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ROLE OF INDIGENOUS FUNGAL SPECIES IN THE DEGRADATION OF SOME COMMONLY USED DYES

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ABSTRACT

Different native fungal strains were isolated from the dye effluent and were acclimatized and screened for the degradation experiments against five commonly used dyes viz. Methyl Red, Methyl Orange, Erichrome Black, Crystal Violet and Malachite Green. RGL and MRL values for these bacterial strains against the dyes were evaluated by keeping them on solid culture media or Dye Modified Media (DMM). The relative decolorization potential of bacterial strains was determined by growing them in liquid media and its modifications. From the screening experiments several fungal isolate strains emerged as 'Degrader strains' that possess a good deal as they displayed good values of MRL and RGL against various dyes in DMM. It was found that these degrader strains were *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus tereus and Penicillium sp.* that displayed a good deal of decolorization against all the dyes tested. Best results were obtained for *Aspergillus niger* followed by *Aspergillus flavus* against all the tested dyes. Methyl Orange and Methyl Red dyes were the most accessible dyes for degradation. *Aspergillus niger* displayed a maximum decolorization percentage of 79% and 78% against Methyl Orange and Methyl Red dye respectively.

Keywords: Degradation, Rich Growth Limit, Maximum Resistance Level, Decolorization percentage.

INTRODUCTION

Synthetic dyes find use in a wide range of industries such as textile dyeing, paper printing, cosmetics and pharmaceuticals [1]. Approximately 10,000 different dyes and pigments are used in industries and over 7 × 105 tons of these dyes are annually produced world-wide. Due to inefficiencies of the industrial dyeing process, 10 - 15% of the dyes are lost in the effluents of textile units, rendering them highly coloured. There are some reports about the negative effects of textile dyes, especially azo dyes, towards aquatic life and humans.

For example, the discharge of those colored wastewaters into rivers and lakes leads to a reduction of sunlight penetration in natural water bodies which in turn decrease both photosynthetic activity and dissolved oxygen concentration. This will create anaerobic conditions thereby killing aerobic marine organism. Furthmore, textile dyes pose serious health threats to human due to their carcinogenicity and lead to mutagenic and toxic effects on organism [2-4].

Currently, various treatment methods exist Biological processes, such as biodegradation, bioaccumulation and biosorption, have received increasing interest due to their cost, effectiveness, ability to produce less sludge and environmental benignity [5,6]. Up till now, several reports have been published on the microbial decolorization/removal of synthetic dyes [7-9] i.e. effects on organism.

Bioremediation constitutes the use of natural biota and their processes for pollution reduction; it is a cost effective process and the end products are nonhazardous [10]. Microbial communities are of primary importance in bioremediation of metal contaminated soil and water, because microbes alter metal chemistry and mobility through reduction, accumulation, mobilization and immobilization [11]. Microbial-metal transformations represent a key component to metal cycling in natural systems [12,13].

Fungi are known to tolerate and detoxify metals by several mechanisms including valence transformation, extra and intracellular precipitation and active uptake [14]. Fungi can accumulate metal by physico-chemical and biological mechanisms including extracellular binding by metabolites and polymers, binding to specific polypeptides, and metabolism-dependent accumulation. Filamentous fungi may be better suited for this purpose than other microbial groups, because of their high tolerance towards metals, wall binding capacity, and intracellular metal uptake capabilities [15].

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Study area

The study of screening of native bacterial stains and their evaluation for dye degradation potential was conducted at Tonk district, which is located in north-eastern part of the Rajasthan state between 75°07' to 76°19' east longitude and 25°41' to 26°34' north latitude.

MATERIALS AND METHODS

Isolation and characterization of fungal isolates for the decolorization of carpet dyes

Screening of fungal isolates on solid media against carpet dyes and recovery of the 'Best-degraders'

When native fungal strains were isolated and screened out from the carpet effluent containing these dyes, then, several fungal strains appeared on the solid media (Dye Modified Media = DMM) at the initial local carpet dye concentration of 10 mg/l at 29^0C – 30^0C on solid media containing agar Plates.

