EnvironmentAsia
The international journal published by the Thai Society of Higher Education Institutes on Environment

Available online at www.tshe.org/ea/index.html
EnvironmentAsia 10(2) (2017) 147-161
DOI

Mycoremediation of Textile Dyes: Application of Novel Autochthonous Fungal Isolates

Sweety [a, b], Sharad Vats [b], Manoj Kumar [c], Shivesh Sharma [d], Vivek Kumar [e] and Shri K. Garg [a]

[a] *Division of Biosciences, College of Applied Education and Health Sciences, Meerut, India*
[b] *Department of Bioscience & Biotechnology, Banasthali University, Banasthali, Rajasthan, India*
[c] *Amity Institute of Microbial Technology, Amity University Uttar Pradesh, Noida, India*
[d] *Department of Biotechnology, MLN National Institute of Technology, Allahabad, India*
[e] *Department of Microbiology, Himalayan School of Biosciences, Swami Rama Himalayan University, Swami Ram Nagar, Jollygrant, Dehradun, India*

Abstract

Four fungal isolates *Trichoderma virens, Phlebiopsis cf. ravenelii, Talaromyces stipitatus, Aspergillus niger* originally isolated from the textile dye contaminated soil of Meerut (U.P). India. They were used for the decolorization studies of selected textile azo dyes under laboratory conditions. Out of total 74 isolates, selected four fungal strains were picked on the basis of primary screening carried out using agar layer decolorization method. Decolorization efficiency of textile dyes was studied at an interval of 3, 5, 7 and 9 days at temperatures 20, 25, 30 and 40°C using five synthetic dyes viz. Xylene cynol FF, Brilliant blue R, Aniline Blue, Orange G II and Crystal violet. Decolorization study was carried out under shaking and stationary conditions at pH 4.0, 5.4, 6.5, and 8.0. The results obtained showed that *Trichoderma virens* and *Aspergillus niger* were more efficient then *Phlebiopsis cf. ravenelii* and *Talaromyces stipitatus.* Highest biodegradation activities of dyes by these aboriginal fungal isolates were observed at pH 5.4 after 9 days of incubation. Maximum decolorization 99.84 % was achieved by *Aspergillus niger*, followed by *Trichoderma virens*. This is the first report where the bioremediation aspects of *Phlebiopsis cf. ravenelii* and *Talaromyces stipitatus* has been revealed.

Keywords: bioremediation; decolorization; native fungi; textile dyes

1. Introduction

Dyes may be defined as chemicals that, when applied to a substrate provides considerable coloring capacity and are widely employed in the textile, pharmaceutical, food, cosmetics, plastics, photographic and paper industries (Zollinger, 1987; Carneiro *et al*., 2007). The rapid growth of industries and urban development has led to the production of various types of synthetic chemicals, including textile dyes, which are being employed in routine life (Ali and El-Mohamedy, 2012). All over the world, more than 7×10^5 metric tons of man-made dyes are manufactured, out of it; due to inefficient processing methods, nearly 10-15 % of non-utilized dyes are poured into the wastewater channels after fabric dyeing and washing processes (Rajamohan and Karthikeyan, 2006).

Regular entry of dyes in wastewater channels repeatedly leads to surface and groundwater contamination in adjoining area. Accordingly, utilization of these waters may cause great health hazard (Arlt *et al*., 2002). Such as, an occurrence of bladder tumors has been frequently reported among dye industry workers compared to the general population (Suryavathi *et al*., 2005). The toxic effects of the azo dyes may result from the direct action of the agent itself or of the aryl amine derivatives generated during reductive biotransformation of the azo bond (Rajaguru *et al*., 1999). The azo dyes entering the body by ingestion can be metabolized to aromatic amines by the azoreductases of intestinal microorganisms. If the dyes are nitro, they can be metabolized by the nitroreductases produced by the same microorganisms (Umbuzeiro *et al*., 2005).

The synthetic dyes are difficult to remediate owing to their chemical structure. The reason behind is that the dyes are manufactured to resist change due to chemical and light fading (Nigam *et al*., 2000). Other factors which cause major hindrance in biological treatment of synthetic dyes are their high water solubility, high molecular weight and complex structure having fused aromatic ring, which causes the dyes to retain color for long duration and also prevent the permeation into biological cell membranes (Rani *et al*., 2014).

A variety of physicochemical treatments have been devised previously for the dyes and textile wastewater (Zaharia *et al*., 2011). However, these suffered from some serious drawbacks in terms of their limited applications or their high cost. Besides, chemical treatments created an additional chemical load in water bodies that eventually resulted in sludge disposal problems (Carneiro *et al*., 2007). Several factors determine the technical and economic feasibility of each single dye removal technique. These include; dye type and its concentration, wastewater composition, operation costs (energy and material), environmental fate and handling costs of generated waste products. A very small amount of dye in water (10-50 mg/L) is highly visible and reduces light penetration in water systems, thus causing a negative effect on photosynthesis (Cooper, 1993; Vandevivere *et al*., 1998).

Various treatment methods for removal of dyes from industrial effluents like chemical coagulation using alum, lime, ferric chloride, ferric sulphate and electro coagulation are very time consuming and costly with low efficiency. Among the numerous water treatment technologies, research interest in the fungal bioremediation due to their greater biomass compared to the bacteria, has increased significantly for decolorization and degradation of synthetic dyes (Ashoka *et al*., 2002; Kanmani *et al*., 2011; Shahid *et al*., 2013; Rani *et al*., 2014). Keeping the above in view, the main objectives of the problem were to screen and employ the selected potential aboriginal textile dye contaminated soil fungal species capable of decolorizing and detoxify the textile dyes using solid and liquid media. The reason for selecting aboriginal cultures was that the organism is well accustomed with contaminated environment.

2. Materials and Methods

2.1 Chemicals and media

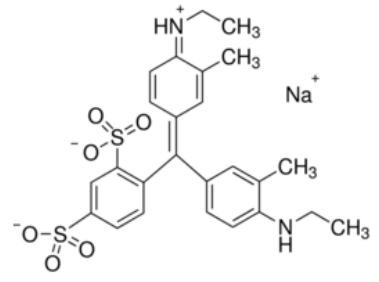
All the chemicals, dyes and media such as Potato Dextrose Agar (PDA) and Potato Dextrose broth (PDB) were procured from Himedia, Mumbai, India.

2.1.1 Dyes

The dyes used in this study were: Xylene cynol FF, Brilliant Blue R, Orange G, Aniline Blue and Crystal Violet, the commercial names, chemical structure and the maximum wavelength (λ max) of these dyes are illustrated as follows;

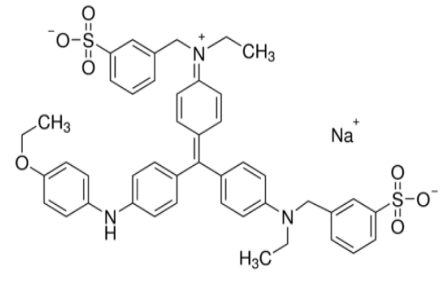
Xylene cynol FF

Sodium hydride-4-{(Z)-[4-(ethylamino)-3-methylphenyl] [(4E)-4-(ethylimino)-3-methyl-2, 5-cyclohexadien-1-ylidene] methyl}-6-hydroxy-1, 3-benzenedisulfonic acid (1:1:1), Molecular formula: $C_{25}H_{27}N_2NaO_6S$, λ max-615.



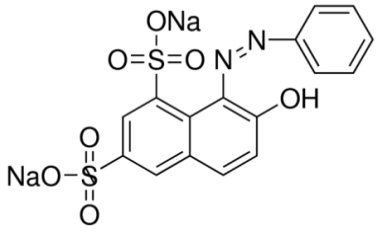
Brilliant Blue R

Chemical name: sodium;3-[[4-[[4-(4-ethoxyanilino) phenyl]-[4-[ethyl-[(3 sulfonatophenyl) methyl] azaniumylidene]cyclohexa-2, 5-dien-1-ylidene] methyl]-N-ethylanilino] methyl] benzenesulfonate, Molecular formula: $C_{45}H_{44}N_3NaO_7S_2$, λ max-595



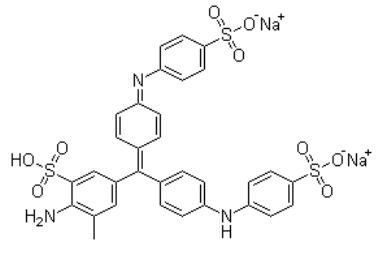
Orange G

Chemical Name: disodium; (8Z)-7-oxo-8-(phenylhydrazinylidene) naphthalene-1, 3-disulfonate, Molecular formula: $C_{16}H_{10}N_2Na_2O_7S_2$, λ max-476



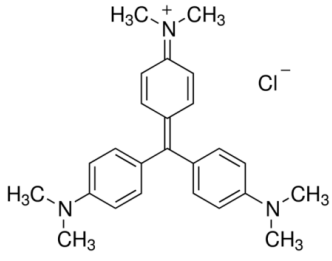
Aniline blue

Chemical Name: Disodium hydrogen aminomethyl [[4-[(sulphonatophenyl) amino] phenyl] [4-[(sulphonatophenyl) imino] cyclohexa-2,5-dien-1-ylidene] methyl] benzenesulphonate, Molecular formula: $C_{32}H_{25}N_3O_9S_{3\text{-}2}Na$, λ max-595



Crystal violet

Chemical Name: Tris (4-(dimethylamino)phenyl) methylium chloride, Molecular formula: $C_{25}N_3H_{30}Cl$, λ max-585



2.1.2 Preparation of dye solution

The stock solutions of dyes ranging from (0.01% - 0.1%) were prepared by dissolving weighed dyes in distilled water for further studies.

