02	549nm
Direct Green-B [5422-17-3]	Diamine Green; Renol Green B; Dianil Green B; Direct Green G; Direct Green 6; Direct Green B	812.7	614nm

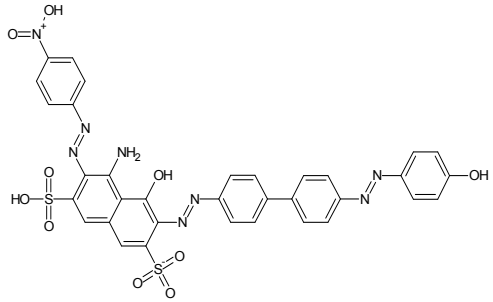
Figure 1
Structure of the Dyes used in the Study



(a) Reactive Violet-2RL



(b) Acidic Blue-G



(c) Direct Green-B

(iv) Preliminary screening

The fungal isolates were screened on relevant solid media containing 100mg/ml and incubated for 3 - 7 days in dark. The fungal colonies, which exhibit zone of clearance was chosen for further study.

(v) Characterization of competent fungal isolates

Fungi were identified according to their morphological characters by Agharkar Research Institute, Pune, India.

Screening

(vi) Fungal growth assay

The textile sludge contains organic matter, chemical nutrients and relative amount of heavy metals and aromatic dyes. In spite of decolorization, maximum fungal biomass production was monitored by fungal growth assay. The mycelial agar plugs (~2 mm) of one-week-old culture grown on Czapek Dox Agar at 28^{o}C was cut from the colony margin under sterile conditions and transferred onto the centre of the experimental plates for each replicate and un-inoculated plate as control. These plates were incubated at room temperature (28^{o}C) in dark and observed for 7days. The experiments were performed in triplicate for each culture.

(vii) Dye-agar plate assay

Dye - agar plate assay was carried out to ensure the potency of decolorization for competent fungal isolates on Czapek Dox Agar medium containing 100mg/ml of dyes. Plates were inoculated with ~2 mm of fungal disc (actively growing region) was transferred onto the centre of the experimental plates for each replicate under aseptic conditions and incubated at room temperature (~28^{o}C) in dark and un-inoculated agar plates were served as control. The clear zone formed around the mycelial disc indicates decolorization; the size of the decolorized halo was measured for every 24hrs.

(viii) Statistical analysis

Fungal growth and dye - agar plate assay were performed in triplicate and the results are reported as mean ± SD (Standard Deviation) by using statistical analysis tool Graph Pad Prism Version 5.04 and association of fungi with decolorization was assessed by linear regression model.

RESULTS AND DISCUSSION

Twelve fungal strains (Figure 2) were isolated from textile sludge and primarily screened by dye-agar plate assay for the dye was chosen for the study. Some dyes inhibit fungal growth because of their chemical structure. Among twelve fungal strains, six fungal strains showed clear zone. Table 2 illustrates the efficiency of fungal isolates for decolorization of selected synthetic dyes.

