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RESEARCH ARTICLE

A study on the influence of different concentrations of dyes Basic fuchsine and Congo red on soil Mycobiota to developed the consortium for decontamination of dye from industry effluents

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Abstract

The present study was aimed to isolate the fungal species capable of tolerating dye pollution. Also the study was conducted to select fungal strain that might be able to remediate the basic fuchsine and congo red dyes from the polluted water. It has been found that the soil treated with different concentrations of basic fuchsine and congo red dyes had shown significant differences in mycodiversity. Soil samples treated with different concentration of these dyes were screened for fungal isolates. Soil fungi were isolated by using dilution plating method and were identified by using two standard manuals of soil fungi. The present research studied the potential of fungal strains, for their tolerance against basic fuchsin and congo red dyes, isolated from dye treated soils for use on removal of dyes. The screening conducted found many strains that presented good tolerant properties, including *Aspergillus flavus, Aspergillus niger* and *Penicillium glabrum* that could survive in BF and CR treated soils in a reasonable extent maximum 500 ppm concentration in the present work and their population were isolated throughout the period of study. From the viewpoint of environmental sustainability, the use of such fungal species for removal of dyes from industrial wastewater can be proved as an ecofriendly and cost-effective approach. Depending on the results, the fungal strains appear as efficient candidates for further biotechnological research on the decontamination of dye based industry effluents. For a better remediation, the combined effects of different types of fungi on the growth of other need to be considered during the selection of efficient fungal strains.

Keywords: Basic Fuchsin, Congo red, Soil mycobiota, Dye-tolerant fungi, environmental sustainability.

Introduction

Soil is a reservoir of nutrients, and hence, considered as highly nourished habitat for the growth of millions of microorganisms including fungi. Soil contains various types of microorganism including fungi, which are the most important and highly dominant group representing a major section of kingdom fungi (Ali-Shtayeh and Jamous 2000,Rane and Gandhe 2006, Tyagi and Charaya 2017,Selim *et al.* 2021). Out of the soil colonizing fungi many fungal strains are capable of living like saprobes, accomplishing their diverse stages of life cycle under soil. Fungi are known to serve as the universal decomposers by breaking down almost all the organic material existing in nature. Undoubtedly, dyes are major pollutants, with diverse chemical structures, of all kind of ecosystems entering through the various means. Dyes are produced by a variety of industries like paper and pulp, textiles, leather, cosmetics, plastic, tannery, food–processing and pharmaceutical companies (Singh *et al.* 2019,Elfeky *et al.* 2019,Jiang *et al.* 2020).

Dye containing effluents of textile industries is highly responsible of creating dye polluted soil and water ecosystems. Due to the malignant and persistent nature of dyes, a huge quantity of discharged dyes causes the contamination of water which eventually make whole the environment polluted (Crini 2006). The presence of dyes and their derivatives or their degradation products in water,

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even in very low amount, can also cause the severe human health disorders like mutations and cancers (Ledakowicz and Pazdzior 2021). A study has postulated that the presence of dyes in wastewater leads suffering from bladder tumor in higher number of peoples working in dye industry than in general population (Suryavathi*et al*. 2005). The effects of dyes on human health can also be detrimental in various other ways such as skin irritation, itchy or blocked noses, sneezing and sore eyes, gastrointestinal irritation and sometimes they may carcinogenic (Suryavathi *et al.* 2005,Santhi *et al.* 2009). The dyestuff, especially those in current use, has been designed to resist fading as well as light-induced oxidation (Nigam *et al.* 2000; Casieri *et al.* , 2008). However, the enhanced dye contamination of soil and water ecosystems has compelled the demand for clean-up strategies. It is therefore a responsibility and necessity to mitigate any risk on human health by treating the industrial effluent. Recently, different alternatives of eco-friendly remediation process have been practiced for the removal of dyes from the environments. These alternative methods of remediation adopt the use of biomass of bacteria, fungi and plants. In the last two to three decades using different parameters, the practicability of these bio-resources for effective bioremediation has been well experimented by many researchers (Tan *et al.* 2009). Many workers suggested that the microorganisms sometimes affect soil ecosystem more speedily than any abiotic component (Titljanova and Tesarova, 1991) and play essential roles in the nutrient recycling. Excess amount of dyes released in industrial effluent can also be a cause of low survival or damage of the beneficial microorganisms. Hence, the microbial community may be useful as a highly sensitive biological indicator of soil disturbance and process of remediation (Gremion *et al.* 2004, Hemanth *et al.* 2016). Several fungal strains isolated from soil have been reported to adopt dye tolerance strategies which include extracellular or intracellular dye sequestration and precipitation, suppressed influx and enhanced dye efflux, production of intra or extracellular enzymes, dye binding to cell walls and complexation. In addition, the contaminants released by industrial effluent shift the growth, reproducibility and activity of microorganisms and also affects the fertility of the soil by performing several physico-chemical processes. Keeping in mind these things, in this study the main focus is on to isolate the mycobiota of soil treated by different concentration of the dyes *i.e.* Basic fuchsin (BF) and Congo red (CR) to observe the tolerance of isolated species on the basis of their abundance. The study was also aimed to select the highly tolerant individuals (fungi) for testing of their capability for biosorption of dyes.