2.2 Sources of fungal isolates

Out of 74, four fungal isolates *Trichoderma virens, Phlebiopsis cf. ravenelii, Talaromyces stipitatus*, *Aspergillus niger* were isolated from the textile dye contaminated sites of Meerut (U.P). Identification of these soil fungal strains was done previously based on their morphological characters (Yao *et al*., 2009), which were further confirmed by molecular studies.

2.3 Screening of fungi for dye decolorization activities

Seventy four dye contaminated soil fungal strains were screened for their ability to degrade dyes using the tube overlay method (Shahid *et al*., 2013). Initially, the fungal strains were grown on culture plates pre filled with Potato Dextrose Agar (PDA) and incubated at room temperature for 14 days. Following incubation, mycelial agar plugs (~5 mm^2) were cut approximately 5 mm from the colony margin and inoculated on test tubes (in triplicates) containing 5 mL of PDA overlaid with 1 mL of PDA with 0.01% (w/v) respective textile dye. All culture tubes were incubated at room temperature (~25°C) and observed weekly for up to four weeks. Clearing of the overlaid dye indicates full decolorization (+++). Partial dye decolorization (++) was indicated by less dye intensity in comparison with the control (uninoculated PDA overlaid with PDA + 0.01% dye). All the four fungal strains were selected on the basis of full or maximum (+++) decolorization.

2.4 Decolorization of dyes in solid medium (Tube overlay method)

The four selected dye contaminated soil fungal isolates were further tested for their ability to decolorize dyes on PDA and Sabouraud Dextrose Agar (SDA) medium, Himedia, Mumbai, India. This was done to select which medium support better growth and dye decolorization activities of selected fungal isolates. Initially, all the three fungal strains were grown as previously described. Following incubation, fungal mycelial agar plugs (~5 mm^2) were cut approximately 5 mm from the colony margin and inoculated on test tubes (in triplicates) each pre-filled with 2 ml of the Potato Dextrose Agar (PDA) medium, supplemented separately with either with following dye 0.01 % (w/v) xylene cynol FF, brilliant blue R, orange G, Aniline blue, crystal violet, respectively (López *et al*., 2006). The culture tubes were then incubated at room temperature (~ 25°C). The growth of the fungi and its ability to decolorize the dye were observed weekly up to four weeks. The depth of dye decolorization (in mm) indicated by clearing of the dye was then measured. Data of this study is not mentioned; rather this method was used to finally screen the fungal strains for further studies.

2.5 Assay for the dye decolorization activities of fungi in liquid media

The spores and mycelia were then dislodged using a flame sterilized inoculating loop. Then, 10 µL of the inoculum were added on culture vials (in triplicates) pre filled with 25 mL Potato Dextrose Broth (PDB) supplemented with 0.01% of either one of the following dyes: xylene cynol FF (XCFF), brilliant blue R (BBR), orange G-II (OG II), Aniline blue (AB), crystal violet (CV). Three sets were prepared and were incubated either under constant agitation/shaking (100 rpm, Yorko Scientific Orbital Shaker) or under stationary/without shaking condition (Rani *et al*., 2014). All culture vials were incubated at temperatures 20, 25, 30 and 40°C for 9 days and all assays were performed in triplicate. Growth and dye decolorization were noted every day. Following culture for 9 days, the culture filtrates were decanted and subjected to spectrophotometric analysis. Absorbance maxima of the tested dyes were read and the extent of dye decolorization by the soil fungal strains on liquid media was calculated using the formula below:

$$\text{Percent dye decolorization (\%)} = \frac{\text{Absorbance control} - \text{Absorbance inoculated} \times 100}{\text{Absorbance control}}$$

Finally, the mycelial biomass were harvested on clean Petri plates and observed directly and observed under a microscope for the biosorption of dyes.

2.6 Laccase enzyme assay (Guaiacol assay method)

Guaiacol (2 mM) in sodium acetate buffer (10 mM pH 5.0) was taken as substrate for laccase assay. The intense brown color development due to oxidation of guaiacol by laccase was correlated to its activity often read at 450 nm. The reaction mixture contained 3ml acetate buffer, 1ml guaiacol and 1ml enzyme source and enzyme blank contained 1ml of distilled water instead of enzyme source was incubated at 30ºC for 15 min and absorbance was read at 450 nm blank using UV spectrophotometer. Enzyme activity was expressed as International Units (IU), where 1 IU is defined as amount of enzyme required to oxidize 1micromole of guaiacol per min. The laccase activity in U/ml is calculated using the extinction coefficient of guaiacol (12,100 M-1 cm -1) at 450 nm by the formula: E.A = (A* V) / (t * e * v), where E.A = Enzyme Activity (U/ml), A = Absorbance at 450nm, V = Total volume of reaction mixture (ml), v = enzyme volume (ml), t = Incubation time (min) and e = Extinction Coefficient (M-1 cm -1) (Bourbonnais *et al*., 1998). The four selected fungal isolates exhibited laccase activity.

2.7 Seed germination bioassay

Effect of bioremediated and untreated dye solution was observed on wheat seed germination. The wheat seeds were sterilized using 0.1 % $HgCl_2$ solution for 50 sec, washed 6-7 times with sterile distilled water to remove traces of $HgCl_2$. In sterile Petri plates sterile filter paper was kept soaked in bioremediated, untreated dye solution and with sterile distilled water soaked filter paper as control, respectively. Ten wheat seeds were kept in each Petri plate and the experiment was conducted in triplicate. Observation on seed germination was taken for eight days.

2.8 Seed germination bioassay in pots

The mustard seeds were grown in pots under greenhouse conditions. Appropriate moisture level was maintained in pot soil using treated (bioremediated) and untreated dye solutions, tap water was used as control.

2.9 Bacterial toxicity

Effect of bioremediated and untreated dye solution was observed on bacterial growth by measuring zone of inhibition. Log phase cells of *E. coli*, 0.1 ml of 10^{-8} were evenly spreaded on Petri plates and sterile filter paper discs impregnated with bioremediated, untreated dye solution and sterile distilled water were kept on the seeded bacterial cells at equidistance and pressed lightly and kept at 30°C for 48 h, observation for zone of inhibition was observed, if any (Kumar, 2011).

3. Results

The dyes evaluated in the present study were chosen as representatives of dyes widely used commercially by the textile industry. Dye decolorization studies were carried out till 9 days; observations were taken on 3rd, 5th, 7th and 9th day. Maximum decolorization was observed on 7th day in all 5 studied dyes, however on 9th day slightly more decolorization (1-2%) was observed. Among all fungus strains studied, *Aspergillus niger* emerged as potential decolorizer as it exhibited maximum decolorization on 5th day compared to other fungus.

Out of 74 soil fungal isolates, four fungal isolates were selected after comprehensive screening process using selected textile dyes biodegradation process for further studies. The decolorization of textile dyes were achieved by inoculating the dye solution by *Trichoderma virens, Phlebiopsis cf. ravenelii, Talaromyces stipitatus, Aspergillus niger*. Using the technique of tube overlay method the screening of fungal isolates was carried out using five textile dyes. It was observed that fungal isolate *Phlebiopsis cf. ravenelii* showed maximum decolorization in BBR (Brilliant Blue R) followed by AB (Aniline Blue) , XCFF (Xylene cynol FF) and CV (Crystal Violet). Minimum decolorization was observed by OGII (Orange G II). Similar results were obtained with *Aspergillus niger*. On the other hand *Trichoderma virens* exhibited little variation in decolorization, here the maximum decolorization was observed in Aniline Blue followed by Brilliant Blue R and crystal violet. The decolorization of Orange GII and Xylene cynol FF was almost similar. Interestingly fungal isolate *Talaromyces stipitatus* also exhibited similar results.