Fungal Strains Isolated from Textile Sludge

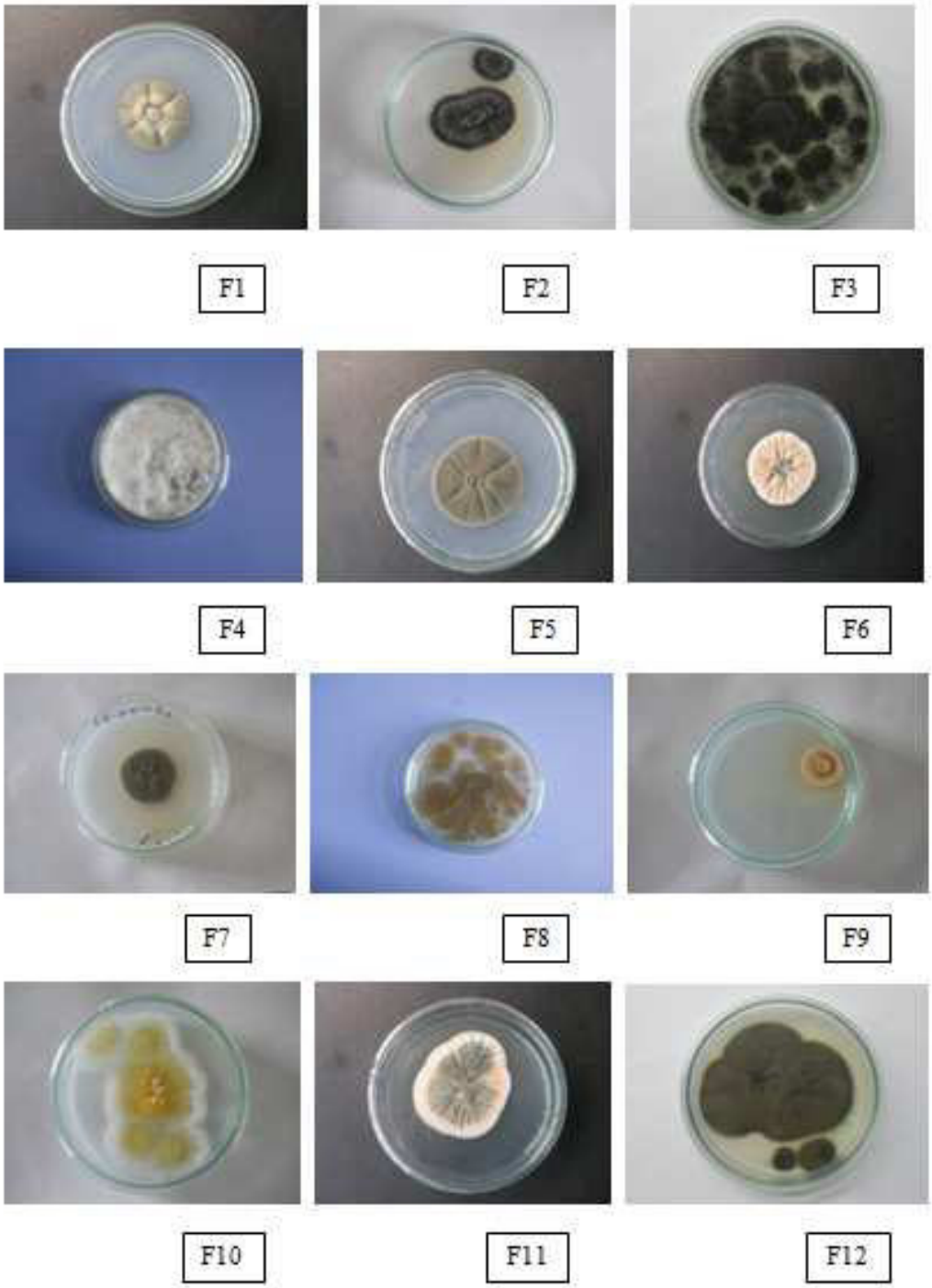


Figure 2
Twelve fungal strains were isolated from textile sludge and cultured in Czapek Dox Agar.

Table 2
Preliminary Screening: Decolorizing efficiency of fungal isolates

FUNGAL ISOLATE. NO.	REACTIVE VIOLET-2RL	DIRECT GREEN-B	ACIDIC BLUE-G
F1	**+**	**+**	**+**
F2	**+**	**+**	**+**
F3	—	+	—
F4	**+**	**+**	**+**
F5	**+**	**+**	**+**
F6	+	—	—
F7	**+**	**+**	**+**
F8	**+**	**+**	**+**
F9	—	—	+
F10	—	—	—
F11	—	+	—
F12	—	—	—

(+) - Clear Zone observed, (—) - No Clear Zone.

Decolorization efficiency of six fungal isolates are identified as *Aspergillus flavus* > *Aspergillus fumigatus* > *Penicillium purpurogenum* > *Penicillium aurantiogriseum Dierckx* > *Lecanicillium lecanii* > *Cladosporium sphaerospermum penz*., by Agharkar Research Institute, Pune, are listed in Table 3 and the fungal isolates are showed in Figure 3.

Table 3
Identification of Fungal Strains based on Microscopic Observations and Cultural Characteristics

