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Screening of Textile Dye Impacted Soils for Novel Dye Decolorizing Fungi Employing Various Nutrient Media

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Abstract: This study aims to isolate and enumerate fungi from soil samples previously polluted with textile dyes from two important textile hub of Tamilnadu, India. All the isolated cultures (39 nos) were studied for their dye tolerant nature in Sabouraud's dextrose agar medium amended with 14 different dyes. Around 10 fungal isolates which revealed good pattern of growth in the plates were again screened for their efficiency in a liquid media (Sabouraud's dextrose broth) again in the presence of 14 dyes and the decolorization efficiency were calculated in percentage. A potent fungal isolate was chosen which showed maximum color removal efficiency in the broth media in the presence of 14 dyes. Further the selected isolate was studied for their dye utilization capacity in various nutrient media comprising various ingredients which revealed that minimal media with less media composition established a better performance in decolorization.

Key words: Isolation · Fungi · Dye tolerance · Media · Decolorization

INTRODUCTION

Colored wastewater from textile industry is considered to be the most polluted in all industrial sectors and over 100,000 different textile dyes and pigments are in common use and the total world organic colorant production is more than 7 x 10^7 tons/year [1, 2]. These dyestuffs find its application in textile, cosmetic, pharmaceutical and leather industries but are of primary importance to textile manufacturing whose wastewaters may contain a variety of these pollutants [3].

Nearly 10,000 garment manufacturers and 2100 bleaching and dyeing industries are concentrated in cities like Tirupur and Karur in Tamilnadu, Ludiyana in Punjab and Surat in Gujarat. Tirupur, a neighbour district of Coimbatore is located at the bank of river Noyyal, a tributary to river Cauvery. The quality of Noyyal river water and climatic condition of Tirupur has been ideal for dyeing operation of yarn and fabric since long time. Presently there are 712 dyeing and bleaching industries in Tirupur that generate 87,000 m^3/d of wastewater [4].

Fungal systems appear to be most appropriate biological agent in the treatment of colored and metallic effluents [5] and are able to degrade a wide variety of recalcitrant organo pollutants [6], including various types of dyes and decreases toxicity [7] and has aroused interest in using them in bioremediation [8]. Thus the present study was planned with the following objective as follows: a) To isolate fungal strains from various textile dye contaminated sites; b) To screen the dye decolorizing efficiency of isolated fungi against 14 different dyes in SDA medium; c) To check their decolorization pattern in the presence of various dyes in broth medium and d) To identify the most influencing nutrient media for decolorization by potent fungal isolate in selected dyes.

MATERIALS AND METHODS

Study Area: Dye contaminated industrial sites located in Coimbatore (11°00' North latitude 77°00' East longitude) and Tirupur (11°05' North latitude 77°20' East longitude) districts of Tamilnadu, India was subjected as study area.

Collection of Soil Samples: At the study area, surface soil was removed and 20-50 g of soil from the depth of 2-3 cm then soil samples were collected with the help of sterilized spatula and kept in sterile polyethylene bags. The samples were transported to the laboratory in an icebox and processed with in 6 hrs of collection.

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Plating Technique: One gm of soil sample was added to 99 ml of sterile distilled water in a 250 ml Erlenmeyer flask and kept in a mechanical shaker at 120 rpm for 15 mins. Serial dilutions up to 10^{-4} was done. Sabouraud's dextrose agar (SDA) medium was prepared, the pH was adjusted to 5.5 and sterilized at 15 lbs for 121°C for 15 mins. To this, filter sterilized Streptomycin solution (50 mg/L of streptomycin) was added and thoroughly mixed to avoid bacterial growth. One ml aliquots of 10^{-4} dilution was pipetted out into sterilized petridishes and about 15 ml of Sabouraud's dextrose agar medium was pour plated in duplicate. The dishes were then rotated clockwise and anti-clockwise for uniform distribution of the samples. The solidified plates were incubated at 30°C in an incubator for five days.

Sabouraud's Dextrose Agar medium (9)	
Peptone	: 10.0 g
Dextrose	: 40.0 g
Agar	: 20.0 g
Distilled water	: 1000 ml
pH	: 5.5±0.2

Enumeration and Isolation of Fungi: After incubation, the total number of fungal colonies was enumerated and the fungal populations were expressed as cfu/g of soil. The isolated individual colonies were transferred to Sabouraud's dextrose agar slants and incubated in an incubator at 30°C.