Dye decolorization in liquid medium displayed various inclinations which were almost comparable or similar to that of tube overlay method (keeping brevity in mind, data of tube overlay is not mentioned). In this method highest decolorization was performed by *Phlebiopsis cf. ravenelii* in BBR (99.77%) followed by AB (98.63%), XCFF (60.1%), CV (52.56%) and least 29.54% in OGII under shaking condition. Similar trend was observed under stationary condition. (Fig. 1(a) and 1(b))

On the other hand *Aspergillus niger* exhibited highest decolorization (99.69%) in AB followed by BBR (99.56%), CV (79.61%), XCFF (78.03%) and least

in OGII (77.27%) under shaking condition. Interestingly this fungus exhibited slight variations under stationary condition, the highest decolorization (89.16%) was observed in BBR followed by AB (77.10%), OGII (59.45%), XCFF (58.23%) and (47.93%) in CV (Fig 2(a) and 2(b)).

Decolorization studies with *Trichoderma virens* showed maximum decolorization (99.6%) with dye BBR followed by AB (99.29%), XCFF (90.02%), CV (85.16%) and least (53.93%) in OG II under shaking condition. Under stationary condition slight differences were observed where the maximum decolorization (88.14%) was observed in XCFF followed by AB (80.81%) and least in OG II (50.14%) (Fig 3(a) and 3(b)).

With reference to *Talaromyces stipitatus* under shaking condition highest decolorization (99.56%) was observed in dye AB followed by BBR (99.33%), (53.64%) in CV, (30.46%) in OG II and least in XCFF (27.41%). Under stationary condition highest decolorization (79.82%) was observed in BBR followed by AB (74.86%) and trend for rest of the dyes were similar to shaking condition (Fig. 4(a) and 4(b)).

Effect of different temperature on decolorization of textile dyes under shaking and stationary condition by all four fungal isolates exhibited that highest decolorization was observed at 25ºC followed by 30ºC. This was observed using dye concentration

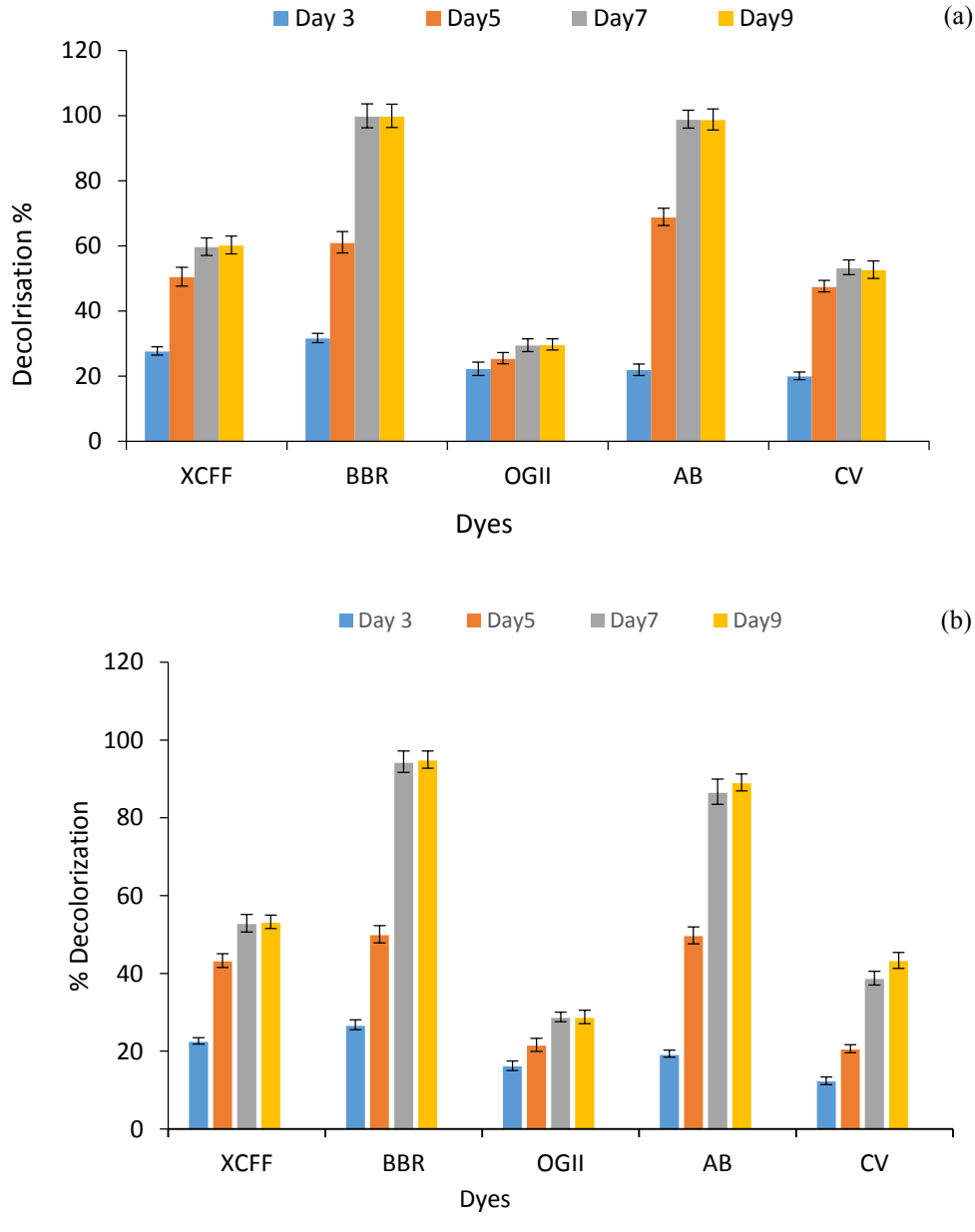


Figure 1. (a) Bioremediation of dyes (0.01%) with *Phlebiopsis cf. ravenelii* under shaking condition at pH 5.4 and 25°C at different time interval and (b) bioremediation of dyes (0.01%) with *Phlebiopsis cf. ravenelii* under stationary condition at pH 5.4 and 25°C at different time interval.

0.01%, here maximum decolorization was observed (98.83%) under shaking condition with dye AB. With increase in temperature and increasing dye concentration the rate of decolorization decreased, however still highest decolorization rate was observed at 25ºC (Figs. 5-8). Enzyme activity of tested fungal soil isolates was also observed, all the four fungal isolates exhibited laccase production.

The toxicity level of treated dye was also checked by wheat and mustard seed germination studies. It was observed that germination percentage was higher upto 90% by treated dye, while the untreated (control) dye inhibited the germination of wheat seeds (Fig. 9). Similar results were also obtained where mustard seeds were grown on pots under green house conditions (Fig. 10). Appropriate moisture level was maintained in pot soil using treated and untreated dye solutions. It was observed that in treated dye pot germination was early and plants height and health was better then untreated. Microbial bioassay study showed that untreated dye (control) inhibited the growth of *E. coli* on Pteriplates by forming a zone of inhibition, while the treated dye did not show any zone of inhibition (Fig. 11). Data of zone of clearnce and seed germination is not mentioned due to concision.