In the present study five standard dyes were chosen Methyl Red, Methyl Orange, Crystal Violet, Erichrome Black and Malachite Green. When these degraders were subjected to higher concentration of carpet dyes (20-1000 mg/l), they showed variable sustainability in adaptation experiment, and confirmed by different dye removal efficiencies. A measurement of rich growth limit (RGL) was also done on the degrader strains; RGL is the fungal growth that was obtained almost half in circumference with any amount of dye compared to that growth without dyes on agar plates in 8 days. Each isolate gave a different RGL, below its maximum resistance level (MRL) for each dye (Table 1).

Decolorization of carpet dyes by degrader fungal isolates in liquid media under static conditions

The screened degrader fungal strains were also assessed for their decolorization potential with different carpet dyes (in liquid media under standard concentration of 100 mg/l) in static condition at 29^0C for 8 days.

RESULTS

Selection of degrader-strains is based upon the concept that a strain was considered better if it decolorized the dyes. The decolorization was monitored by the Zone of decolorization on solid media, and confirmed by the spectrophotometric analysis done in the liquid nutrient media. Out of the various fungal strains, only five strains were categorized as best degraders, which were based upon their adaptation and degradation capabilities. These two characteristics are in turn dependent upon their Rich Growth limit (RGL) and Dye Removal efficiency, (DRE).

The indentified and screened 'best-degraders' were- *Aspergillus niger, Aspergillus flavus, Aspergillus tereus, Aspergillus fumigatus* and *Penicillium spp*.

Among the fungal degrader strains, *Aspergillus niger* has been proven as the best degrader against all the dyes tested, followed by *Aspergillus flavus.*The most accessible dye for degradation was Methyl Orange followed by Methyl red and Malachite Green was the most difficult dye to degrade.

Best decolorization results were obtained for *Aspergillus niger* against Methyl Orange dye (79%) followed by results on Methyl Red dye (78%) by the same strain.

Table 1. Maximum Resistance Level (MRL) and Rich Growth Limit (RGL) values for indigenous fungal strains tested against tested dyes on solid Dye Modified Media (DMM)

S. No.	Isolated fungal strain	MRL and RGL values of tested dyes														
		Methyl Red			Methyl Orange			Crystal Violet			Erichrome Black			Malachite Green		
		MRL (mg/L)	RGL (mg/L)	Rank	MRL (mg/L)	RGL (mg/L)	Rank	MRL (mg/L)	RGL (mg/L)	Rank	MRL (mg/L)	RGL (mg/L)	Rank	MRL (mg/L)	RGL (mg/L)	Rank
1.	*A.niger*	600	400	1	650	500	1	600	400	1	600	450	2	250	>100	1
2.	*A.flavus*	550	350	2	600	500	2	600	350	2	650	450	1	200	>100	2
3.	*A.fumigatus*	500	350	3	600	450	3	550	300	3	550	400	3	150	>100	3
4.	*A.tereus*	350	200	4	450	300	5	400	250	4	550	400	3	100	50	4
5.	*Penicillium spp.*	400	250	5	500	350	4	400	250	4	450	300	4	200	>100	2

Table 2.Decolorization percentage of fungal strains against all the tested dyes in liquid DMM media

Sl.No.	Name of the fungal degrader strain	Decolorization percentage of tested dyes				
		Methyl Red	Methyl Orange	Crystal Violet	Erichrome Black	Malachite Green
1.	*A.niger*	78	79	74	72	40
2.	*A.flavus*	74	77	70	78	38
3.	*A. fumigatus*	60	66	61	65	20
4.	*A. tereus*	55	59	54	64	18
5.	*Penicillium spp.*	55	64	50	54	25

Figure 1. MRL and RGL values for indigenous fungal strains tested against all the tested dyes in Dye Modified Media (DMM)

