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Isolation and Evaluation of Indigenous Soil Fungi for Decolourization of Textile Dyes

¹N.S. Raju, ¹G.V. Venkataramana, ²S.T. Girish, ³V.B. Raghavendra and ¹P. Shivashankar

¹Department of Studies in Environmental Science Manasagangotri,
University of Mysore Karnataka, India 570006

²Department of Studies in Botany and Microbiology, Yuvaraja's College University of Mysore, India

³Department of Studies in Applied Botany and Biotechnology, Manasagangotri, University of Mysore, India

Abstract: Decolourization of textile dyes from fungi *Aspergillus flavus*, *Fusarium oxysporum*, *Fusarium moniliforme* and *Trichoderma harzianum* isolated from the soil samples around the textile distillery industries of Nanjangud, Karnataka, India were tested for their efficacy in decolourization of Textile dyes, Orange3R, Blue3R, YellowGR, BlackRL and T blue. It was found that all the four fungal species were found to be efficient in decolouring textile dyes. Among the four fungal species *A. flavus* was more efficient followed by *F. oxysporum*, *F. moniliforme* and *T. harzianum*. The percent decolourization of black RL and T blue was more when compares to Orange3R, Blue3R and YellowGR.

Key words: Textile dyes, decolourization, *Aspergillus*, *Trichoderma*, *Fusarium* species

INTRODUCTION

Textile industries have been using synthetic dyes intensively because of their ease and cost effectiveness in synthesis. The textile dyes are highly reactive and there fore during processing it is difficult to treat. The commonly used dyes by the textile industry are azodyes (Orange3R YellowGR and BlackRL). Antroquinone (Blue3R) and copper phthalocyanine (T blue). The textile industries utilize large volume of water in its wet processing operation and there by generates substantial quantities of waste water is the principle route by which dyestuffs enter the soil environment (Elliott, 1999). During the past decade, the use of microbiological degradation methods have been under active development in textile and dyestuff industry (Knapp *et al.*, 1995). Among the microorganisms bacteria are the most commonly used for various bioremediation process. As par as fungi are concerned their reports on bioremediation of textile dyes from the environment by fungi are scanty. There are few reports of using fungi especially white rot fungi is the most commonly used fungi for bioremediation. In the environment there are many microorganisms specially fungi which are abundant but their potential is not utilized completely. The ability of fungi to transport a wide variety of hazardous chemicals has aroused interest in using them in bioremediation (Brar *et al.*, 2006). As can be seen from the literature *Phanerochaete chrysosporium*, basidiomycetes fungus is the only choice organism

reported to be potent decolourizer of the effluent (Krik *et al.*, 1992). In the present study an attempt has been made to utilize the common soil inhabiting fungi isolated indigenously from the soil polluted with textile industry for decolorization of textile dyes.

MATERIALS AND METHODS

The soil samples were collected near the places where the effluents are discharged from the factories such as Sayilakshmi Textiles, Jemini Distillery and South India Paper Mills situated around Nanjanagud Town Mysore, Karnataka, India.

Soil samples were subjected to serial dilution to get dilutions and inoculated onto the sterile petri dishes which consist of sterile, cool, molten, Czapek -Dox agar medium and then we followed the spread plate method and kept the plates for incubation at 30±1 °C for 4 days. Fungal colonies were identified using steriobinocular microscope and with the help of standard manuals.

Decolourization study: The dye samples were collected by the textile industry named by Saayilakshmi Textile Industries situated in Nanjanagud area. The fungal species which are identified as *Aspergillus flavus*, *Fusarium moniliforme*, *Fusarium oxysporum* and *Trichoderma harzianum*. The isolated fungi were tested for its ability to decolourize textile dyes. Textile dyes, orange3 R ($\lambda_m = 493$ nm), Blue 3R ($\lambda_m = 572$ nm),

yellow GR ($\lambda_m = 413$ nm), Black RL ($\lambda_m = 574$ nm) and T. blue ($\lambda_m = 664$ nm) were used at 200 mg L^{-1} concentration (Devi and Kaushik, 2005).

Fifty milliliter of C- limited Czapek-Dox sterile medium was amended separately with each of the textile dyes and subsequently inoculated with 2% (v/v) fungal spore suspension containing $2.5 \times 10^6 \text{ cfu mL}^{-1}$ (colony forming unit) spores. The flasks were incubated at $30 \pm 1^\circ\text{C}$ for 8 days samples drawn at 2 days intervals for observation. Samples were centrifuged at $10000 \times g$ for 10 min decolourization was assessed by measuring absorbance of the supernatant with the help of spectrophotometer at wave length maxima (λ_m) of respective dye. Two control flasks (dye + medium without inoculums and medium with inoculums without dye) were maintained.