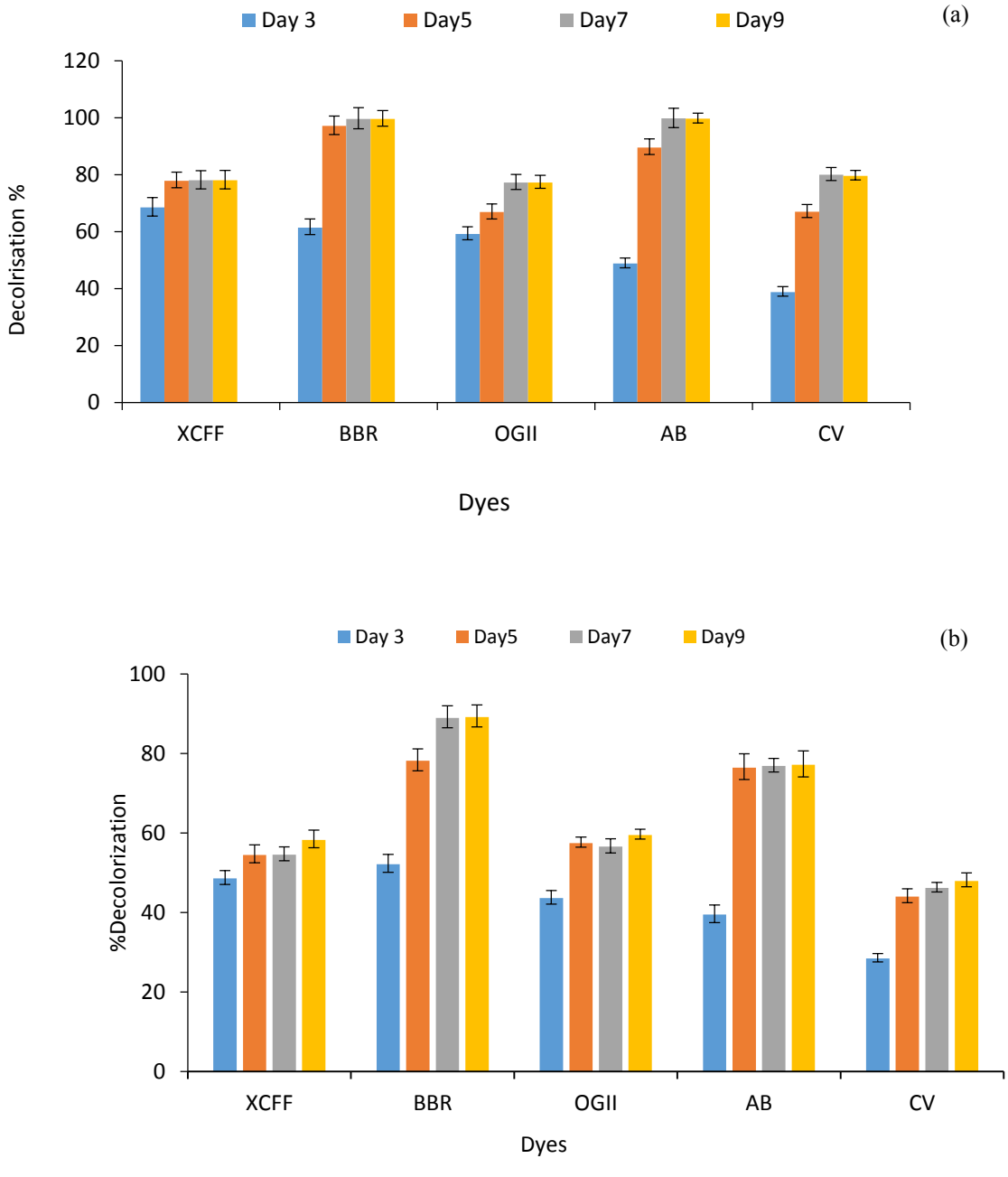


Figure 2. (a) Bioremediation of dyes (0.01%) with *Aspergillus niger* under shaking condition at pH 5.4 and 25°C in different time interval and (b) bioremediation of dyes (0.01%) with *Aspergillus niger* under stationary condition at pH 5.4 and 25°C in different time interval.

4. Discussion

Use of textile dye contaminated soil fungi, could offer a much inexpensive and competent alternative means of treating the heavily contaminated textile dyes wastewaters. Though, even the qualitative assays using tube overlay method are powerful tools in screening fungi for extracellular enzyme production, they are not conclusive in that a negative reaction is not an absolute confirmation of an organism inability to produce a particular enzyme (Abdel-Raheem and Shearer, 2002). Hence, the tube agar overlay method (López *et al*., 2006) only provides an easier and quicker method to screen a large number of fungal isolates for further studying dye decolorization activity.

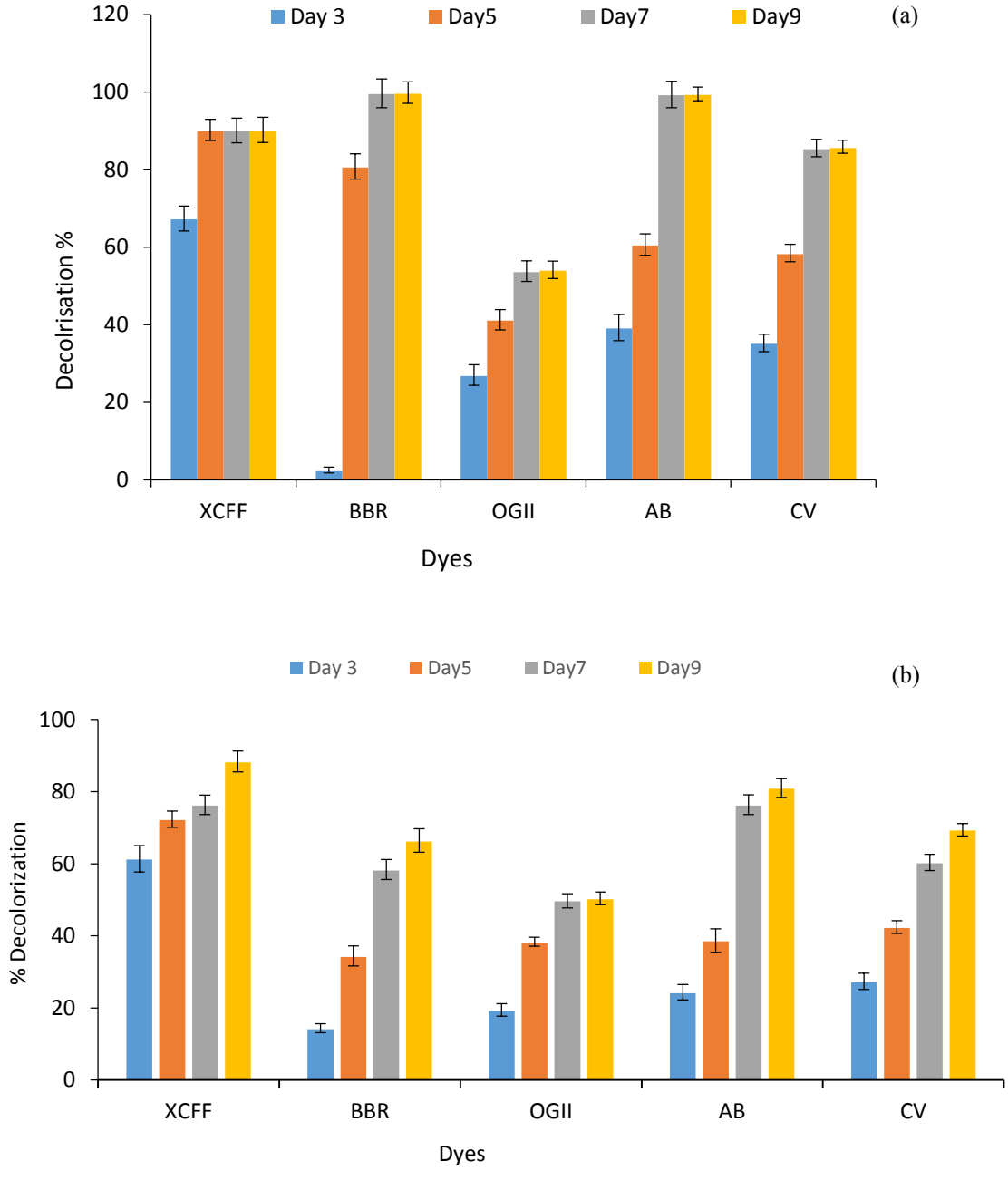


Figure 3. (a) Bioremediation of dyes (0.01%) with *Trichoderma virens* under shaking condition at pH 5.4 and 25°C in different time interval and (b) bioremediation of dyes (0.01%) with *Trichoderma virens* under stationary condition at pH 5.4 and 25°C in different time interval.

Fungi decolourization and detoxification of synthetic dyes according to their life state are of two types, type 1: application of living cells to biodegrade and biosorb dyes. The major mechanism involved here is biodegradation because the organism produce the lignin modifying enzymes, such as laccase, manganese peroxides to mineralize synthetic lignin of dyes (Stolz, 2001; Wesenberg *et al*., 2003). The use of type 2: dead cells (non-living fungal biomass) which only adsorb dyes and it does not produce enzymes. The bio-sorption mechanism involves physico-chemical interactions such as adsorption, deposition and ion-exchange (Park *et al*., 2007; Husseiny, 2008).

A diminish in toxicity is the target of all environmental studies, consequently bioremediation ought to be characterized not only as decolorization processes, but the elimination or reduction of toxicity must also be attained. In this context, the applications of live fungal biomass are reported to produce more benefits for dye effluent treatment. Therefore, we have used the type-1 state of fungi, i.e. living cells for dye bioremediation. The aboriginal viable cells are not well affected by toxic wastes, do not require a continuous supply of nutrients and they can be regenerated and reused for many cycles (Plácido *et al*., 2016), as they are acclimatized to that particular environment.