Purification of Fungal Isolates by Single Hyphal Tip Method [10]: Sabouraud's dextrose agar was prepared, sterilized at 15 lbs for 121°C for 15 mins and dispensed onto sterile petridishes. After solidification, peripheral mycelia from the slants were carefully lifted, streaked on to SDA plates and incubated at 30°C for 5 days. After incubation, the colonies were observed for hyphal developments. The peripheral tip of the mycelial growth was taken from the plates, reinoculated onto SDA medium and incubated at 30°C for 5 days.

Preparation of Inoculum: Purified fungal isolates grown in plates were taken after good growth of mycelial matt and 0.6 cm diameter mycelial discs were cut with a flame sterilized cork-borer and used as inoculum.

Preparation of Dye Solution: Commercial grade dyes were purchased from a local textile industry, Coimbatore and some laboratory grade was purchased from HiMedia and used without further purification. One gm of the dye powder was dissolved in de-ionized water and made up to 1000 ml to give 1000 mg/L. From the stock solution, required concentrations of dye solutions were prepared and filter sterilized using 0.45ì membrane filter. The dyes used in the present study are listed in Table 1.

Table 1: Dyes used for the study

S.No.	Dyes	λ_{max} (nm)
1.	Congo red	500
2.	Reactive orange 86	460
3.	Reactive blue 13	600
4.	Reactive green	613
5.	Reactive blue 222	610
6.	Reactive blue 140	660
7.	Reactive blue 220	612
8.	Reactive red 195	545
9.	Reactive yellow 84	354
10.	Reactive orange 72	388
11.	Reactive brown 18	468
12.	Reactive yellow 17	351
13.	Reactive brown 36	473
14.	Reactive blue 4	495

Decolorization in Sabouraud's Dextrose Agar (SDA) Medium: Sabouraud's dextrose agar medium was prepared in Erlenmeyer flasks and the pH was adjusted to 5.5 and sterilized at 15 lbs for 121°C for 15 mins. All the fourteen dyes at 50 mg/L concentration individually were filter sterilized, added to Sabouraud's dextrose agar medium, thoroughly mixed and poured on to petriplates in aseptic condition. After solidification, 0.6 cm diameter mycelia of the isolated fungal strains were inoculated in SDA medium with individual dyes at 30°C. SDA plates containing dyes without fungal inoculum were maintained as control. After 5 days of incubation, the plates were examined for fungal growth and a clear zone of decolorization. Based on the size of the clear zone around the colonies, the decolorization pattern by the fungal strains were categorized under good decolorization (+++), moderate decolorization (++), less decolorization (+) and no decolorization (-).

Decolorization in Sabouraud's Dextrose Broth (SDB) Medium: Sabouraud's dextrose broth medium was prepared to a final volume of 50 ml in a 100 ml Erlenmeyer flask in triplicate and the pH was adjusted to 5.5 and sterilized at 1atm pressure for 15 mins. All the fourteen dyes at 50 mg/L concentration were filter sterilized and added to SDB medium individually. The fungal strains which exhibited good decolorization in all the 14 dyes were selected and grown in SDA medium for 5 days. After good growth, 0.6 cm diameter mycelia were cut and inoculated into the broth medium. The flasks were incubated in a thermostat controlled shaker at 120 rpm at 30 °C for 48 hrs. Appropriate control flasks containing dye without inoculum were also maintained.

Selection of Dyes and Dye Decolorizing Fungus: Strain number 9 (HM11) showed maximum decolorization of Congo red and Reactive blue 140 when compared to other isolates and dyes tested. Hence

strain number 9 (HM11) and the two dyes namely Congo red and Reactive blue 140 were selected for further studies.

Identification of Fungi by Moist Chamber Technique [11]: Sabouraud's dextrose agar medium was poured in sterile petriplates as a thin film. The medium was cut into squares (0.5 cm x 0.5 cm) using sterile scalpel. Each agar block was transferred to the moist chamber aseptically. The four sides of the agar piece were inoculated with the fungal isolate and a clean cover slip was placed on the agar block and kept for incubation for five days at 30 °C. After incubation, the cover slip was carefully removed with fungal mycelium, a drop of lactophenol cotton blue stain [12] was added and the coverslip was placed on a new clean glass slide and was examined under microscope and the morphological features of the fungi were recorded. Manuals carrying the description of fungi were consulted for identification [13-18].