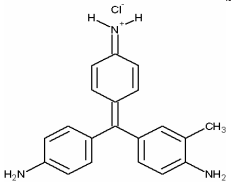
Characteristics of used dyes

Basic fuchsin

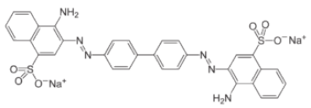
Basic fuchsin is a cationic triaminotriphenylmethane dye with a chemical formula of $C_{20}H_{19}N_3$·HCl. It is composed of a mixture two hydrochlorides such as rosaniline and pararosaniline hydrochlorides containing about 88% of rosaniline hydrochloride, calculated on the dried basis (Ghada and El-Sheekh. 2013). Basic fuchsin has been applied widely in coloring various materials including orlon, paper, cotton, or leather (Bayramoglu *et al.* , 2009) and commonly used in gram-staining and in a mixture with phenol for acid-fast staining of disease causal organism of tuberculosis *i.e. Mycobacterium*, in the procedure known as Ziehl-Neelsen (Horobin and Kiernan 2000, Clark, 1973). A fluky ingestion of Basic fuchsin may be detrimental or may produce serious damage to human health and can cause cancer, eye irritation and systemic harm by entering into the blood-stream through cuts, abrasions or lesions.

Congo red

It is a sodium salt of benzidinediazo-bis-1-naphthylamine-4-sulfonic acid; a diazo dye having chemical formula $C_{32}H_{22}N_6Na_2O_6S_2$. It produce red color in alkaline solution and blue in acid solution and used especially as an indicator and as a biological stain. It is highly toxic to a variety of microorganisms and other life forms and is suspected as carcinogenic and mutagenic factor. It also represents a significant effluent problem along with related dyes from textiles, printing and dyeing, paper and pulp, food, rubber, and plastic industries. The structural stability of Congo red makes it highly resistant to biological degradation, and possibly its bright color and toxicity is entirely undesirable in the environment and also harmful to all the biological forms.



Basic fuchsin



Congo red

Material and Methods

Isolation of fungi from dye-treated soil:

The soil sample was collected only one time from Botanical Garden, Maharaj Singh College, Saharanpur, Uttar Pradesh, India in April, 2017 at the depth of about 15 centimeter with the help of a sterile trowel, removing the upper soil containing extraneous litter or organic matters. The collected soil sample was then dried and crushed thoroughly to achieve a powdery form. The 63 sterilized pots of 150 ml capacity, each containing 100 gm of sampled soil were employed in the present study. Out of these 63 pots, 9 pots were treated with 25 ml of distilled water at a regular interval of five days for a total duration of 45 days. These nine pots treated with distilled water served as control. The remaining 54 pots were treated with different concentrations of basic fuchsin (BF) and Congo red (CR) dye solutions. The 18 pots were treated separately with 100 ppm solutions of both the dyes in a way as 9pots for basic fuchsin (BF) and 9 pots for

congo red (CR).Similarly another 18 pots were treated with 250 ppm solutions of BF and CR dyes. In the last, remaining 18 pots were also treated with 500 ppm solutions of BF and CR dyes in similar way.

After 15 days, out of the 9 pots of soil treated with distilled water (control), the soils of 3 pots were mixed thoroughly to obtain an expected mixture of treated sample. The 9 pots treated with three concentrations (each 3 of 100 ppm, 250 ppm and 500 ppm) of BF dyes were processed to obtain their separate composite soil samples. Similarly the 9 pots treated with different concentrations of CR (each 3 of 100 ppm, 250 ppm and 500 ppm) were also mixed and obtained their composite soil samples. Each of the obtained composite soil samples was then analyzed for Mycobiota, using dilution plate method (Waksman and Fred, 1922). 20 gm of soil from a composite soil sample were transferred into 200 ml of distilled water in a 500 ml conical flask. To get a suspension with almost equal distribution of fungal propagules, the conical flasks containing soil samples were stirred well for 30 minutes. Under the sterile conditions, 10 ml of this suspension is transferred immediately to a 250 ml conical flask containing 90 ml of distilled water to prepare a soil suspension of 10^{-2} dilution. Thus prepared suspension was used to prepare further serial dilutions as 10^{-3} and 10^{-4}. From the suspension of each of the dilutions, 1 ml aliquots were transferred to each of a set of three petri dishes filled with 20 ml of sterilized, cooled and solidified Potato Dextrose Agar (PDA) medium containing 30 mg of Rose Bengal and 30 mg of Streptomycin (for 1 liter medium). All the petri dished containing culture medium and inocula were incubated at 25±2° C for a period of 7 Days. Similar treatments were given to the suspensions of 10^{-3} and 10^{-4} dilutions. The incubated petri dishes were observed from third day of the inoculation so as to observe the growth and characters of fast growing fungal colonies appeared in the first two to three days. To obtain the axenic cultures and to facilitate an accurate identification, some of the inocula from petri dishes were transferred aseptically to another petri dishes containing fresh PDA (Choudhary *et al.* 2017). Identifying with the help of two standard manuals of soil fungi (Gilman 1944, Gilman 1966,Nagamani *et al.* 2006), a complete record of the fungal isolates and their species was maintained (Kumar and Charaya. 2012, Tyagi and Charaya 2017, Singh *et al.* 2021). Also the composite samples of BF and CR dye treated soils after 30 days and 45 days were obtained from the soils treated with their different concentrations(100, 250 and 500 ppm) processed in similar way. The procedure for identification and records of isolates was further repeated again. Also the mycobiota of dye treated soils was assessed after 30 and 45 days treated soils.

