

Studies On the Role of Fungal Strains in Bioremediation of Dyes Isolated from Textile Effluents

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Abstract: Fungal strains are widely used for the cleaning of soil, sediments, groundwater, surface water, and the ecosystem. The presence of extracellular enzymes in fungi facilitates the process of bioremediation of textile dyes. This study was conducted to observe the quality of water being released from textile dyes industries and also the capability of some fungal strains which can remediate these dyes by showing the tendency of their resistance. Samples of water were collected from the polluted area surrounding the textile dyeing industries in Lahore. In the process of isolation, Potato Dextrose Agar (PDA) medium was used to verify the fungal growth. Fungal strains were purified, and the morphological characterization of fungal strains was carried out at 10X and 100X by using a magnification microscope. The fungal strains, such as *Aspergillus niger*, *Aspergillus oryzae*, and *Aspergillus flavus* were identified. The stress of four types of dyes was given to each fungal strain. The results showed that *Aspergillus oryzae* was one of the most stable, non-toxic, and resistant fungal species against the high stress of dyes as compared to other species.

Keywords: *Aspergillus oryzae*, textile industries, PDA medium, bioremediation, morphological characterization.

Introduction

The global uprise of urbanization and industrialization is closely linked to the increase of wastewater released from the industries and municipal remains which have potential effects on the environment. This wastewater consists of a high level of contaminants which includes heavy metals, hydrocarbons, pharamaseicticals dyes and different pesticides (Mrozik, 2021). Water is an essential aspect of human life, which includes: community health, production of food, economic durability, and ecosystem biodiversity. However, when its biological and physical attributes are changed due to the addition of pollutants then it becomes chronically ill for drinking and irrigation (Kløve et al., 2017). The textile industry is one of the sectors developing rapidly worldwide (Rather et al., 2019) which produces synthetic chemicals of significant concern in the form of synthetic dyes and their intermediates (Zhao et al., 2019). In Pakistan, the textile industry is considered as one of the most essential and largest sectors for carrying the per capita income of the country (Ahmad et al., 2016). Alongside, the harmful impact of the textile industry on the environmental conditions is also worth thinking about. A large amount of textile processed water with high pollutants is being discharged on daily basis polluting the groundwater. It is hazardous because about 70% of the people in Pakistan rely on groundwater for household purposes (Awan, 2016). In Asia, Pakistan is ranked as the 8th largest exporter of

textile products because at the moment Lahore, Karachi, Sialkot, and Faisalabad sectors are the major contributor to exporting textile goods. Unfortunately, these are also producing significant pollutants being released into the water bodies (Shumaila and Tahir 2020). Thus, the removal of organic dyes which are persistent in their nature from the wastewater is a huge challenge for the textile industries (Sultan, 2017; Zahid, 2018; Koutsos et al., 2018). Various processes for removal of these dyes have been applied and tested including carbon absorption, membrane filtration, activated sludge treatment, but these are found less effective. The decolourization of textile dye effluents through aerobic and anaerobic bacteria, fungi, and some physical-chemical methods are economical in this regard. Biotechnological applications do not only remove colour but also completely degrade resistant dyes from the environment (Bhatia et al., 2017). Bioremediation has been effectively applied for clean-up of soil, surface water, groundwater, soil, sediments as well as ecosystem restoration (Sala-Comorera et al., 2019). Bioremediation is generally taken up with the help of bio-stimulation involving the addition of natural or engineered micro-organisms to increase the desired catalytic capabilities for getting better results (Azubuike et al., 2016). Most of the bacterial and fungal strains have been found very effective for removing dyes of such industrial wastewater. The bacteria like *Deinococcus radiodurans* have the potential to consume and digest ionic mercury and toluene from highly

radioactive nuclear waste. Similarly, *Pseudomonas putida* are helpful in the bioremediation of toluene, naphthalene. The fungal strains like *Phanerochaete chrysosporium*, the lignin-degrading -rot fungus, exhibits strong potential for bioremediation of pesticides, dioxins, TNT, dyes (Taha et al., 2018). Lladó et al., (2017); Dwivedi (2018) also reported the mechanism of working of fungal mycelium grown in the polluted area against the dyes present in that region (Jones et al., 2018). The aim of this study was also to examine the behaviour of selected fungal strains towards the dyes present in the wastewater and their ability to neutralize or show resistant behaviour towards the contaminants as a higher prevalence of contaminants coming out from textile industries have become a grave issue.