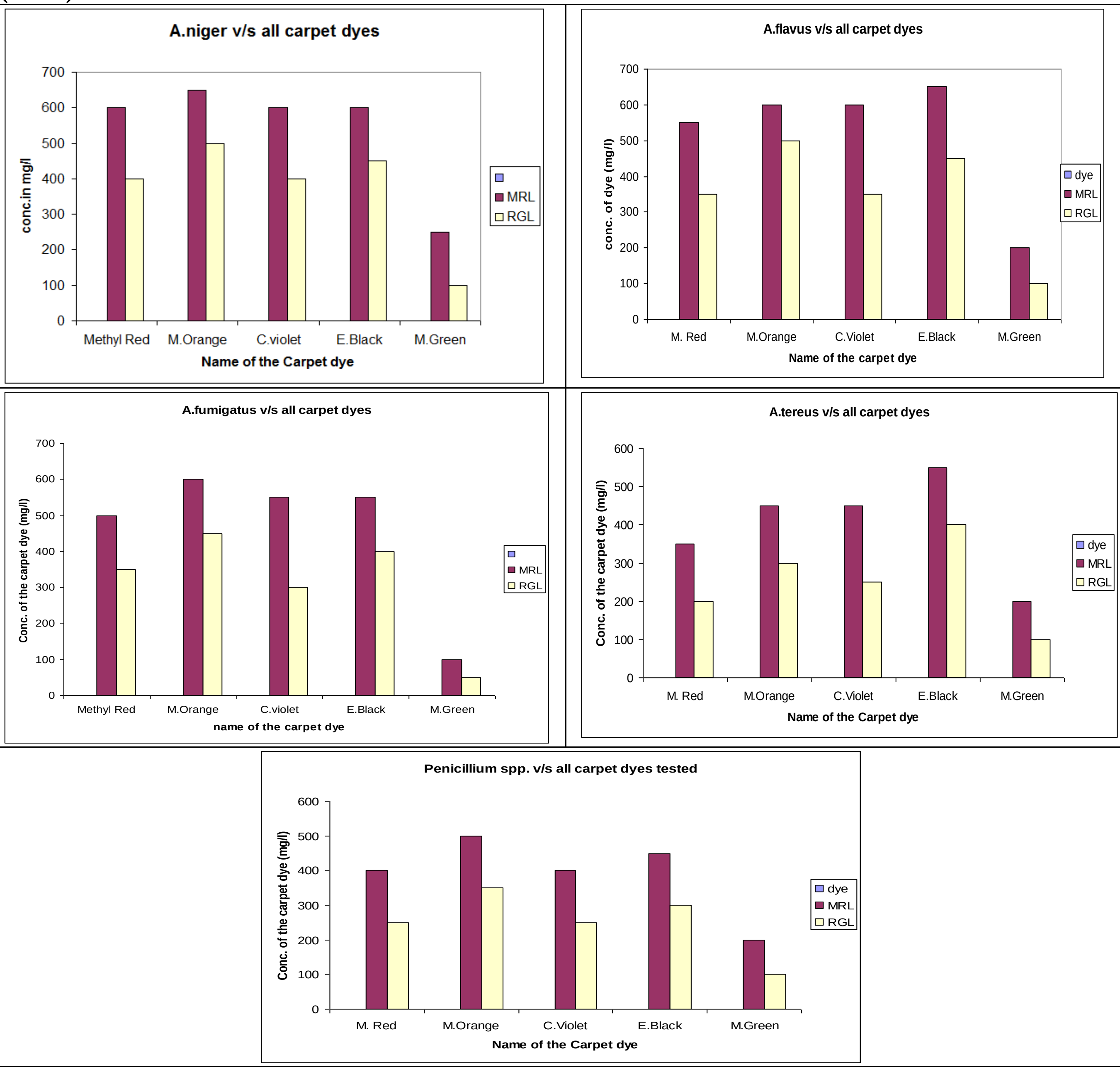
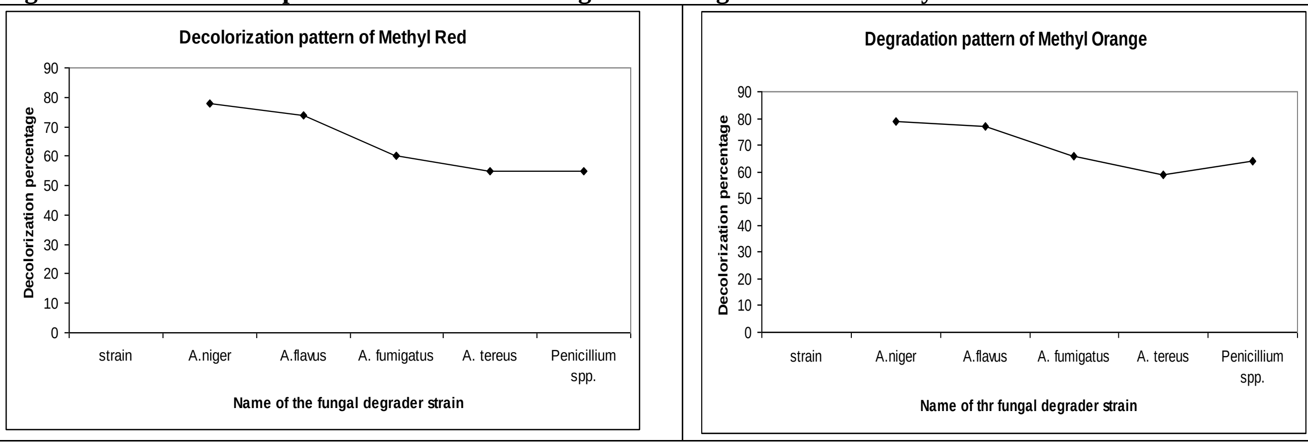
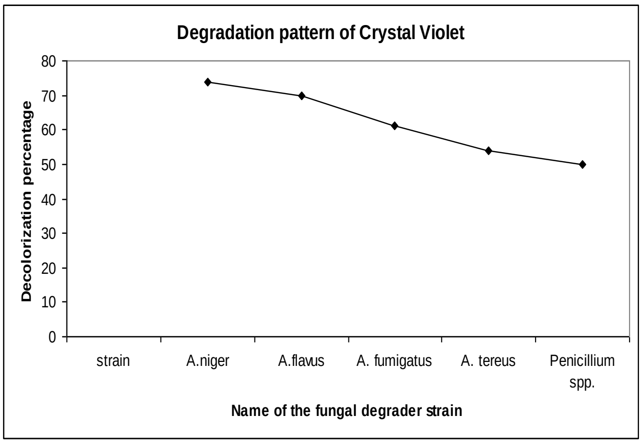