Results and Discussion

In the present study, a total of 59 fungal species were isolated from the soil treated with different concentrations (*i.e.* 100 ppm, 250 ppm and 500 ppm) of basic fuchsin and congo red dyes as well as from the control (treated with distilled water) soil. The treatment was applied from 15 days to 45 days. The mycobiota of the dye treated soil was found influenced greatly in terms of species abundance and diversity after under treatment of 15 day, 30 days and 45 days. Some genera had shown a big fluctuation of appearance and disappearance under different concentrations of dyes as well as in different treatment periods whereas some genera/ species were found to be highly tolerant towards stressed conditions created by high concentrations of dyes. The tolerant fungal isolates even more or less in number were found dominating the culture plates after all the treatment intervals.

It was observed that 19 genera of fungal isolate were found to highly tolerant that showed survival towards the highest concentrations (500 ppm in this study) of dyes. Among the isolated species (59), most of the isolates were belonging to class ascomycetes and the remaining were of Zygomycetes, Hyphomycetes, Deuteromycetes, and Basidiomycetes; and with less numbers some isolated species belongs to Oomycetes, Dothideomycetes and Saccharomycetes.

Under treatment of dyes after 15 days, for the isolation and identification the fungal cultures were raised by inoculating the dye treated soils on PDA in petri dishes. A total of 53 fungal species were isolated from the soil treated with different concentrations (*i.e.* 100 ppm, 250 ppm and 500 ppm) of basic fuchsin and congo red dyes as well as from the control soil. The total 28 species of 11 genera could be isolated from control soil while from the BF and CR dye treated soils 31 species (14 genera) and 27 species (12genera) could be isolated. Out of these 53 species, 10 species were common in control soil and basic fuchsin and congo red dye treated soils after 15 days. These include *Aspergillus fischeri, A. flavus*, *A. fumigatus*, *A. niger*, *Fusarium poae*, *Penicillium glabrum, Rhizopus oryzae*, *Trichoderma reesei, Trichoderma lignorum* and *Penicillium digitatum*. It was found that the dyes concentration did not showed any appreciable inhibitory effect on fecundability of tolerant fungi. It was found that the number isolates of *A. flavus* was decreased under treated soil, but it showed a great tolerance towards the different concentrations of basic fuchsin and congo red dyes. Its existence in petri dishes was remained constant through into the 45th day treated soils. It clearly means that the fungus *A. flavus* is highly capable to tolerate a high concentration of both the dyes used in the study.

Interestingly another *Aspergillus* species *i.e. A. fumigatus*, that was very low (1.89%) in its abundance in control soil, showed a great tolerance towards dyes. It was found existing in petri plates cultured from all three dye treated soils after 15 days. However it was absent in 250 ppm of basic fuchsin treated soil and in 100 ppm of congo red treated soils in the same treatment period. Surprisingly, it showed the huge

occupation (56.19%) under the 500 ppm basic fuchsin dye treated soil. It was also found occupy the petri plates by 18.96% and 15.38% of the isolates in 250 ppm and 500 ppm of congo red dye treated soils respectively.

Among the Penicillia, *P. glabrum* were dominated the culture plates in both control as well as dye treated soils after 15 days while it was absent in all the congo red treated soils after same period of treatment. It showed remarkable appearance in control soil with maximum percentage of 18.65%. Further, after treatment of 15 days its maximum percentage was recorded 37.58% in 250 ppm basic fuchsin treated soil. *P. glabrum* showed good abundance and contributed a big fraction to the myco-community in cultures plates. Also the fungus was found dominating the petri plates by 21.10%, and 4.23% in100 ppm and 500 ppm basic fuchsin treated soils respectively.

Another dominant genus of the isolated fungi was *Trichoderma* which is represented by 8 species. The species of *Trichoderma* were also found dominating the petri plates in different concentrations of dyes while they did not showed a throughout consistency. Its different species identified as *T. asperellum*, *T. atrovride*, *T. citrinoveride*, *T. harzianum*, *T. lignorum*, *T. resei*, *T. veride* and *Trichoderma* sp. The species of the genus *Trichoderma* were found possessing lesser tolerance capacity against the different concentrations of basic fuchsin and congo red dyes. From the discussion it is explicit that *Trichoderma* could not be considered as tolerant fungal isolates.

When we took a look at *Mucor*, these were found not dominated the soil mycobiota in both the control as well as in dye treated soils. It was only *Mucor heimalis* that was appeared in control soil with very low percentage while it showed a little tolerance against 100 ppm basic fuchsin treated soils with frequency of 0.91% and 0.84% in 100 and 250 ppm basic fuchsin dye treated soil respectively. The genus *Mucor* was completely absent in the petri plates of congo red dye treated soils after same period of treatment.