The exclusion of textile dye color is significant in the possible application of effluent soil fungal organisms as bioremediation agents in wastewater treatment plants and in runoff waters. Thus, it is essential to test dye contaminated soil fungal strains for dye decolorization in liquid medium. In this study the tested fungi remediated and decolorized the dye solution ranging from 29 to 99% within 9 days of incubation. Similar results have been reported by Rani *et al*. (2014), where the contaminated soil fungi *Aspergillus niger* and *P. chrysosporium* bioremediated Nigrosin, Basic fuchsin and Malachite green within 6 days up to 90%.

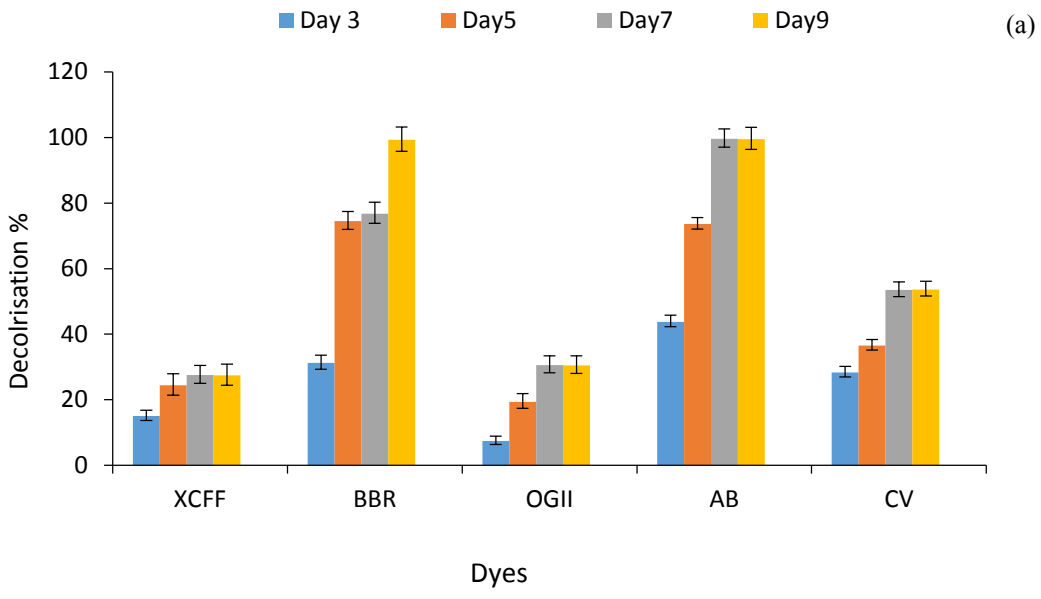
Use of *Phlebiopsis* sp. and *Talaromyces* sp. as dye remediator has been studied in this work and the significant decolorization may be accredited to either through the production of extracellular enzymes such as laccase and or by biosorption by the fungal biomass. Laccase production by the soil fungal species has been reported earlier (Stolz, 2001; Wesenberg *et al*., 2003; Rani *et al*., 2014). A study on enzyme excretion by test fungi was done to know, whether this enzyme is being produced by the test fungi and if yes then enzyme plays a role in decolorization or biodegradation of textile dyes, as reported by several authors (Stolz, 2001; Wesenberg *et al*., 2003; Rani *et al*., 2014).

The soil fungi possess ligninolytic enzymatic activities and play a significant role in the degradation of lignocellulose in soil ecosystems (Okino *et al*., 2000). Lignin degrading enzymes are involved not only directly in the degradation of lignin in their natural ecosystem but also in the degradation of numerous xenobiotic compounds, including textile dyes (Christian *et al*., 2005). Furthermore, ligninolytic enzymes have also been reported to oxidize many recalcitrant substances such as chlorophenols, polycyclic aromatic hydrocarbons (PAHs), organophosphorus compounds, and phenols (Wesenberg *et al*., 2003).

Likewise, the selected fungal strains also exhibited promising decolorization activities against tested dyes (Figs. 1-8). Vasdev (2011) exhibited that Malachite green was readily degraded in liquid culture by *Aspergillus flavus, Aspergillus solani* and some white rot fungi within six days up to 96%, support the work reported in this study. Results of the dye biodegradation by soil fungi in this work using spectrophotometric analysis were even comparable with the percent dye decolorization exhibited by the recognized bioremediator white rot fungus *Trametes versicolor* and *Pleurotus ostreatus* (Yao *et al*., 2009), and even *Phanerochaete chrysosporium* (Bumpus and Brock, 1988).

Observations of literature reveals that in many cases a rapid uptake of the dyes is first observed, being followed by a gradual assimilation through enzymatic means (Wang and Yu, 1998). Considering that, during the early contact the interaction between dye and organism was predominantly biosorption, i.e., removing the entire dye molecule without leaving toxic sub products in the water. This process avoids the appearance of toxic metabolites from the molecules of degraded dyes, like aromatic amines such as benzidine among other and which cause great evils in the environment (Schneider *et al*., 2004). In our study also, the fungal mycelium initially absorbed the dye and slowly deolorized it, as shown in Fig. 12.

Biosorption of dyes occur essentially either through complexation, adsorption by physical forces, precipitation, entrapment in inner spaces of fungal mycelium, ion exchange due to surface ionization, and by formation of hydrogen bonds (Yeddou-Mezenner, 2010). Due to an increased cell-to-surface ratio, fungi have greater chance of physical contact with the environment, compared to bacteria. Thus, some fungi have revealed better dye adsorption potential compared even to that of activated charcoal (Fu and Viraraghavan, 2002). Additionally, it is not uncommon



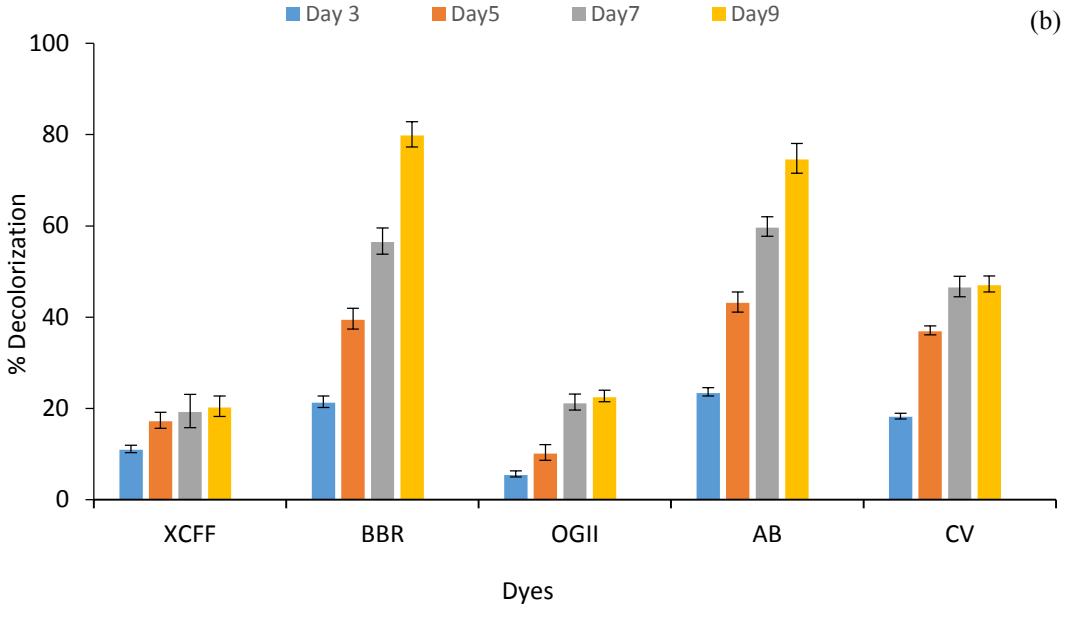


Figure 4. (a) Bioremediation of dyes (0.01%) with *Talaromyces stipitatus* under shaking condition at pH 5.4 and 25°C at different time interval and (b) Bioremediation of dyes (0.01%) with *Talaromyces stipitatus* under stationary condition at pH 5.4 and 25°C at different time interval.