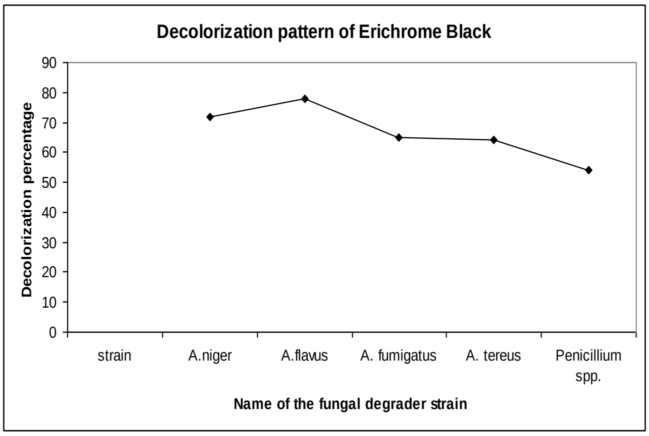
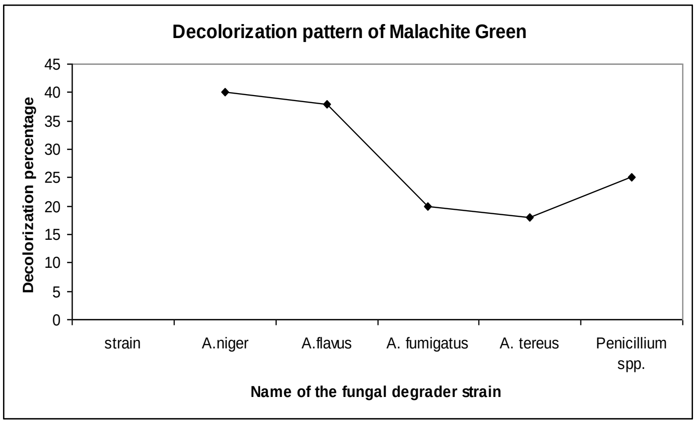