However the genus *Rhizopus* was represented by two species *R. oryzae* and *R. stolonifer*, only *Rhizopus oryzae* were found somewhat dominating the culture plates. Despite appeared in control soil with appreciable number of isolates (1.83%), it was occurred in the dye treated soils with few number of isolates. It was appeared only in 100 ppm basic fuchsin and 500 ppm basic fuchsine dye treated soils and also in100 ppm congo red dye treated soil.

From the discussion it is clear that the genus *Aspergillus*, *Penicillium* and *Trichoderma* were dominated the soil mycobiota of both type of soils *i.e.* control soil as well as in the soils treated with different concentrations of basic fuchsin and congo red dyes. It was also noticed that the *Aspergilli* and *Penicillia* were more dominant as compared to *Trichoderma*. Out of the *Aspergillus* species, *A. flavus*, *A. fumigatus* and *A. niger* were realized as highly tolerant isolates among all since these were present in almost all the soil samples treated with different concentrations of both the dyes whereas of the *Penicillia* the species *P. glabrum* had shown the greater tolerance to the dyes followed by *P. chrysogenum*.

After 30 days treatment a total of 35 species of 12 genera were recorded (Table 2). Of the 35 species, control soil contains 14 species of 5 genera whereas basic fuchsin and congo red dye treated soils contains 27 species (of 12 genera) and 17 species (of 9 genera) respectively. After 30 days seven species were found common in control and dye treated soils. These include *Aspergillus flavus*, *A. fumigatus*, *A. niger, Penicillium chrysogenum, Penicillium glabrum*, *Rhizopus oryzae* and *Gliocladium* sp. It can be easily said that dyes have made the conditions adverse to many fungal species since some genera were completely disappeared after 30 days of treatment as compare to those after 15 days. The soil was dominated by *Aspergillus, Penicillium* and *Mucor* species mainly. The control soil was dominated mainly by *A. flavus* (20%), *Penicillium chrysogenum* (18.09%) and *A. niger*(18.09%) followed by *Penicillium glabrum* (12.38%) and *Aspergillus fumigatus* (10.47%). The other isolated species were found each contributing near 4% or low in the control soil.

The species of *Aspergillus* and *Penicillium* again showed the dominance in the soils treated with different concentrations of basic fuchsin and congo red dyes. *P. glabrum* was found the most dominating species with 68.78% of the isolates in 100 ppm BF dye treated soil, 40.38 % in 500 ppm CR dye-treated soil, 33.33 % of the isolates in 250 ppm CR dye-treated soil and 10.65% of the isolates in 250 ppm BF dye-treated soil after 30th day, followed by *Penicillium chrysogenum* (29.87% of the isolates in 500 ppm BF dye-treated soil), *Aspergillus fumigatus* (26.31% in 100 ppm CR dye treated soil), *Aspergillus niger* (23.07% of the isolates in 500 ppm CR dye-treated soil), *Aspergillus flavus* (19.23% of the isolates in 500 ppm CR dye-treated soil) and *Trichoderma reesei*(13.61% of the isolates in 250 ppm BF dye treated soil). The isolates of these species were also reported sporadic in different samples of BF and CR dye treated soils. Other species contributed as much as 8 to 9 % of the isolates in all the dye treated soils.

After 45 days treatment a total of 35 species of 14 genera were recorded (Table 3). Out of these 35 species the control soil contain 12 species of 5 genera whereas the BF and CR dye treated soils contain 23 species (10 genera) and 18 species (8 genera) respectively. It can be easily said that dyes have made the conditions less adverse as compare to those after 30 days, some species reappeared. The dyes treated soil was dominated by *Penicillium* species followed by *Aspergillus, Fusarium* and *Trichoderma* species mainly. The control soil was dominated mainly by *A. niger* (26.15%), *A. fischeri* (18.46%) and *Trichoderma lignorum* (18.46%) followed by *Aspergillus candidus* (8.46%) and *Gliocladium* sp. (7.69%).

Table 1: Mycoflora after 15 Days Treatment of Distilled water (Control) and dyes (Basic Fuchsine and Congo Red) TI= Total Isolates, PI= Percentage of Isolates

Quantitative and qualitative distribution of Mycobiota in soil– control as well as treated with 100ppm, 250ppm and 500ppm concentration of Basic Fuchsin and Congo Red over a period of 45 days obtained by dilution plate method.