Taxonomic Determination of the Fungal Isolate: Confirmation of taxonomic identification of the fungal isolate was carried out at Fungus Identification Service, Mycology and Plant Pathology Laboratory, Agharkhar Research Institute, Pune, India. The potent dye decolorizing fungal isolate was found to be *Aspergillus niger* and was designated as *Aspergillus niger* HM11 and used for further study.

Dye Decolorization Efficiency of the Selected Fungi in Various Broth Medium: Various broth media (Sabouraud's Dextrose broth [9]; Minimal medium [19]; Kirk's basal salts broth [20]; Malt Extract broth [9]; Malt Yeast broth [21]; Czapek-Dox broth [9] and Mineral Salts broth [9] were used.

Fifty ml of the corresponding medium was prepared in 100 ml conical flasks and pH of the medium was maintained at 5.5 and sterilized at 1 atm for 15 mins. To one set of flasks, filter sterilized dye Congo red and to another set of flasks, the dye Reactive blue 140 was added at a concentration of 50 mg/L. After cooling, the flasks were inoculated with 0.6 cm fungal mycelial spores and incubated at 30°C at 120 rpm. Medium with dyes and without the inoculum were also maintained as control. After 48 hrs of incubation, growth and percent decolorization was measured. Experiments were carried out in triplicate.

Measurement of Decolorization: After incubation, the samples were centrifuged at 10,000 rpm for 15 min and the suspended biomass was separated. The absorption spectra of the clear supernatant were recorded at λ_{max} of various dyes (Table 1) using a spectrophotometer (UV-Vis 3210, Hitachi, Japan). Medium containing dyes without the inoculum was taken as control and the experiments were carried out in triplicate. The initial and final absorbance values obtained were then used to calculate e percentage decolorization of the dye

$$\% \text{ decolorization} = \frac{\text{Initial absorbance value - Final absorbance value}}{\text{Initial absorbance value}} \times 100$$

Measurement of Growth: The mycelial pellet was separated from liquid medium and washed twice with distilled water over a preweighed Whatman no.1 filter paper. The filter paper with fresh mycelial pellets was dried in hot air oven at 80 °C for four hours, weighed and the growth in terms of biomass was expressed in g/L dry weight.

RESULTS AND DISCUSSION

Distribution of Fungi in Soil Samples: The increased demand for textile products enhanced the functions of both manufacturing industries and dye-consuming industries. The release of coloured effluents from these industries into the environment is undesirable since many dyes and their breakdown products are toxic and/or mutagenic to life [22]. Despite the existence of a variety of chemical and physical treatment processes, the environment friendly approach in treating textile dyeing effluents has stimulated interest in exploring novel means. Soil samples collected from twelve contaminated sites were subjected for the enumeration of fungi and the results are presented in Table.2. Soil samples collected near textile dyeing plant in Sample III showed the maximum of 8.0 x 10^4 cfu/g fungal counts followed by Sample IV (5.0 x 10^4 cfu/g). The count of fungal colony was found to be less (1.0 x 10^4 cfu/g) in soil sample V.

In Sabouraud's Dextrose Agar Medium: All the 39 fungal strains isolated from various soil samples were screened for their dye decolorization efficiency in Sabouraud's dextrose agar medium amended with selected fourteen dyes individually and the results are given in Table 3. Ten fungal isolates (3, 6, 9, 10, 11, 18, 19, 21, 27 and 37)

Table 2: Distribution of fungi in the soil samples contaminated with textile dyes

S.No	Source of soil from various dye contaminated sites	Abbreviation	Fungal colony (cfu x 10^4/g)
01.	Sample I	TDUP	2.0
02.	Sample II	TDUT	4.0
03.	Sample III	TDTPA	8.0
04.	Sample IV	TDPS	5.0
05.	Sample V	CAV	1.0
06.	Sample VI	TUA	4.0
07.	Sample VII	ODSL	2.0
08.	Sample VIII	ODS	2.0
09.	Sample IX	TDUM	3.0
10.	Sample X	TDUA	3.0
11.	Sample X1	TDPT	2.0
12.	Sample XI	TPP	3.0

Population expressed in cfu per gram of sample, where cfu is the colony forming unit.

strains exhibited good decolorization (+++) against various dyes tested and hence these fungal strains were selected and tested for their decolorization efficiency in Sabouraud's dextrose broth medium. In general, the novel dye decolorization capacity of any microorganism can be achieved by exposing it to chemically diverse dye stuff to analyze its capacity in broad gamut. In this regard, in the present study, initial screening of dye decolorization performance of the fungal strains isolated was done in the presence of various dyes. In line with our studies, reports mentioning several usage of dyes against various microorganisms were carried out by researchers [23, 24].