Materials and Methods

Sample Collection

Samples from three textile mills in Lahore were collected and stored at 4°C for the observation of microbial and fungal growth by following the method of LaRocco et al. (2015). For determination of suspended solid, 1.5 ml from the sample of each textile industry was centrifuged at 12000 rpm for 5 min in a sterilized Eppendorf tube and the supernatant was discarded.

Media preparation for fungal strain isolation

Potato dextrose agar (PDA) 20 g was dissolved in 500 ml of distilled water in a 1000 ml flask for the preparation of standard media for fungal growth. About 20 µg/1000 ml of streptomycin was added to avoid bacterial interference and autoclaved for 15 minutes at 121°C by following Mohammed and Badawy (2017). The medium was poured into 10 Petri dishes in a laminar flow of biosafety level 2 and was turned on for 15 minutes before use.

Fungal Growth Observation

Samples preserved at 4°C were put in the incubator at 30°C for four days. After four days, fungal growth was observed in all samples. Each colony from the parental plate was picked with a sterilized inoculating loop and separated into different plates with medium to achieve extensive fungal growth and were named F1, F2, and F3. The plates were taken out from the incubator to observe the fungal growth following the methodology of Ueki et al. (2017).

Preparation of textile dye stock solution

Four different types of dyes Remazol red RGB, Remazol golden yellow, Remazol dark blue Remazol black dye were used. For this 0.4 grams of each dye was dissolved in 15mL of distilled water in disposable test tubes with four factors and were put in the autoclave for 15 minutes at 121°C by using the method

of Harry-asobara and Kamei (2019). Control was also prepared by dissolving 10 mg of PDA in 250 ml of sterilized water. 5 µg/250 ml of streptomycin was also added in the medium volume to avoid bacterial interference.

Media preparation for dye stress and fungal strains

About 10mg of PDA was dissolved in 250ml of distilled water in the flask of 1000ml. 5 µg/250 ml of streptomycin was added in the medium to avoid bacterial interference. PDA media was put in the autoclave at 121°C for 15min and was placed in 9 Petri undercounting from 1 to 9 by following the method of Wilke et al., (2018). Remazol red dye was added in the media containing plates 1, 2, 3 in one group while 4,5,6 were put in the second group and 7,8,9 were placed in the third group. Inoculated fungal strain F1 was put in the first group of plates, F2 in the second group plates, and F3 in the third group of Petri plates were observed.

Growth analysis of fungi in medium containing textile dyes

After two days of incubation, fungus growth was observed, and the diameter of fungus F1, F2, and F3 against medium containing the dye was measured by using the method of Agrawal and Chanyal (2017) and stress on fungal biomass as well. They are found resistant to dyes. Morphological characters were also measured by taking the material from F1, F2, and F3 and was put under 10X and 100X magnification with the help of the microscope by adopting the procedure of Luo et al. (2017).

Table.1: Physicochemical Analysis of Samples

Parametrs	Sample-1	Sample-2	Sample-3
Proteins (mg/dl)	500	100	30
Nitrates (mg/l)	3.5	0.00	2.5
Sugar(mg/l)	Nil	150	Nil
pH	9	8	9
Color	Purple	Yellow	Light Red
Suspended, Soluble or Insoluble	Nil	Nil	Nil

Result and Discussion

Fungal Growth analysis

The physicochemical properties of samples have been given in Table.1 which reflects the growth pattern of fungal strains in the dyes. In this regard, stock solutions of four different dyes named Remazol red dye, Remazol golden yellow dye, Remazol deep blue dye, and Remazol black dye were prepared separately to check the resistance of three fungal strains of *Aspergillus niger*, *Aspergillus oryzae* and *Aspergillus flavus*. Each fungal strain was tested against each dye by maintaining the concentration constant by following the method of Abd El-Rahim et al. (2017). As shown in Figure 1 (A, B, C) and Table.2, the *Aspergillus*