Fungal Species	*Control*		*Basic Fuchsine*						*Congo Red*					
		100 ppm		*250 ppm*		*500 ppm*		*100 ppm*		*250 ppm*		*500 ppm*		
	TI	*PI*	*TI*	*PI*	*TI*	*PI*	*TI*	*PI*	*TI*	*PI*	*TI*	*PI*	*TI*	*PI*
Alternaria alternata	10	3.05	2	1.83	3	2.01	1	0.84	–	–	–	–	–	–
Alternaria brassicicola	–	–	–	–	–	–	2	1.69	–	–	–	–	–	–
Arthrinium euphorbiae	–	–	–	–	–	–	–	–	–	–	2	3.44		
Aspergillus candidus	3	0.91	–	–	–	–	–	–			3	5.17		
Aspergillus fischeri	21	6.42	–	–	–	–	1	0.84	23	27.71	–	–	–	–
Aspergillus flavus	28	8.56	8	7.34	22	14.76	7	5.93	3	3.61	4	6.89	3	11.11
Aspergillus fumigatus	6	1.83	22	18.80	–	–	68	57.62	–	–	11	18.96	4	14.81
Aspergillus humicola	–	–					6	5.08	–	–	–	–	–	–
Aspergillus nidulans	–	–			2	1.34	1	0.84	–	–	–	–	–	–
Aspergillus niger	18	5.50	16	13.67	16	10.74	3	2.54	13	15.66	3	5.17	2	7.41
Aspergillus ochraceous	5	1.52	–	–	–	–	–	–	2	2.41	4	6.89	–	–
Aspergillus sydowi	1	0.31	–	–	–	–	–	–	–	–	–	–	–	–
Aspergillus tamari	9	2.75	–	–	–	–	–	–	3	3.61	1	1.72	3	11.11
Aspergillus terreus	15	4.58	1	0.91	4	2.68	1	0.84	–	–	–	–	–	–
Aspergillus ustus	3	0.91	2	1.83	–	–	–	–	–	–	–	–	–	–
Candida albicans	–	–	–	–	–	–	–	–	1	1.20	–	–	–	–
Cladosporium cladospoiroides	–	–	–	–	–	–	–	–	1	1.20	–	–	–	–
Cladosporium herbarum	13	3.97	–	–	4	2.68	–	–	–	–	–	–	–	–
Cladosporium oxysporum	–	–	–	–	1	0.67	–	–						
Colletotrichum sp	–	–	–	–	–	–	–	–	1	1.20	–	–	–	–
Curvularia clavata	3	0.91	–	–	–	–	–	–	–	–	–	–	–	–
Curvularia lunata	12	3.67	–	–	2	1.34	2	1.69	–	–	–	–	–	–
Fusarium moniliforme	–	–	–	–	–	–	3	2.54	–	–	–	–	–	–
Fusarium oxysporum	19	5.81	3	2.75	21	14.09	–	–	–	–	–	–	–	–
Fusarium poae	5	1.52	–	–	4	2.68	–	–	1	1.20	–	–	02	7.41
Fusarium sp.	8	2.44	–	–	3	2.01	–	–	–	–	–	–	–	–
Geotrichum candidum	12	3.67	1	0.91	9	6.04	2	1.69	–	–	–	–	–	–

Gliocladium sp.	10	3.05	02	1.83	–	–	03	2.54	–	–	–	–	–	–
Humicola grisea	–	–	–	–	–	–	1	0.84	–	–	–	–	–	–
Microsporum sp.	–	–	1	0.91	–	–	–	–	–	–	–	–	–	–
Mucor fragilis	7	2.14	–	–	–	–	01	0.84	–	–	–	–	–	–
Mucor heimalis	1	0.31	1	0.91	–	–	01	0.84	–	–	–	–	–	–
Mucor plumbeus	–	–	–	–	–	–	–	–	05	6.02	–	–	–	–
Mucor racemosus	–	–	–	–	–	–	3	2.54	–	–	–	–	–	–
Penicillium chrysogenum	17	5.19	–	–	–	–	–	–	1	1.20	3	5.17	–	–
Penicillium citrinum	3	0.91	–	–	1	0.67	–	–	–	–	–	–	–	–
Penicillium digitatum	–	–	–	–	–	–	1	0.84	2	2.41	6	10.34	–	–
Penicillium glabrum	61	18.65	23	21.10	56	37.58	5	4.23	–	–	04	6.89	–	–
Penicillium spinulosum	–	–	–	–	–	–	–	–	–	–	–	–	5	18.51
Penicillium sp.	–	–	–	–	1	0.67	–	–	–	–	–	–		–
Pithomyces medicus	–	–	–	–	–	–	2	1.69	–	–	–	–	–	–
Pythium elongatum	–	–	–	–	–	–	–	–	–	–	–	–	1	3.70
Pythium middletonii	–	–	–	–	–	–	–	–	5	6.02	–	–	1	3.70
Pythium sp.	–	–	–	–	–	–	–	–	1	1.20	1	1.72	1	3.70
Rhizopus oryzae	6	1.83	1	0.91	–	–	4	3.39	1	1.20	–	–	–	–
Rhizopus stolonifer	–	–	–	–	–	–	–	–	2	2.41	2	3.45	–	–
Rhizopus sp.	1	0.31	–	–	–	–	–	–	–	–	–	–	–	–
Stachybotrys parvispora	–	–	–	–	–	–	–	–	–	–	–	–	1	3.70
Trichoderma atroviride	–	–	–	–	–	–	–	–	–	–	–	–	1	3.70
Trichoderma citrinoviride	–	–	–	–	–	–	–	–	–	–	–	–	3	11.11
Trichoderma lignorum	–	–	26	23.85	–	–	–	–	17	20.48	3	5.17	–	–
Trichoderma ressei	–	–	–	–	–	–	–	–	1	1.20	10	17.24	–	–
Trichoderma viride	22	6.72	–	–	–	–	–	–	–	–	–	–	–	–
Trichoderma sp.	8	2.44	–	–	–	–	–	–	–	–	–	–	–	–
No. of Species	28	–	14	–	15		21	–	18	–	15	–	12	–
Total Isolates	327	–	109	–	149	–	118	–	83	–	58	–	27	–
Shannon Diversity index (H)	2.8254		1.589		1.959		1.825		1.591		2.452		2.322	
Simpson's Diversity index (D)	1.366		6.0459		4.975		2.901		6.105		9.785		9.018	