Figure 2. Decolorization pattern of the selected fungal strains against the tested dyes









DISCUSSION

In the present study, the dye degradation potential of five native fungal strains has been revealed viz. *Aspergillus niger, Aspergillus flavus, Aspergillus tereus, Aspergillus fumigatus and Penicillium spp*. It is well known that Fungi thrive well in inhospitable habitats and environmental extremes because of their enzyme system [16]. Fungi are involved in the bio degradation of undesirable materials or compounds and convert them into harmless, tolerable and useful products [17] Fungi are recognized for their superior aptitudes to produce a large variety of extracellular proteins, organic acids and other metabolites, and for their capacities to adapt to severe environmental constraints [18,19]. Fungal systems appear to be most appropriate in the treatment of colored and metallic effluents [20].

In a study, the decolorization of a reactive dye, Drimarene Blue K_2RL was performed using immobilized fungal strain *Aspergillus niger SAI* [21].
Similar results were obtained in the present study where it was noticed that *Aspergillus niger* was the most potent strains in terms of decolorization of all the dyes.

In another study the decolorization activity of various native fungal strains was tested, and it was found that *Aspergillus ochraceus, Aspegillus terreus, Aspergillus niger, Fusarium moniliforme and Penicillium citrinum were* capable for degradation of the dyes, Methylene Blue, Gentian Violet, Crystal Violet, Cotton Blue, Sudan Black, Malachite Green, Methyl Red and Corbol Fushion [22]. Likewise in the present study, the degradation of five most commonly used dyes viz. Methyl Red, Methyl Orange, Crystal Violet, Erichrome Black and Malachite Green was successfully performed using the indigenous species of fungi where the genera of *Aspergillus* and *Penicillium* emerged out as most dominant degraders. Decolorization and biodegradation of textile dyes such as Crystal Violet and Malachite Green have also been studied using *Fusarium solani (Martius) saccardo*, and this strain could decolorize a maximum of 98% for Crystal Violet and 96% for Malachite Green [23]. Similiarly in the present study, Crystal Violet and Malachite Green dyes were degraded using the native fungal strains.

In another study, Comparison of various indigenous fungal isolates for their dye decolorization capacity was done for model dye Acid Red 151, the employed strains were *Aspergillus niger SA1, Aspergillus flavus SA2* and *Aspergillus tereus SA3* and it was found that the best decolorization was observed with 2% fungal inoculum in all three fungal isolates [24]. In the present study also, the various strains of *Aspergillus* have been proven as the best degraders against all tested dyes. It is to be believed that the ability of these fungi to degrade such a range of organic compounds results from the relatively non-specific nature of their lignolytic enzymes, such as lignin peroxidase(LiP),manganese peroxidase(MnP) and laccase. These enzymes have unique catalytic properties like LiP catalyzes the oxidation of non-phenolic aromatic compounds such as Veratryl alcohol, while MnP oxidises Mn^{+2} to Mn^{+3}, which is able to oxidize many phenolic compounds [24].

CONCLUSION

Therefore it can be concluded from the present study that the drained dye waste water should be treated with the indigenous fungal strains under optimum conditions so that the toxic recalcitrant dyes should be converted down into colourless, less harmful or harmless products that have little or no disastrous effect on the local microbial fauna and flora that are inhabitating the sinks or local water reservoirs of the area.

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