niger had more meaningful growth in a medium without dye. There was a gradual increase in mean growth (4.1 to 8.5) from the 3rd to the 6th day. After the 6th day, there was no increase in growth further. Petri plate containing Remazol red dye showed less growth as compared to control, which indicated fungal inhibition due to dye. Fungal diameter after 6th day (5.8 cm) in Remazol red dye was comparatively more than that of 3rd day (2.5 cm). An increase in diameter indicated that *A. niger* showed resistance against Remazol red dye. While in the case of *Aspergillus oryzae* as shown in Figure 1 (A, B, C) and Table.2, has more meaningful growth in a medium without dye. There was a gradual increase in mean growth (3.3 to 8.1 cm) from the 3rd to the 6th day. After the 6th day, there was no further increase in growth. Petri plate containing Remazol red dye showed less growth as compared to control, which indicated fungal inhibition due to dye. Fungal diameter after 6th day in Remazol red dye (6.3 cm) was comparatively more than that of 3rd day (2.3cm). An increase in diameter indicated that *A. oryzae* showed resistance against Remazol red dye. As shown in Figure 1 (A, B, C) and Table.2, *Aspergillus flavus* was the highest mean growth in medium without dye. There was a gradual increase in mean growth (2.7 to 6.9 cm) from the 3rd to the 6th day. Petri plate containing Remazol red dye showed very little growth as compared to control, which indicated the highest fungal inhibition due to dye. Fungal diameter after 6th day in Remazol red dye (4.7 cm) is comparatively more than that of 3rd day (1.9 cm). A slight increase in diameter indicates that *A. flavus* showed very little resistance against Remazol red dye. Therefore, it is concluded that *Aspergillus oryzae* showed the highest growth in Remazol red dye while *Aspergillus niger* showed growth but slightly lesser than that of *Aspergillus oryzae*. *Aspergillus flavus* showed the lowest growth rate. Thus *Aspergillus oryzae* was the most resistive fungal strain against remazol red dye.

Association of Dyes and fungal strains

As shown in Figure 1 (A, B, C) and Table.2 *Aspergillus niger* was having more meaningful growth in medium without dye. There was a gradual increase in mean growth (4.1 to 8.5) from the 3rd to 6th day, respectively. After the 6th day, there was no further increase in growth in the medium with dye. Petri plate containing Remazol golden yellow dye showed less growth as compared to control, which indicated fungal inhibition due to dye. Fungal diameter after 6th day (6.9 cm) in Remazol golden yellow dye was comparatively more than that of 3rd day (3.7 cm). An increase in diameter indicated that *A. niger* showed resistance against Remazol golden yellow dye. In the case of

Aspergillus oryzae growth as shown in Figure 1 (A, B, C) and Table.2 *Aspergillus oryzae* has more meaningful growth in a medium without dye.