Table 2: Mycoflora after 30 Days Treatment of Distilled water (Control) and dyes (Basic Fuchsine and Congo Red) TI= Total Isolates, PI= Percentage of Isolates

Quantitative and qualitative distribution of Mycobiota in soil– control as well as treated with 100ppm, 250ppm and 500ppm concentration of Basic Fuchsin and Congo Red over a period of 30 days obtained by dilution plate method.

Fungal Species	*Control*		*Basic Fuchsin*						*Congo Red*					
			100 ppm		*250 ppm*		*500 ppm*		*100 ppm*		*250 ppm*		*500 ppm*	
	TI	*PI*	*TI*	*PI*	*TI*	*PI*	*TI*	*PI*	*TI*	*PI*	*TI*	*PI*	*TI*	*PI*
Alternaria alternata	–	–	02	0.97	07	4.14	02	2.59	–	–	–	–	–	–
Alternaria brassicicola	–	–	08	3.90	16	9.46	–	–	–	–	–	–	–	–
Arthrinium euphorbiae	–	–	–	–	12	7.10	–	–	–	–	–	–	–	–
Aspergillus candidus	02	1.90	–	–	–	–	–	–	–	–	–	–	–	–
Aspergillus fischeri	05	4.76	–	–	–	–	–	–	–	–	05	9.26	–	–
Aspergillus flavus	21	20	14	6.83	10	5.92	05	6.49	10	17.54	17	31.48	10	19.23
Aspergillus fumigatus	11	10.47	09	4.39	40	23.67	19	24.67	15	26.31	01	1.85	04	7.69
Aspergillus humicola	–	–	02	0.97	–	–	–	–	01	1.75	–	–	–	–
Aspergillus nidulans	02	1.90	06	2.92	–	–	01	1.29	–	–	–	–	–	–
Aspergillus niger	19	18.09	07	3.41	08	4.73	07	9.09	06	10.52	08	14.81	12	23.07
Aspergillus ochraceous	–	–	–	–	–	–	–	–	02	3.51	–	–	–	–
Aspergillus tamarii	–	–	–	–	–	–	–	–	01	1.75	–	–	01	1.92
Aspergillus terreus	–	–	–	–	–	–	01	1.29	–	–	–	–	–	–
Aspergillus ustus	01	0.95	–	–	–	–	3	3.89	–	–	–	–	–	–
Aspergillus sp.	–	–	–	–	01	0.59	–	–	–	–	–	–	–	–
Curvularia lunata	–	–	03	1.46	02	1.18	–	–	–	–	–	–	–	–
Fusarium moniliforme	01	0.95	–	–	–	–	–	–	–	–	–	–	–	–
Fusarium oxysporum	–	–	04	1.95	09	5.32	–	–	03	5.26	–	–	–	–
Fusarium sp.	–	–	–	–	–	–	01	1.29	–	–	–	–	–	–
Geotrichum candidum	–	–	03	1.46	–	–	01	1.29	–	–	–	–	–	–
Gliocladium sp.	03	2.86	–	–	–	–	01	1.29	–	–	–	–	01	1.92
Mucor fragilis	–	–	–	–	03	1.77	–	–	–	–	–	–	–	–
Mucor heimalis	–	–	03	1.46	01	0.59	01	1.29	–	–	–	–	–	–
Mucor plumbeus	–	–	–	–	–	–	–	–	02	3.51	–	–	–	–
Mucor racemosus	–	–	–	–	02	1.18	–	–	–	–	–	–	–	–

Penicillium chrysogenum	19	18.09	–	–	07	4.14	23	29.87	01	1.75	–	–	–	–
Penicillium citrinum	01	0.95	01	0.48	–	–	–	–	–	–	–	–	–	–
Penicillium glabrum	13	12.38	141	68.78	18	10.65	04	5.19	–	–	18	33.33	21	40.38
Penicillium sp.	04	3.81	01	0.48	–	–	–	–	–	–	–	–	–	–
Pythium sp.	–	–	–	–	–	–	–	–	04	7.01	01	1.85	02	3.84
Rhizopus oryzae	03	2.86	01	0.48	–	–	08	10.39	01	1.75	02	3.70	01	1.92
Rhizopus stolonifer	–	–	–	–	02	1.18	–	–	–	–	–	–	–	–
Trichoderma lignorum	–	–	–	–	08	4.73	–	–	05	8.77	02	3.70	–	–
Trichoderma ressei	–	–	–	–	23	13.61	–	–	06	10.52	–	–	–	–
No. of Species	14	–	15	–	17		14	–	13	–	08	–	08	–
Total Isolates	105	–	205	–	169	–	77	–	57	–	54	–	52	–
Shannon Diversity index (H)	2.180		1.324		2.403		1.5501		2.018		1.619		1.5669	
Simpson's Diversity index (D)	7.251		2.069		8.766		5.628		7.111		4.111		3.8392	

Table 3: Mycoflora after 45 Days Treatment of Distilled water (Control) and dyes (Basic Fuchsine and Congo Red). TI= Total Isolates, PI= Percentage of Isolates

Quantitative and qualitative distribution of Mycobiota in soil– control as well as treated with 100ppm, 250ppm and 500ppm concentration of Basic Fuchsin and Congo Red over a period of 45 days obtained by dilution plate method.