The percentage decolourization was calculated using following formula.

$$\% \text{ Decolourization} = \frac{\text{Initial OD} - \text{Final OD}}{\text{Initial OD}} \times 100$$

RESULTS AND DISCUSSION

The soil samples collected from several industries of Nanjanagud where the textile industries are located were screened for fungi subjecting to serial dilution and plating methods. The species identified by its different characteristics and the structural arrangements using stereo microscope with the help of standard manual. (Table 1). The different fungal colony shows different structures with different colors. The *Aspergillus* *Trichoderma* and *Fusarium* species were commonly present in the polluted soil samples of all the industries where the soil is collected. The incidence of fungi in the polluted soils depends on the availability of nutrient, oxygen and biological, chemical and physical features of the pollutant.

The decolourization efficiency of *A. flavus* and *F. oxysporum*, *F. moniliforme* and *T. harzianum* was monitored at periodic intervals by measuring the Optical Density after 2, 4, 6, 8 days of incubation. It is noticed that there was decrease in the OD in all the four species in all the four colours as the incubation period increased (Table 2). Among the four species *A. flavous*

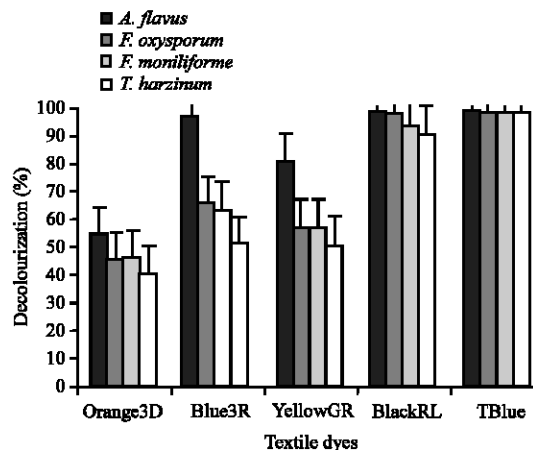


Fig. 1: Percent decolourization of textile dyes by species of *Aspergillus*, *Fusarium* and *Trichoderma*

was more effective followed by *F. oxysporum*, *F. moniliforme* and *T. harzianum*. The percentage of decolourization of colours by fungi was also calculated. It was found that all the fungi were found to be efficient decolourizer of Blue the decolourization of dye amounted to 99.0, 98.8, 98.59 and 98.03%, respectively within 8 day. Orange was recalcitrant to decolourization. The OD from an initial value of 1.729 was reduced only to 0.831, from 1.729 and 1.839 to 1.0, 1.851 to 1.001 and 1.886 to 1.113 by *A. flavous*, *F. moniliforme*, *F. oxysporum* and *T. harzianum* respectively. %decolourization was 54.51, 45.16, 45.92 and 40.98 respectively (Fig. 1). The % decolourization of Yellow colour is slightly higher than orange colour and the % decolourization of Black colour is similar to Blue colour (Fig. 1).

As par as *F. moniliforme*, *F. oxysporum* and *T. harzianum* is concerned there is hardly any literature is available about their use for decolourization of dyes is concerned. In our study it is proved that these fungi are also showed the potential to degrade the dyes which is equal to the *A. flavous*. There are several advantages in using these species for bioremediation. *T. harzianum* has the potential to grow fast and it is non pathogenic. In addition to decolorize the dyes the fungi especially *Trichoderma* species is known to process the complete set of enzymes required to breakdown cellulose to glucose. It is believed that in the decolourization of reactive dyes is due to the action of azo-reductase enzymes (Sani and Banerjee, 1999) isolated three microorganisms from soil samples amended with color effluent and found that they have great potential to bio-transform the tri-phenyl-methane dyes. Decolonization of textile dyes by different enzymes has been reported earlier. Copper containing phthalocyanine dye

Table 1: Fungal colonies identified in different industrially polluted soils

Organisms	Sayilakshmi textiles	South indian			
		Reid and taylor	paper mills	Jemini distilleries	Zenith textiles
<i>Aspergillus flavous</i>	+	+	+	-	+
<i>Aspergillus niger</i>	+	+	+	+	+
<i>Fusarium moniliforme</i>	+	-	+	+	-
<i>Fusarium oxysporium</i>	+	-	+	+	+
<i>Tricoderma harzianum</i>	+	+	+	-	+

+: Present - : Absent

Table 2: Optical density of dyes treated with fungal species at different intervals of incubation