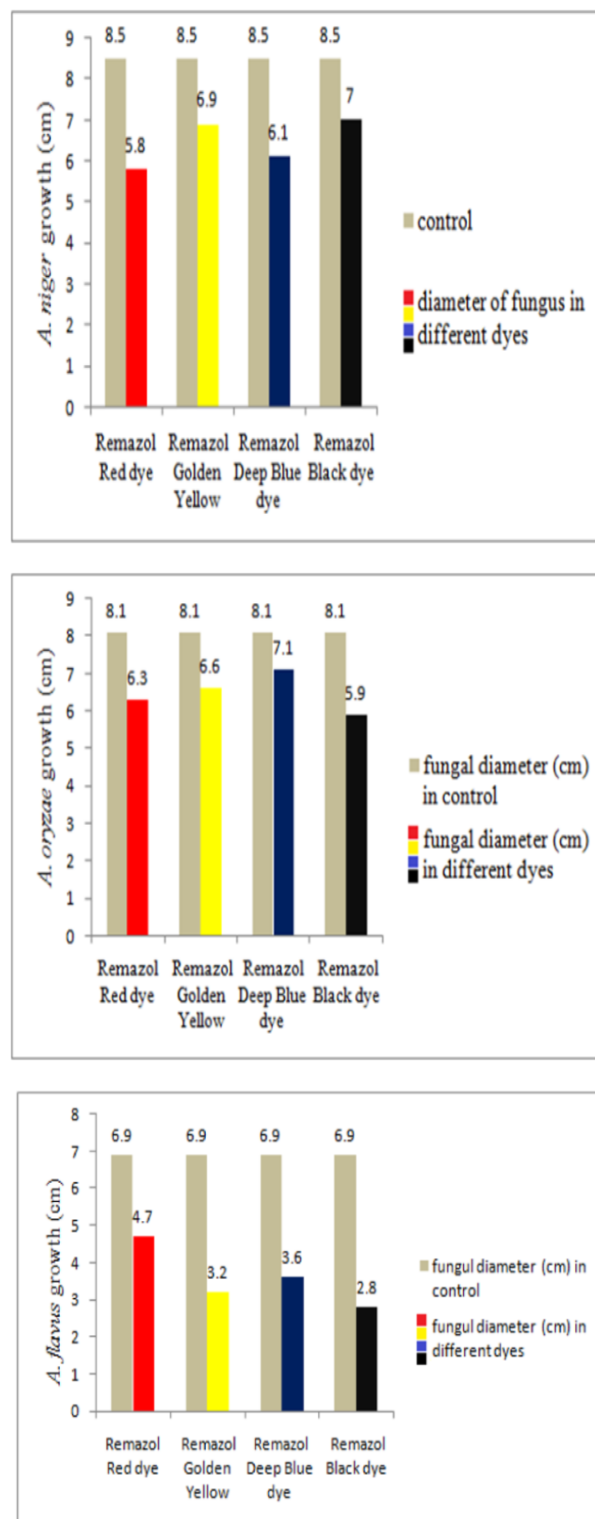


Fig. 1 (A, B, C). *Aspergillus oryzae*, *Aspergillus niger* and *Aspergillus flavus* diameter and different dyes.

Table 2. Growth of *Aspergillus niger*, *Aspergillus oryzae* and *Aspergillus flavus* at a constant concentration of Remazol Red dye, Remazol Golden Yellow, Remazol Deep Blue dye and Remazol Black dye.

Fungus	Dyes	Mean diameter of fungus (cm) (Control)		Mean diameter of fungus (cm) (dye)		Inhibition (%)	Resistance (%)
		3 rd day	6 th day	3 rd day	6 th day		
<i>Aspergillus niger</i> F1	Remazol Red	4.1	8.5	2.5	5.8	31.76	68.24
	Remazol Golden yellow	4.1	8.5	3.2	6.9	18.82	81.18
	Remazol Deep Blue	4.1	8.5	2.9	6.1	28.24	71.76
	Remazol Black	4.1	8.5	3.4	7.0	17.65	82.35
<i>Aspergillus oryzae</i> F2	Remazol Red dye	3.3	8.1	2.3	6.3	22.22	77.78
	Remazol Golden Yellow	3.3	8.1	2.5	6.5	19.75	80.25
	Remazol Deep Blue	3.3	8.1	2.7	7.1	12.34	87.65
	Remazol Black dye	3.3	8.1	2.1	5.9	27.16	72.83
<i>Aspergillus flavus</i> F3	RemazolRed dye	2.7	6.9	1.9	4.7	31.84	68.12
	Remazol Golden Yellow	2.7	6.9	1.6	3.2	53.62	46.38
	Remazol Deep Blue	2.7	6.9	1.7	3.6	47.82	52.17
	Remazol Black	2.7	6.9	1.4	2.8	59.42	40.58

There was a gradual increase in the mean growth (3.3 to 8.1 cm) from the 3rd to the 6th day. After the 6th day, there was no further increase in growth in the dye medium. Petri plate containing Remazol golden yellow dye showed less growth as compared to control, which indicated fungal inhibition due to dye. Fungal diameter after 6th day in Remazol golden yellow dye (6.5 cm) was comparatively more than that of 3rd day (2.5 cm). An increase in diameter indicated that *A. oryzae* showed resistance against Remazol golden yellow dye. *Aspergillus flavus* growth has also been shown in Figure 1 (A, B, C) and Table.2 *Aspergillus flavus* which showed the highest mean growth in medium without dye. There was a gradual increase in the mean growth (2.7 to 6.9 cm) from the 3rd to the 6th day. Petri plate containing Remazol red dye showed very little growth as compared to control. Fungal diameter after 6th day in Remazol red dye (3.2 cm) was comparatively more than that of 3rd day (1.6 cm). A slight increase in diameter indicated that *A. flavus* was very less resistant to Remazol golden yellow dye. *Aspergillus flavus* growth in response to Remazol deep blue dye has been shown in Figure 1 (A, B, C) and Table.2, where the change is shown after the 3rd and 6th days in response of the dye with a gradual mean of growth from 2.7cm to 6.9 cm (Fig. 7). Petri plate containing Remazol deep blue dye showed very little growth as compared to control, which indicated the highest fungal inhibition due to dye. Fungal diameter after the 6th day in Remazol deep blue dye (3.6 cm) was comparatively more than that of the 3rd day (1.7 cm).where *Aspergillus flavus* showed the lowest growth rate. Thus, *Aspergillus oryzae* was the most resistive fungal strain against Remazol deep blue. Similarly, *Aspergillus flavus* growth after the 3rd and 6th day in response to Remazol black dye was also found as the highest mean growth in medium without dye. There was a gradual increase in mean growth (2.7 to 6.9 cm) from the 3rd to the 6th day. Petri plate containing Remazol black dye showed very little growth as