Fungal Species	*Control*		*Basic Fuchsine*						*Congo Red*					
			100 ppm		*250 ppm*		*500 ppm*		*100 ppm*		*250 ppm*		*500 ppm*	
	TI	*PI*	*TI*	*PI*	*TI*	*PI*	*TI*	*PI*	*TI*	*PI*	*TI*	*PI*	*TI*	*PI*
Alternaria alternata	–	–	01	0.39	14	9.72	–	–	–	–	–	–	–	–
Alternaria brassicicola	–	–	–	–	21	14.58	–	–	–	–	–	–	–	–
Arthrinium euphorbiae	–	–	–	–	–	–	–	–	–	–	01	2.08	–	–
Aspergillus candidus	11	8.46	–	–	–	–	–	–	–	–	–	–	–	–
Aspergillus fischeri	24	18.46	04	1.58	–	–	–	–	–	–	03	6.25	07	14.58
Aspergillus flavus	09	6.92	27	10.71	07	4.86	04	5.13	08	17.02	05	10.41	–	–
Aspergillus fumigatus	–	–	58	23.01	61	42.36	14	17.95	14	29.78	03	6.25	–	–
Aspergillus humicola	02	1.54	–	–	–	–	–	–	–	–	–	–	–	–
Aspergillus nidulans	–	–	–	–	01	0.69	–	–	–	–	–	–	–	–

Aspergillus niger	34	26.15	26	10.31	08	5.55	21	26.92	04	8.51	03	6.25	16	33.33
Aspergillus ochraceous	1	0.77	01	0.39	–	–	–	–	–	–	–	–	01	2.08
Aspergillus tamarii	–	–	02	0.79	–	–	–	–	01	2.12	17	35.41	–	–
Aspergillus terreus	7	5.38	03	1.19	01	0.69	–	–	–	–	–	–	–	–
Aspergillus ustus	–	–	07	2.77	01	0.69	–	–	–	–	–	–	–	–
Beltrania rhombica	–	–	–	–	–	–	–	–	–	–	01	2.08	–	–
Chaetomium globosum	–	–	02	0.79	–	–	–	–	–	–	–	–	–	–
Cladosporium herbarum	–	–	–	–	–	–	02	2.56	–	–	–	–	01	2.08
Curvularia clavata	–	–	03	1.19	01	0.69	–	–	–	–	–	–	–	–
Curvularia lunata	–	–	03	1.19	09	6.25	–	–	–	–	–	–	01	2.08
Fusarium moniliforme	–	–	01	0.39	01	0.69	–	–	–	–	–	–	–	–
Fusarium oxysporum	–	–	11	4.36	01	0.69	–	–	–	–	–	–	05	10.41
Fusarium poae	–	–	–	–	–	–	–	–	–	–	–	–	02	4.16
Fusarium sp.	–	–	–	–	01	0.69	–	–	–	–	–	–	–	–
Gliocladium sp.	10	7.69	–	–	01	0.69	–	–	–	–	–	–	–	–
Mucor heimalis	–	–	04	1.58	–	–	08	10.25	–	–	–	–	–	–
Mucor varians	01	0.77	–	–	–	–	–	–	–	–	–	–	–	–
Penicillium chrysogenum	1	0.77	–	–	–	–	14	17.95	–	–	–	–	02	4.16
Penicillium digitatum	06	4.61	–	–	–	–	–	–	01	2.12	–	–	01	2.08
Penicillium glabrum	–	–	98	38.89	17	11.80	06	7.69	15	31.91	14	29.16	01	2.08
Pythium middletonii	–	–	–	–	–	–	–	–	–	–	–	–	01	2.08
Pythium sp.	–	–	–	–	–	–	–	–	–	–	–	–	01	2.08
Rhizopus oryzae	–	–	–	–	–	–	09	11.54	–	–	–	–	–	–
Trichoderma harzianum	–	–	–	–	–	–	–	–	–	–	–	–	09	18.75
Trichoderma lignorum	24	18.46	–	–	–	–	–	–	04	8.51	–	–	–	–
Trichoderma ressei	–	–	–	–	–	–	–	–	–	–	01	2.08	–	–
No. of Species	12	–	16	–	15		08	–	07	–	09	–	13	–
Total Isolates	130	–	252	–	144	–	78	–	47	–	48	–	48	–
Shannon Diversity index (H)	2.025		1.686		1.843		1.890		1.607		1.714		1.711	
Simpson's Diversity Index (D)	6.30		4.366		4.295		1.340		4.269		4.281		6.703	