In Sabouraud's Dextrose Broth Medium: The above selected fungal strains were further tested in Sabouraud's dextrose broth medium amended with all the fourteen dyes individually. The dye decolorization was monitored after a period of 48 hrs and the decolorization pattern is tabulated below (Table 4). Among ten isolates tested for their decolorization efficiency in all the fourteen dyes, isolate Number 9 showed maximum decolorization of five dyes namely Congo red (94.8%) followed by Reactive blue 140 (93.1%), Reactive yellow 84 (88.8%), Reactive blue 220 (87.9%) and Reactive blue 13 (82.1%). Hence isolate Number 9 was chosen for further study to evaluate its dye decolorizing potential against Congo red and Reactive blue 140 which were decolorized to the maximum in the previous experiment.

Selection of Dyes and Dye Decolorizing Fungus: Strain number 9 (HM11) showed maximum decolorization of Congo red and Reactive blue 140 when compared to other isolates and dyes tested. Hence strain number 9 (HM11) and the two dyes namely Congo red and Reactive blue 140 were selected for further studies.

Identification of the Fungal Isolate: Isolate Number 9 was identified as *Aspergillus* sp. by employing Lactophenol cotton blue staining method and further taxonomic identification of the fungal isolate at Agharkhar Research Institute, Pune revealed that it was *Aspergillus niger* HM11.

Selection of Growth Medium for Decolorization of Selected Dyes by A.niger HM11: Various broth media such as Sabouraud's dextrose broth, Minimal broth, Kirk's basal salts broth, Malt extract broth, Malt yeast broth, Czapek-Dox broth and Mineral salts broth were used to study the influence of liquid media on growth and decolorization of Congo red and Reactive blue 140 by *A. niger* HM11. The results are presented as Figures 1 and 2.

Dye removal by the fungus occurs either through bioaccumulation or biodegradation [25]. In this study, decolorization efficiency of *Aspergillus niger* HM11 strain was assessed against various dyes (Congo red, Methyl orange, Methylene blue, Brilliant green, Reactive blue 222, Reactive blue 140, Reactive blue 220, Reactive red 195, Reactive yellow 150, Reactive orange (Orange HEZN), Reactive brown18, Reactive yellow, Reactive brown36 and Reactive blue MR). *A.niger* HM11 decolorized almost all the dyes tested with a maximum of Congo red (99.6%) and Reactive blue 140 (99.5%). Interestingly, the initial experiments established that there was a varied and a different pattern of decolorization performance when exposed to various dyes in agar medium whereas the pH of the medium and incubation temperature for all the dyes was kept constant.

Biologically (Fungi) mediated decolorization/ degradation of different textile dyes has been found effected and influenced under different physicochemical conditions, including pH [26], temperature [27], carbon source [28] nitrogen source [29] and concentrations of different dyes [30]. Maximum percent decolorization of Congo red was observed in Sabouraud's dextrose broth medium (94.8%) followed by Minimal broth media (94.2%) and their corresponding biomass pattern was found to be 5.87 g/L and 5.85 g/L. Similarly, maximum percent decolorization of Reactive blue 140 was observed in Sabouraud's dextrose broth medium (94.3%) and Minimal broth medium (94.0%). The biomass of 5.85 g/L and 5.81 g/L were recorded in Sabouraud's dextrose broth medium and in Minimal medium respectively.