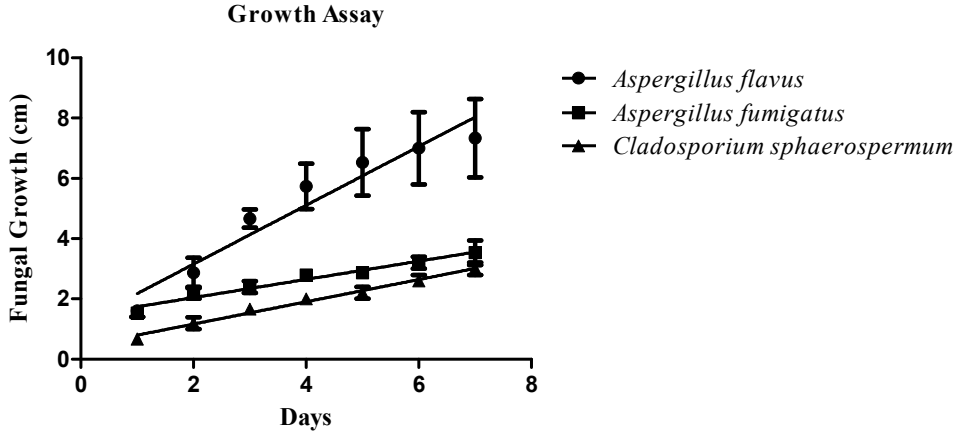
STRAIN NO.	ORGANISMS IDENTIFIED	ACCESSION NUMBER
F1	*Aspergillus flavus*	NFCCI -1957
F2	*Aspergillus fumigatus*	NFCCI - 1993
F4	*Penicillium purpurogenum*	NFCCI - 2823
F5	*Penicillium aurantiogriesum*	NFCCI - 942
F7	*Lecanicillium lecanii*	NFCCI - 2824
F8	*Cladosporium sphaerospermum*	NFCCI - 2730

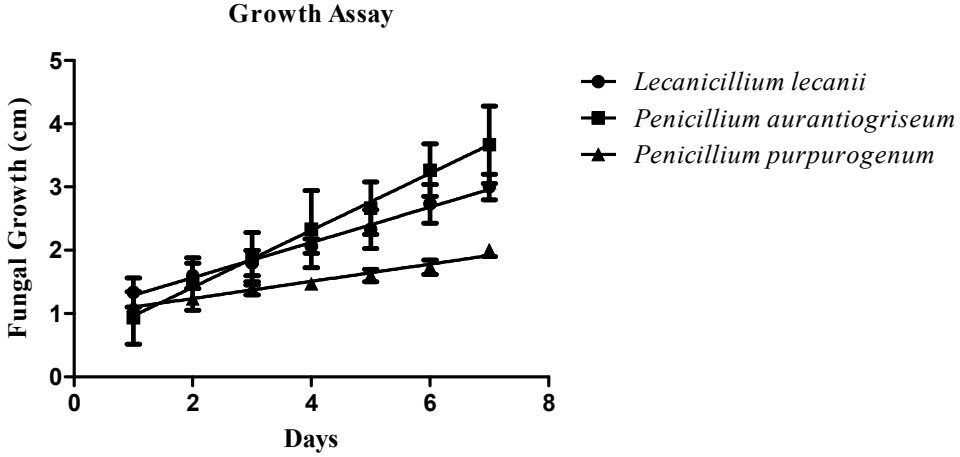
Competent Fungal Isolates for Selected Synthetic Dye Decolorization

Figure 3
Six fungal isolates have Decolorizing potential for three selected synthetic dyes.

Selected fungal strains were tested for their growth and decolorization efficiency are shown in Graph 1. Maximum fungal growth was seen in *Aspergillus flavus* of diameter 7.30±1.30. Dye decolorization by fungi during growth on solid medium has been widely employed to identify the degradation potential of dyes. In plate assay, maximum decolorization was showed by *Aspergillus flavus* in Reactive Violet-2RL (6.20±1.58) and *Aspergillus fumigatus* in Acidic Blue-G (5.33±0.42) and Direct Green-B (4.73±0.23).

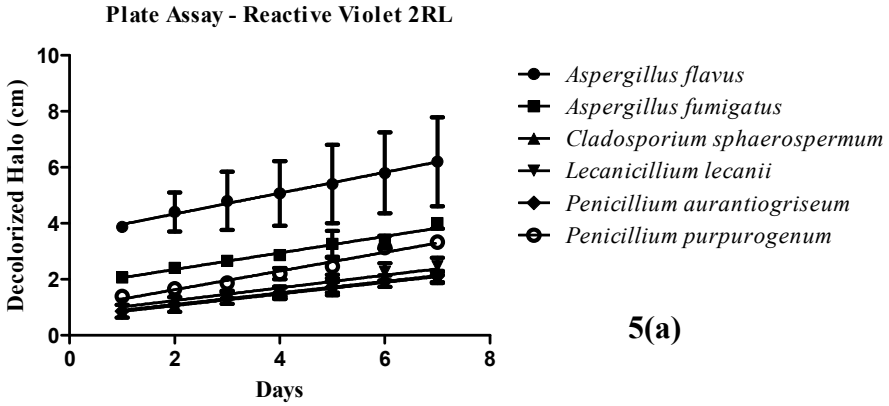
Graph 1
Growth Assay for Six Isolates in Czapek Dox Agar