Textile dye	Organisms	Optical density after incubation days				
		0	2	4	6	8
Orange3R	<i>Aspergillus flavus</i>	1.729	1.327	1.205	0.903	0.831
	<i>Fusarium oxysporum</i>	1.839	1.406	1.362	1.190	1.000
	<i>Fusarium moniliforme</i>	1.851	1.521	1.211	1.112	1.001
	<i>Trichoderma harzianum</i>	1.886	1.761	1.438	1.245	1.113
Blue3R	<i>Aspergillus flavus</i>	0.629	0.411	0.110	0.032	0.022
	<i>Fusarium oxysporum</i>	0.629	0.462	0.399	0.251	0.215
	<i>Fusarium moniliforme</i>	0.854	0.754	0.612	0.499	0.312
	<i>Trichoderma harzianum</i>	0.800	0.701	0.621	0.511	0.391
YellowGR	<i>Aspergillus flavus</i>	1.139	0.872	0.515	0.299	0.218
	<i>Fusarium oxysporum</i>	1.139	0.835	0.575	0.477	0.485
	<i>Fusarium moniliforme</i>	1.201	1.011	0.854	0.611	0.512
	<i>Trichoderma harzianum</i>	1.232	1.012	0.902	0.798	0.604
BlackRL	<i>Aspergillus flavus</i>	0.893	0.353	0.105	0.025	0.012
	<i>Fusarium oxysporum</i>	0.893	0.294	0.136	0.028	0.017
	<i>Fusarium moniliforme</i>	0.912	0.423	0.301	0.101	0.061
	<i>Trichoderma harzianum</i>	0.901	0.541	0.358	0.110	0.085
T Blue	<i>Aspergillus flavus</i>	0.620	0.312	0.038	0.022	0.006
	<i>Fusarium oxysporum</i>	0.620	0.231	0.035	0.013	0.009
	<i>Fusarium moniliforme</i>	0.712	0.312	0.102	0.051	0.010
	<i>Trichoderma harzianum</i>	0.691	0.425	0.201	0.091	0.011

(200 mg L⁻¹) was found to be completely decolorized within 7 days by *Phanerochaete chrysosporium*. In one study, *Aspergillus flavus* and *A. niger* were found to be efficient decolorizer of T. Blue (Cu-phthalocyanine dye) to the extent of 99% within 8 days of incubation suggesting that like *P. chrysosporium* (Conneely *et al.*, 1999) these species can also be employed. We observed maximum decolorization of BlackRL followed by Blue3R (anthraquinone dye) and relatively to less extent of Yellow GR and Orange 3R (Azo dyes) by *A. flavus*. The possible mechanism of decolorization may be biosorption, which is dependent on functional groups in the dye molecule and in fungal biomass, which may also be playing role in the biosorption of dye (Fu and Viraraghavan, 2002).

Under anaerobic conditions, azo-reductases usually cleave azo dyes into the corresponding amines, many of which are mutagenic and/or carcinogenic (O' Neill *et al.*, 2000; Chivukula and Renganathan, 1995; Chung and Stevens, 1993) Furthermore, azo reductases have been shown to be very specific enzymes, thus cleaving only azo bonds of selected dyes (Zimmermann *et al.*, 1984).

Excess production of protein in response of dyes and growth in C-limited medium indicated that the fungus utilized the dyes as the sole source of carbon and produced enzymes to degrade the dyes.

Decolorization of dyes by fungi is mainly ascribed to extracellular activity, which is in agreement with results reported previously for *Trametes hispida* (Rodriguez *et al.*, 1999). 1-Amino-substituted anthraquinoid dyes were good substrates for the *T. hirsuta* laccase and they were degraded to a similar extent. Out of the two azo dyes, Direct Blue 71 was the preferred substrate for the

T. hirsuta laccase, which might be due to limited accessibility of the -OH and -NH₂ groups in Reactive Black 5.

However, for smaller substrates, the electronic contribution of substituents on the aromatic ring seemed to be more important than steric effects (Xu, 1996) Electron-donating methyl and methoxy substituents seemed to enhance laccase activity, while electron-withdrawing chloro, fluoro and nitro substituents inhibited oxidation of azophenols and other substituted phenols and phenol analogs by fungal laccases (Chivukula and Renganatham, 1995).

Similar to our present study on decolorization of dye, Kousar and Charya (2002) also reported for fungi such as *Aspergillus niger*, *Fusarium*, *Oxysporum*, *Mucor mucedo* isolated from textile and dye contaminated soils were able to decolorize the dye effluent (Nicola *et al.*, 1998). In the biotechnological approach to colour removal suggested that the microbiological treatment is ideal for colour removal as less sludge is produced in comparison to lower daily running cost on anchored in comparison to chemical treatment.

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