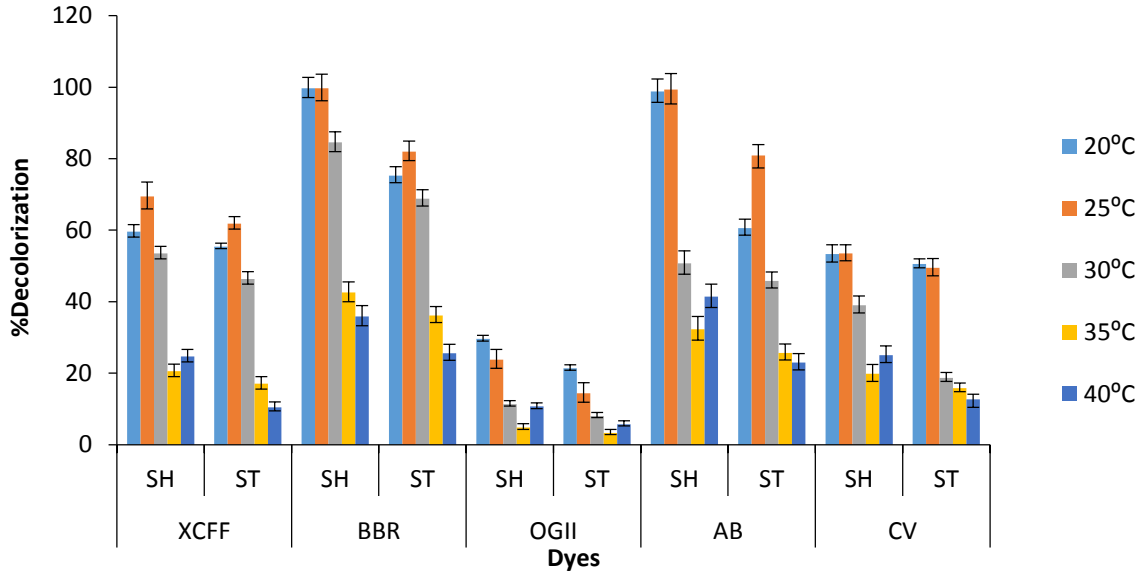


Figure 5. Effect of different temperatures on decolorization of textile dyes (0.01%) at pH 5.4 and under shaking and stationary condition by using *Phlebiopsis cf. ravenelii*

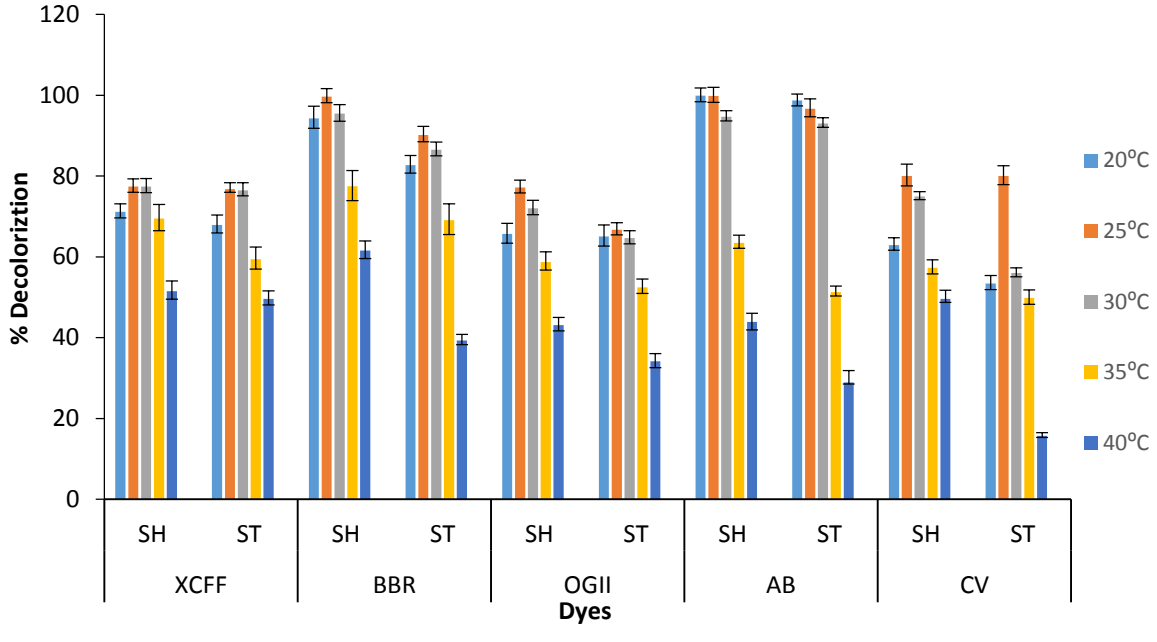


Figure 6. Effect of different temperature on decolorization of textile dyes (0.01%) at pH 5.4 under shaking and stationary conditions by *Aspergillus niger*.

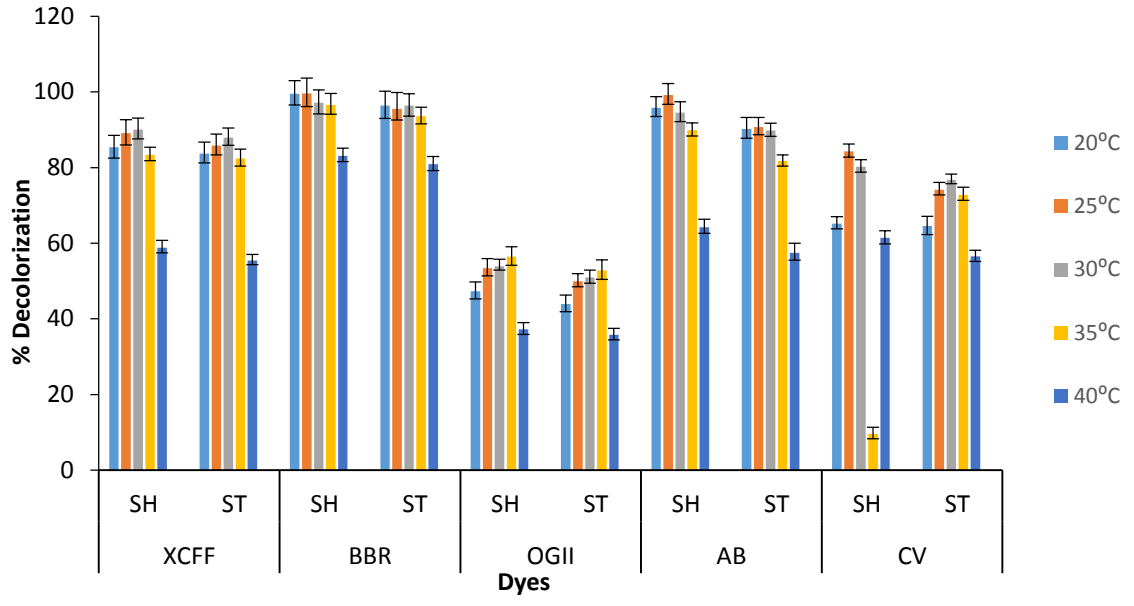


Figure 7. Effect of different temperature on decolorization of textile dyes (0.01%) at pH 5.4 and under shaking and stationary condition by using *Trichoderma virens*

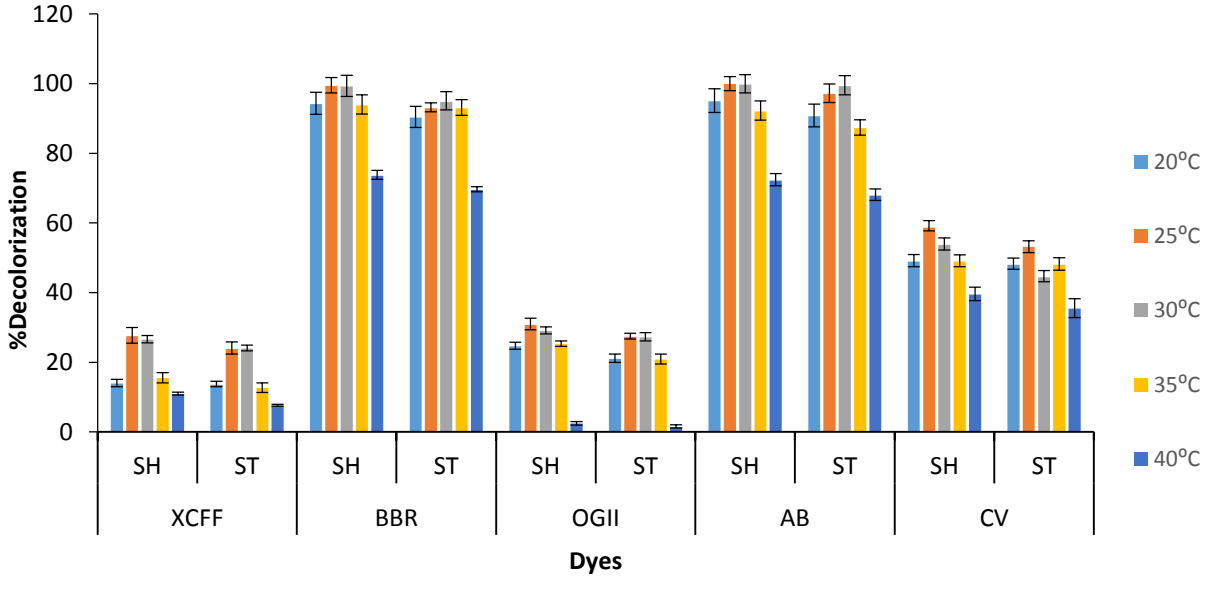


Figure 8. Effect of different temperature on decolorization of textile dyes (0.01%) at pH 5.4 under shaking and stationary condition by *Talaromyces stipitatus*