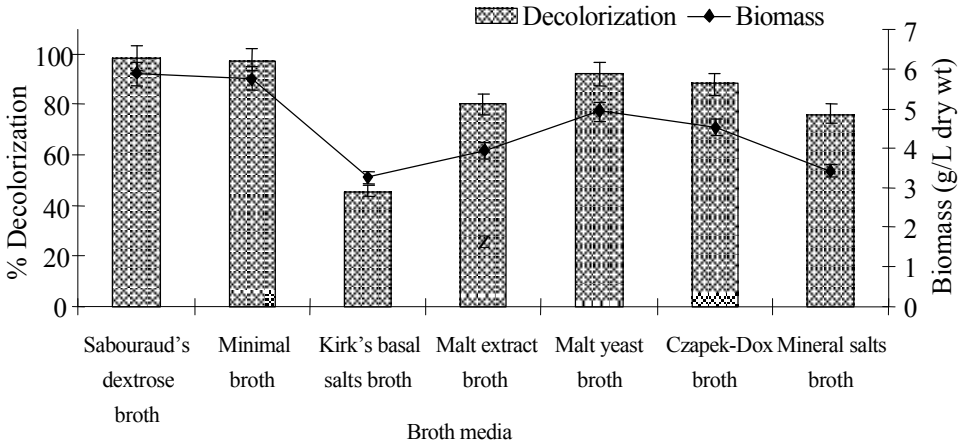

Fig. 1: Influence of broth media on decolorization of Congo red and biomass of *A.niger* HM11

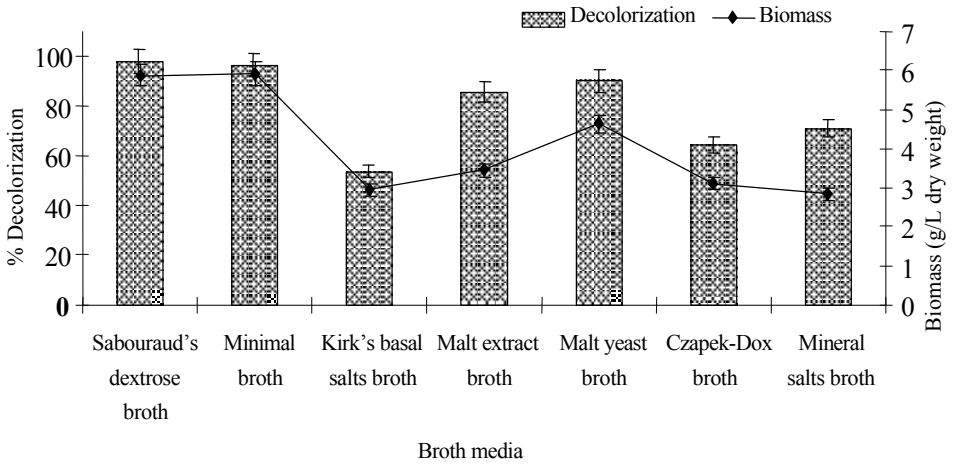

Fig. 2: Influence of broth media on decolorization of Reactive blue 140 and biomass of *A.niger* HM11

Table 3: Decolorizing efficiency of isolated fungi in SDA medium (14 dyes)

Isolate No	Sample	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
1.	TDUP	+	+	-	++	-	++	++	+	+	+	+	+	++	++
2		++	+	-	++	++	+	++	++	+	++	++	++	++	-
3	TDUT	+	+	+	+++	+++	++	++	+	+	++	++	+	+	++
4		+	-	-	+	+	+	++	+	+	+	++	+	+	+
5		+	-	-	++	-	++	++	+	+	++	+	++	+	++
6		+	-	+	+++	+	+	++	+++	++	+	++	++	++	++
7	TDTPA	++	+	-	++	+	+	++	++	-	++	++	++	++	-
8		+	-	-	++	+	+	++	+	+	+	++	+	+	+
9		+++	++	+++	++	++	+++	+++	++	+++	++	++	+++	++	++
10		+	+	+	+++	++	+++	++	++	++	++	++	++	+	+
11		+	+	+	++	++	+	++	+++	++	++	+	+	+	++
12		+	+	-	++	-	++	++	++	+	+	+	+	++	++
13		++	+	+	++	++	+	++	++	++	++	++	++	++	+
14		++	+	-	++	++	+	++	++	+	++	++	++	++	-
15	TDPS	+	+	+	+	++	-	-	-	+	+	+	+	+	+
16		+	+	+	++	+	+	++	-	+	+	+	+	+	+
17		+	+	+	+	+	-	+	-	-	+	-	-	+	+
18		++	+	+	+++	++	+	+++	++	++	+++	++	++	++	+
19		++	+	++	++	+	++	++	++	+++	++	+	++	+	++