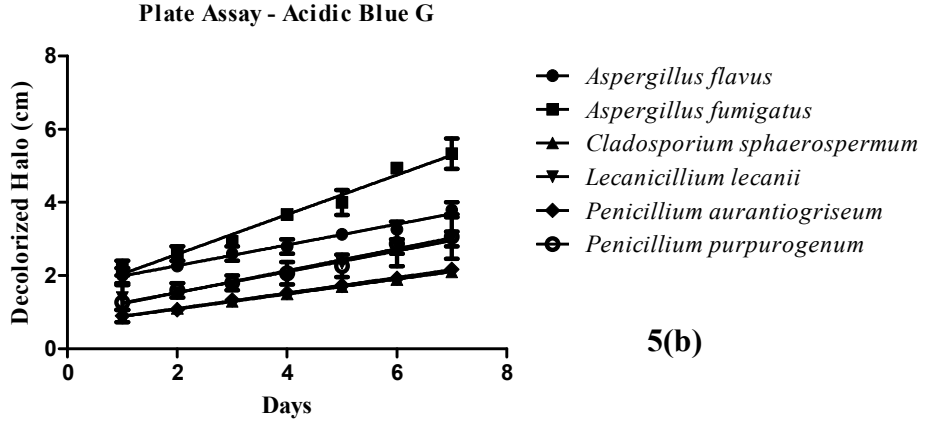


In order to verify the relationship between the fungal growth, and decolorization, a model of linear regression was evaluated for three dyes (Graph 2). The regression analysis reveals, the correlation coefficient (R^2) for fungal growth, R^2 was found to be 0.8334, which is better than the acceptance criteria of 0.9177. The fungal growth and decolorized halo was compared with the fungal isolates by the measurement of the diameter. The linear relationship between the fungal growth and decolorization produces a correlation coefficient for Reactive Violet-2RL (*Aspergillus flavus*), R^2 was found to be 0.3787, Acidic Blue-G and Direct Green-B (*Aspergillus fumigatus*), R^2 was found to be 0.9516 and 0.8595 and p - value is significant (<0.0001) and confirm the goodness of this approach. The development of plate assay holds a great promise and paves a way for new approach for treatment of synthetic dyes in textile industries by submerged fermentation.

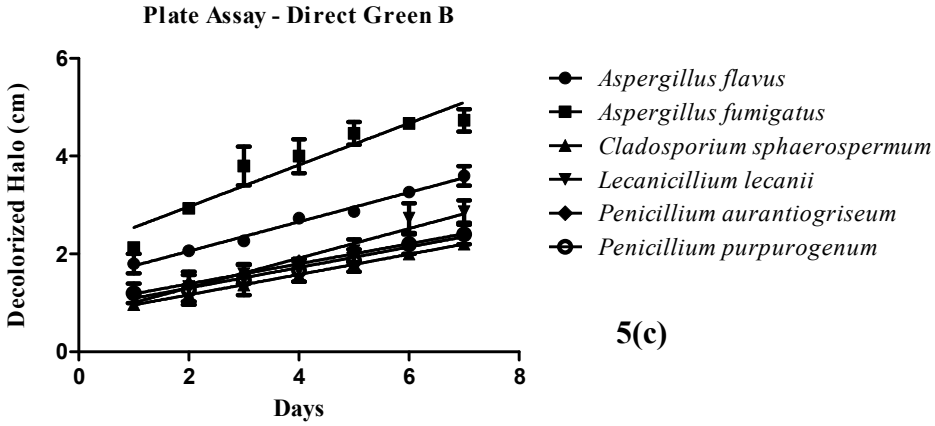
Graph 2
Plate Assay for Decolorization of Synthetic Dyes



5(a)



5(b)



5(c)

5(a): Reactive Violet-2RL, 5(b): Acidic Blue-G, 5(c): Direct Green-B

CONCLUSION

This research paper has demonstrated that isolated fungal strains had excellent potential for decolorization of selected synthetic dyes viz., Reactive Violet-2RL, Acidic Blue-G and Direct Green-B. Fungi have potential role in mineralizing many persistent composites. *Aspergillus flavus* and *Aspergillus fumigatus* showed the greatest extent of dye decolorization in agar plates. This study demonstrate the decolorization of dyes with efficient strains like *Penicillium purpurogenum, Penicillium aurantiogriseum Dierckx, Lecanicillium lecanii and Cladosporium sphaerospermum penz.*, revealed the decolorization of wide range of dyes. These results highlighted the potential strains for use in the treatment of dye-contaminated effluent.

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