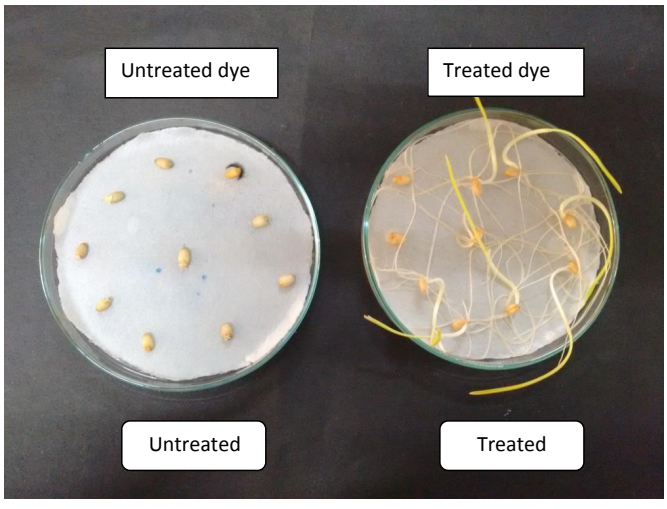


Figure 9. Germination assay of wheat seeds with treated and untreated dye

Figure 10. Germination assay of mustard seeds under pot condition with treated and untreated dye

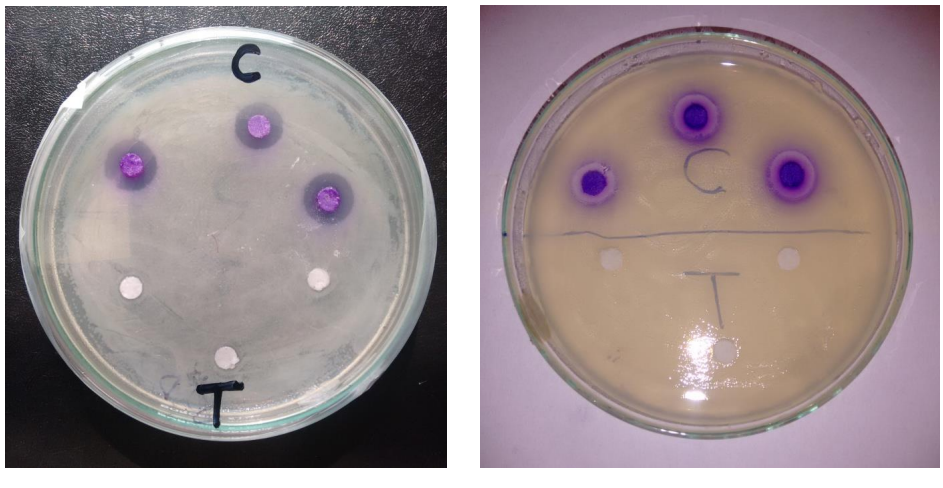


Figure 11. Antimicrobial assay of Basic fuchsin (C = control dye; T = treated dye).

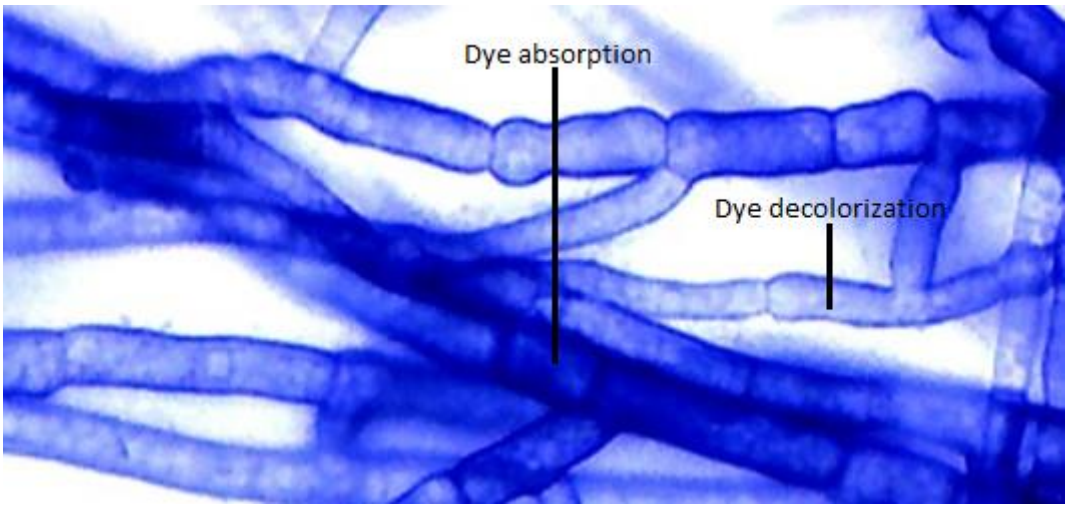


Figure 12. Plate Dye (Aniline blue) absorption and deolorization by *A. niger* hypha

for some fungal species to reveal both enzymatic degradation and biosorption in decolorization of dyes (Park *et al*., 2007; Shahid *et al*., 2013). Thus, it is possible that in addition to extracellular enzymes production, aptitude of dye effluent soil fungi to decolorize synthetic dyes is also coupled with their biosorption potential (Kaushik and Malik, 2009). We have also observed dye absorption by the test fungal mycelium under microscope (1500 ×) (Fig. 12). This may reason for the more efficient textile dye biodegradation by the soil fungal strains (Kirby *et al*., 2000). Therefore, it is possible that the ability of dye contaminated aboriginal fungi to degrade xylene cynol FF, brilliant blue R, orange G, Aniline blue and crystal violet as revealed in this study can also be largely attributed to the degrading enzyme system of organism. Additionally to extracellular enzymes, it is also probable that dye decolorization activity of these fungi could also be attributed to the ability of their mycelia to adsorb or absorb the dye. Bioremediation rate of dyes was higher with individual dyes as compared to dye mixture, which could be due the reason that a mixture of dyes forms more complex structures which become resistant or a sort of xenobiotic for biodegradation (Rani *et al*., 2014).

The detoxification aspects of all the dyes were conclusively confirmed by wheat seed germination and bacterial growth bioassay. Untreated dyes repressed the wheat seeds germination even after four days of incubation, while on the other hand seed germination was observed after 48 h in treated dyes treatments. In the same way, mustard seed germination studies were conducted in pots using treated and untreated dye solution. The treated dye solution plants exhibited growth more or less similar to normal watered mustard plants, while the untreated dye plants showed stunted and poor growth. In this study, we have tested both wheat and mustard seeds (under different growing conditions) to overcome the error of germination ability of single crop seeds. It might be possible that one type of seed may be sensitive or resistant to treated or untreated dye solution. Similarly, filter paper discs impregnated with untreated dye solution exhibited zone of inhibition of microbial growth, while the discs impregnated with treated dyes showed no zone of inhibition. The results of this study suggest that potentially competent fungal strains can be efficiently used for detoxification and bioremediation of harmful textile dyes.

Although successful in bench scales, this methodology needs further improvements to be amplified to real textile dye effluents. The present work is suggested to assist this crucial follow step, mainly with the application of novel aboriginal fungal culture, when considering the advantages.