Table 3: Continue

Isolate No	Sample	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
20	CAV	++	-	+	+	++	+	+	+	+	+ +	+ +	+	+	++
21	TUA	+ +	+	+	+++	+	+ +	+++	+++	+ +	+ +	+	+ +	+	-
22		++	+	+	++	+ +	++	++	++	+ +	+	+ +	+ +	+	-
23		+ +	+	+	+ +	+ +	+	++	+ +	+	+	-	+ +	+	++
24		-	-	-	+	+	+ +	+ +	+	+	+	+	+	+	+
25	ODSL	+	+	-	+	+	+	+ +	+ +	+	+ +	+ +	+	+	+
26		+	-	+	+ +	+	+	+	+	+ +	+ +	+	+	+	-
27	ODS	++	+	++	++	+	++	++	++	+++	+ +	+	+ +	+	+ +
28		++	-	+	+	++	+	+	+	+	+ +	+ +	+	+	++
29	TDUM	+	-	-	+	+ +	+	+ +	+ +	+	+ +	+	+	+ +	+ +
30		-	-	-	+ +	-	+ +	+	+	+ +	+	+ +	+	+	+ +
31		+	+	-	+ +	+ +	-	+ +	+	+	+	+ +	+	+	+ +
32	TDUA	+	-	+	+	+	+	+	+	+	++	++	++	++	++
33		+	+	+	-	+	+	-	+	+	+	+	+	+	+
34		+	+	+	++	+	+	+	+ +	+ +	+	+	+	-	-
35	TDPT	+	+	+	+	+	+	+	+	+	++	++	++	++	++
36		+ +	+	+	++	+ +	+	++	+ +	+ +	+ +	+ +	+ +	+ +	+
37	TPP	+	+	+	++	++	++	++	++	++	+ +	+ +	+++	+ +	++
38		+ +	+	+	++	+	++	++	++	++	++	+	++	++	-
39		-	+	-	++	++	++	++	++	+	+	+	++	++	++

D1 - Congo red D2 - Reactive orange 86 D3 - Reactive blue 13
D4 - Reactive green D5 - Reactive blue 222 D6 - Reactive blue 140
D7 - Blue -220 D8 - Reactive red 195 D9 - Reactive Yellow 84
D10 - Reactive orange 72 D11 - Reactive brown 18 D12 - Reactive yellow 17
D13 - Reactive brown 36 D14 - Reactive blue 4

Table 4: Decolorization of 14 dyes by selected fungal strains in SD broth medium

Isolate No.	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
3	32.1	34.5	21.9	88.9	80.2	67.3	55.4	28.1	27.3	54.3	67.3	22.2	30.3	62.8
6	22.3	5.6	14.89	89.3	19.2	21.7	47.9	90.1	43.9	20.4	59.3	64.3	44.2	48.6
9	94.8	67.4	82.1	54.3	71.9	93.1	87.9	65.4	88.8	58.9	69.2	75.4	58.9	28.9
10	12.4	17.8	22.3	89.8	65.7	88.8	67.9	55.3	49.8	66.0	43.8	52.0	17.6	18.2
11	15.6	17.2	17.2	56.4	62.1	22.1	57.8	87.1	54.3	28.0	18.9	22.4	28.7	67.8
18	67.3	18.7	22.2	87.6	64.3	91.1	23.8	46.9	52.1	87.5	68.9	54.3	49.2	22.1
19	65.7	17.2	55.5	62.4	19.1	66.6	60.1	54.9	88.8	54.3	12.87	73.7	17.8	48.9
21	63.2	32.8	14.6	87.9	15.4	45.7	87.3	87.3	54.8	53.8	21.9	63.7	23.1	8.4
27	63.2	22.2	65.9	63.2	20.3	48.9	52.1	55.8	87.8	57.8	18.7	53.8	17.8	53.2
37	11.8	20.9	21.4	64.3	47.8	69.4	72.4	64.6	60.7	70.5	44.4	89.3	52.7	55.8

CONCLUSION

The soil samples contaminated with textile dyes revealed the presence of fungal colonies and all the 39 fungal strains isolated showed dye decolorizing capability against almost all the fourteen different textile dyes in agar medium. Similarly, most fungal isolates possess dye decolorization capacity in liquid medium also. The potent isolate *A.niger* HM11 resulted maximum decolorization in Congo red and Reactive blue 140 among the dyes tested. Even though SDA and minimal broth medium recorded better results during decolorization of Congo red and Reactive blue 140 as well as supported the growth of the fungus, minimal media was found to be ideal because it has least nutrients which will make the treatment system cost-effective when employed in large scale. Most of the isolated strains were found to posses dye tolerant nature which makes clear that the perspective of these fungal cultures for future biotreatment application in industries.

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