compared to control, which indicated the highest fungal inhibition due to dye. Fungal diameter after 6th day in Remazol black dye (2.8 cm) was comparatively more than that of 3rd day (1.4 cm). A slight increase in the diameter indicated that *A. flavus* showed very slight resistance against Remazol black dye. As shown in Figure 1 (A, B, C) and Table.2, *Aspergillus niger* diameter in the case of Remazol red RGB was lesser as compared to Remazol yellow, Remazol deep blue, and Remazol black dye. Fungal diameter increased in Remazol deep blue as compared to Remazol red. Still, it was less than that of Remazol yellow and Remazol black dye. *A. niger* diameter increases in the presence of Remazol yellow dye as compared to Remazol red and Remazol deep blue dye. The diameter of *A. niger* in Remazol black dye was measured highest as compare to Remazol red, Remazol yellow, and Remazol deep blue. *A. niger* was highly resistant to Remazol black dye. The order of resistance was *Aspergillus oryzae* diameter = Remazol black dye < Remazol red dye < Remazol yellow dye < Remazol deep blue. In the case of *Aspergillus flavus*, the diameter of Remazol black dye was less as compared to Remazol red, Remazol yellow, and Remazol deep blue dye. Fungal diameter increased in Remazol Yellow dye more as compared to Remazol Black dye, but it was lesser than that of Remazol red and Remazol deep blue dye. *A. flavus* diameter increased in the presence of Remazol deep blue dye as compared to Remazol yellow and Remazol black dye. The diameter of *A. flavus* in Remazol red dye was measured highest as compared to Remazol deep blue, Remazol yellow, and Remazol black. *A. flavus* was highly resistant to Remazol red dye and the range of resistance was *Aspergillus flavus* diameter= Remazol black dye < Remazol golden yellow < Remazol deep blue < Remazol red dye. The report of (Mohanty et al. 2016) have also shown the positive behaviour of *Aspergillus* species towards the degradation of the heavy metal during the working biodegradation of polymers

contained in the packing material. They found that fungal strains showed more growth rate on the biphenyl compounds as compared to that of the bacterial strains in an open environment but its activity has also been found suppressive in the presence of polychlorinated biphenyl compound in the liquid material. The present study also depicts the findings of (Vidal-Diez de Ulzurrun et al., 2019) about the stability of fungal stains against some emerging contaminants which are being released in the environment. The observations recorded by (Khan et al., 2019) is much relevant to the present study concerning the resistant capacity of *Aspergillus* and some blue-green algae under a controlled environment. Serge et al., (2018) also found a smooth pattern of growth on agar media but the resistant capacity was found weaker as compared to the bacterial species.

Conclusion

The main focus of this study was to identify the fungal strains that can show resistance against the dyes present in the textile contaminated water. It has been found that fungal strains present in the textile dyeing effluent can grow easily at the normal temperature on the media containing four different dyes. In this regards, *Aspergillus oryzae* growth was high in the Remazol deep blue, *Aspergillus niger* in the Remazol black dye and *Aspergillus flavus* showed the highest resistance for only Remazol black dye. These three fungal species showed good performance for all the tested dyes. At the same time, the stability of *Aspergillus flavus* was also found higher than that of *Aspergillus niger* and *Aspergillus oryzae* against any dye. So in this study, it is concluded that *Aspergillus flavus* can be used as one of the best vectors for remediation of dyes present in the contaminated water on a large scale and on an economical basis.

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