5. Conclusions

The present investigation deals with the decolorization of synthetic dye effluent using four fungal isolates *Trichoderma virens, Phlebiopsis cf. ravenelii, Talaromyces stipitatus, Aspergillus niger*. Highest rate of decolorization was observed with *Aspergillus niger* followed by *Trichoderma virens*. The decolorization of dyes was studied under stationary and shaking conditions; encouraging results were obtained even after 3 days, but maximum decolorization of all the dyes were obtained after 9 days. In this study we have observed higher decolorization under shaking conditions by *T. virens*

and *A. niger*, which could be due to better oxygenation of the fungus culture and regular contact of excreted enzymes with the dye molecules to decolorize it, furthermore agitation also helps the fungus to grow better. Vanishing of dye color may also be owing to the biodegradation of chromophore in dye molecule because of extracelluar enzyme production by fungi along with the absorption and adsorption process. Due to the environmental friendly techniques it utilizes, the bioremediation process has been considered as a soft technology. The cost effectivity and the little or no disruption in the environment make this technology an attractive and alternate method of choice. Further identification and research on novel fungal strains with the aid of molecular biology or genomic techniques will further enhance the practical applications of potential fungal organism and it is predicted that fungal remediation will soon be a dependable, viable and inexpensive dye remediation technology.

References

Abdel-Raheem A, Shearer CA. Extracellular enzyme production by freshwater ascomycetes. Fungal Diversity. 2002; 11: 1-19.

Ali NF, El-Mohamedy RSR. Microbial decolourization of textile waste water. Journal of Saudi Chemical Society 2012; 16(2): 117-23.

Arlt VM, Glatt H, Muckel E, Pabel U, Sorg BL, Schmeiser HH, Phillips DH. Metabolic activation of the environmental contaminant 3-nitrobenzanthrone by human acetyltransferases and sulfotransferase. Carcinogenesis 2002; 23(11): 1937-45.

Ashoka C, Geetha MS, Sullia SB. Bioleaching of composite textile dye effluent using bacterial consortia. Asian Journal of Microbiology, Biotechnology and Environmental Sciences 2002; 4(1): 65-68.

Bourbonnais R, Leech D, Paice MG. Electrochemical analysis of the interactions of laccase mediators with lignin model compounds. Biochimica et Biophysica Acta (BBA) - General Subjects 1998; 1379(3): 381-90.

Bumpus JA, Brock BJ. Biodegrdation of crystal violet by white rot fungus *Phanerochaete chrysosporium*. Applied and Environmental Microbiology 1988; 54(5): 1143-50.

Carneiro PA, Nogueira RFP, Zanoni MVB. Homogeneous photodegradation of C.I. reactive blue 4 using a photo-Fenton process under artificial and solar irradiation. Dyes and Pigments 2007; 74(1): 127-32.

Christian V, Shrivastava R, Shukla D, Modi HA, Vyas BR. Degradation of xenobiotic compounds by lignin-degrading white-rot fungi: enzymology and mechanisms involved. Indian Journal of Experimental Biology 2005; 43(4): 301-12.

Cooper P. Removing colour from dyehouse waste waters - a critical review of technology available. Journal of the Society of Dyers and Colourists 1993; 109(3): 97-100.

Fu Y, Viraraghavan T. Removal of Congo red from an aqueous solution by fungus *Aspergillus niger*. Advances in Environmental Research 2002; 7(1): 239-47.

Husseiny ShM. Biodegradation of the reactive and direct dyes using Egyptian isolates. Journal of Applied Sciences Research 2008; 4(6): 599-606.

Kanmani P, Satish Kumar R, Yuvaraj N, Paari KA, Pattukumar V, Aru V. Microbial decolorization of synthetic dyes and reactive dyes of industrial effluents by using a novel fungus *Aspergillus proliferans*. Water Environment Research 2011; 83(11): 2099-106.

Kaushik P, Malik A. Fungal dye decolourization: recent advances and future potential. Environment International 2009; 35(1): 127-41.

Kirby N, Marchant R, McMullan G. Decolourisation of synthetic textile dyes by *Phlebia tremellosa*. FEMS Microbiology Letters 2000; 188(1): 93-96.

Kumar V. Laboratory manual of microbiology. Scientific Publishers, Jodhpur, India. 2011.

López MJ, Guisado G, Vargas-García MC, Suárez-Estrella F, Moreno J. Decolorization of industrial dyes by ligninolytic microorganisms isolated from composting environment. Enzyme and Microbial Technology 2006; 40(1): 42-45.

Nigam P, Armour G, Banat IM, Singh D, Marchant R. Physical removal of textile dyes from effluents and solid-state fermentation of dye-adsorbed agricultural residues. Bioresource Technology 2000; 72(3): 219-26.

Okino LK, Machado KMG, Fabris C, Bononi VLR. Ligninolytic activity of tropical rainforest basidiomycetes. World Journal of Microbiology and Biotechnology 2000; 16(8): 889-93.

Park C, Lee M, Lee B, Kim SW, Chase HA, Lee J, Kim S. Biodegradation and biosorption for decolorization of synthetic dyes by *Funalia trogii*. Biochemical Engineering Journal 2007; 36(1): 59-65.

Plácido J, Chanagá X, Ortiz-Monsalve S, Yepes M, Mora A. Degradation and detoxification of synthetic dyes and textile industry effluents by newly isolated *Leptosphaerulina* sp. from Colombia. Bioresources and Bioprocessing 2016; 3(6): 1-14.

Rajaguru P, Fairbairn LJ, Ashby J, Willington MA, Turner S, Woolford LA, Chinnasamy N, Rafferty JA. Genotoxicity studies on the azo dye Direct Red 2 using the in vivo mouse bone marrow micronucleus test. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 1999; 444(1): 175-80.

Rajamohan N, Karthikeyan C. Kinetic studies of dye effluent degradation by *Pseudomonas stutzeri* [homepage on the Internet]. 2006 [cited 2015 August 20]. Available from: http://www.eco-web.com/edi/061103.html.

Rani B, Kumar V, Singh J, Bisht S, Teotia P, Sharma S, Kela R. Bioremediation of dyes by fungi isolated from contaminated dye effluent sites for bio-usability. Brazilian Journal of Microbiology 2014; 45(3): 1055-63.

Schneider K, Hafner C, Jäger I. Mutagenicity of textile dye products. Journal of Applied Toxicology 2004; 24(2): 83-91.

Shahid A, Singh J, Bisht S, Teotia P, Kumar V. Biodegradation of textile dyes by fungi isolated from North Indian field soil. EnvironmentAsia 2013; 6(2): 51-57.

Stolz A. Basic and applied aspects in the microbial degradation of azo dyes. Applied Microbiology and Biotechnology 2001; 56(1-2): 69-80.

Suryavathi V, Sharma S, Sharma S, Saxena P, Pandey S, Grover R, Kumar S, Sharma KP. Acute toxicity of textile dye wastewaters (untreated and treated) of Sanganer on male reproductive systems of albino rats and mice. Reproductive Technology 2005; 19(4): 547-56.

Umbuzeiro GA, Freeman H, Warren SH, Kummrow F, Claxton LD. Mutagenicity evaluation of the commercial product CI Disperse Blue 291 using different protocols of the *Salmonella* assay. Food and Chemical Toxicology 2005; 43(1): 49-56.

Vandevivere PC, Bianchi R, Verstraete W. Treatment and reuse of wastewater from the textile wet processing industry: review of emerging technologies. Journal of Chemical Technology and Biotechnology 1998; 72(4): 289-302.

Vasdev K. Decolorization of triphenylmethane dyes by six white-rot fungi isolated from nature. Journal of Bioremedeiation and Biodegradation 2011; 2(5): 61-66.

Wang Y, Yu J. Adsorption and degradation of synthetic dyes on the mycelium of *Trametes versicolor*. Water Science and Technology 1998; 38(4-5): 233-38.

Wesenberg D, Kyriakides I, Agathos SN. White-rot fungi and their enzymes for the treatment of industrial dye effluents. Biotechnology Advances 2003; 22(1-2): 161-87.

Yao MLC, Villanueva JDH, Tumana MLS, Caalimg JG, Bungihan ME, Dela Cruz TEE. Antimicrobial activities of marine fungi isolated from seawater and marine sediments. Acta Manilana 2009; 57: 19-28.

Yeddou-Mezenner N. Kinetics and mechanism of dye biosorption onto an untreated antibiotic waste. Desalination 2010; 262(1-3): 251-59.

Zaharia C, Suteu C, Muresan A. Options and solutions of textile effluent decolourization using some specific physico-chemical treatment steps. Proceedings of 6th International Conference on Environmental Engineering and Management ICEEM'06, Balaton Lake, Hungary, September 1-4, 2011. 121-22.

Zollinger H. Synthesis, properties of organic dyes and pigments. *In*: Color chemistry. VCH Publishers, New York, USA. 1987; 92-102.

Received 25 May 2017
Accepted 7 June 2017

Correspondence to
Dr.Vivek Kumar
Department of Microbiology,
Himalayan School of Biosciences,
Swami Rama Himalayan University,
Swami Ram Nagar
Jollygrant, Dehradun,
India
Email: vivekbps@gmail.com