ANOVA for Table 1

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	5935.333333	53	111.9874214	3.089640724	4.36387E-10	1.380984259
Within Groups	1068.026455	6	178.0044092	4.910995046	8.05374E-05	2.127128348
Error	11526.25926	318	36.2460983			
Total	18529.61905	377				

ANOVA for Table 2

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between groups	8315.752101	33	251.9924879	2.942294341	1.86067E-06	1.495429486
Within Groups	663.4537815	6	110.5756303	1.291094246	0.262883794	2.144597218
Error	16957.68908	198	85.64489432			
Total	25936.89496	237				

ANOVA for Table 3

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	7199.697959	34	211.7558223	3.035477339	6.39295E-07	1.487210154
Within Groups	974.6122449	6	162.4353741	2.328478584	0.033868478	2.143230815
Error	14231.10204	204	69.76030412			
Total	22405.41224	244				

The other isolated species were found contributing near 4 to 6 % or low.

The mycobiota after 45 days was found to have four common species of the only genus *Aspergillus* in control soil and both the dye treated soils. These include *Aspergillus fischeri*, *A. flavus*, *A. niger* and *A. ochraceous*. The mycobiota after 45 days was dominated by *Aspergillus fumigatus*. The species contributed 42.36% of the isolates in 250 ppm BF dye treated soil followed by *P. glabrum* with 36.89% of the isolates in 100 ppm BF dye treated soil, *Aspergillus tamarii* 35.41% of the isolates in 250 ppm CR dye treated soil and *Aspergillus niger* with33.33% of the isolates in 500 ppm CR dye treated soil. Also the appearance of *Aspergillus flavus* was not negligible. The species accounted for 17.02%, 10.71 % and 10.41% of the isolates in 100 ppm CR, 100 ppm BF and 250 ppm CR dye treated soils respectively. However, the species *Alternaria brassicicola* also found dominating the petri plates in 250 ppm BF dye treated soil it was recorded neither from control soil nor from any other dye treated soils after 45 days.

Analysis of variance (ANOVA) has revealed that the treatment with basic fuchsin and congo red dyes had significant negative effect on the qualitative as well as quantitative distribution of fungi in the soil after 15 days (F= 3.08, significant at 0.05 level). Adverse effects of BF and CR dyes on mycodiversity became remarkable as the duration of the treatment increased though this was found to be statistically significant, and hence, results also yielded significant negative effect (F= 2.94; significant at 0.05 level) from 30 days dye treated soil and from 45 days dye treated soil (F= 3.03at 0.05 level). This variation in mycobiota of control soil and dye treated soils over 45 days is also confirmed by the Simpson's and Shannon's diversity indices. The variation in mycobiota of different soils after every treatment periods has also became cleared by diversity indices.

In the present study sought clearly that dyes have significant negative effect of soil mycobiota. The findings revealed that many of the fungal species were not able to tolerate the adverse conditions created by dye treatment. However the mycobiota was not so affected by dye treatments after 15 days the frequencies of fungal isolates was found reduces with increasing treatment periods as well as with increasing dye concentrations. Since many species and genera were disappeared from BF and CR dye treated soils after 30 and 45 days treatments, the species *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger* and *Penicillium glabrum* were found most tolerant among all. Usha *et al.* (2011) confirmed the tolerance capacity of *Aspergillus fumigatus*, *A. niger* and *A. terreus* by protein profiling of the isolated fungal species from the soil polluted with paper and pulp effluent. The revealed high tolerance capacity of *Aspergillus* species in the study is in full agreement with the present work. They had done the analysis of physico-chemical properties of the soil and concluded that the alkaline nature of soil caused by paper and pulp mill effluent can make the interference in stress adaptation of fungi for survival. Some other studies conducted are also evident for

the tolerance capacity of these isolated species towards the dyes and other kind pollution (Salar *et al.* 2012, Kumar and Charaya2012,Shahid*et al.* 2013, Franklin *et al.* 2013,Kaur and Arya 2015,Tyagi and Charaya 2017,Sani and Abdullahi 2017, Ramdass and Rampersad 2021). It is obvious that *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus* and *Penicillium glabrum* isolated from different dye treated soils have evolved panoply of adaptations which includes mechanisms for producing different enzymes and chemicals to overcome competition with other soil organisms and surviving harsh environmental conditions. The present study also depicted that the mycobiota has significantly reduced under the increasing treatment periods and dye concentrations. The results are also confirmed by ANOVA and diversity indices between fungal species and number of isolates in different types of soils (control and dye treated soils). From the above observations it is clear that there is a need to understand the systematic diversity of fungi with the accumulation of dyes and/or other pollutant loads through monitoring.

Conclusion

This study indicated that the mutualistic symbiotic relationship of fungi with soil environment can lead to a sustainable approach for restoration of contaminated soil and water. Some tolerant species of fungi used dyes as their nutrition source, and hence the degradation process can contribute to dye's detoxification. The comparison of different mycobiota isolated from different treatment periods of BF and CR dye treated soils also depicted that the soil mycobiota has potential to withstand dye contaminated conditions. These fungi can degrade not only the dyestuffs but also reduce the toxicity of industrial wastewater.

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