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BIODEGRADATION OF DIAZO DYE DIRECT RED 81 BY FUNGI ISOLATED FROM DYES INDUSTRIAL EFFLUENTS

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Azo dyes industrial effluents are toxic and can cause many hazard effects on the environment. Therefore they have to be treated before their releasing into the environment. The Direct Red 81 (DR81) is one of these azo dyes which can be toxic to human, animals and aquatic environment. The treatment of the synthetic dyes by fungi is an effective and appropriate method. The aim of this study is to isolate fungi capable of decolorizing and degrading the DR81 dye. The ability of thirteen fungi belonging to Aspergillus spp., Penicillium spp., and Trichoderma sp. to degrade DR81 was tested. The optimal conditions for dye decolorization by the most potent isolate were found to be pH 5.0, temperature 25ºC, in shaking and dark conditions after 6 days of incubation and 99.8 mg/L dye concentration. The decolorization percentage, COD and BOD removal percentages were found to be 95.48%, 79.94%, and 77.76%, respectively. The UV-VIS analysis confirmed that the maximum peak of DR81 disappear after the decolorization in the optimum conditions. The phytotoxicity tests on Trigonella foenum-graecum, Vicia faba and Lens culinaris indicated that the toxicity of the dye was reduced after decolorization by the most potent fungi. The results have revealed the potential of the selected fungal isolate in the treatment of DR81 dye

Keywords: *azo dyes, decolorization, direct red 81, fungi, wastewater*

INTRODUCTION

Synthetic azo dyes are extensively used in many industries including leather, textile, paint and plastic. In each year it is estimated that about 7×10^5 tons of dyes are produced. The low-cost and stability of these dyes to sunlight and washing make them used widely[1]. Unfortunately, large portion of these dyes escapes to the environment even when good manufacturing practices were used which cause long term influences on the aquatic environment. Unlike the natural dyes, the synthetic ones are resistant for recycling and remain for long time in the environment[2].

The releasing of the colored effluents from dyeing industries is one of the major environmental problems. Discharged dyes even at very low concentrations have a huge effect on the environment due to turbidity of the water bodies and their high pollution strength. Therefore these dyes must be treated by an appropriate method due to their toxicity and carcinogenicity. Also, as a result of the differences and complexity in the chemical structures of dyes, it is difficult to determine specific traditional, biological, or chemical method that can be selected to remediate them[3-5].

Different studies have showed that azo dyes have mutagenic, carcinogenic, and toxic effects. Also, their biotransformation compounds may causedifferent damages to the organisms[6]. Direct Red 81 (DR81) is a synthetic sulphonated azo dye that belongs to direct dyes that represent more than 50% of the

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dyes used in pulp and textile industries. The sulphonic acid groups of DR81 make them more water-soluble and easier to bind to fibers. This dye is known for its toxicity and carcinogenicity to humans and animals where it may cause harmful effects on skin and eyes. The solubility, charge, and binding affinity of DR81 to cellulose are due to the auxochrome groups[7,8].

Chemical and physical methods for dyes treatment including precipitation, ion exchange, ozonation, irradiation, electro-floatation, electrolysis and oxidation via chlorine are quite expensive and generate large amounts of sludge. Among low-cost and effective alternatives for treatment of dyes effluents are the biological methods.The treatment using microbes is eco-friendly and can lead to mineralization of the dyes. The effectiveness of these methods depends on the adaptability of the microorganisms to the dyes during the treatment. Fungi have shown high adaptability and efficiency in the decolorization of these compounds. Also due to their large surface area and easy separation, fungi offer an efficient system for dyes treatment. Many fungal genera have been investigated for their ability to decolorize dyes either in living or deadform[3,9-12]. The current study aimed to investigate the ability of different fungi isolated from dyes industrial effluent to decolorize DR81 and selection of the most potent isolate.

MATERIALS AND METHODS

Dyes and chemicals

DR81 dye used in this study was kindly provided from dye factory located in Shubra El-kheima, Qalyubia governorate, Egypt. The chemicals used in the present study were of analytical grade. The characteristics of the DR81 dye are in the **Table 1**.

The chemical structure of DR81 dye is in the **Fig. 1.**

Table 1: The characteristics of the DR81 dye.

Parameter	DR81
Molecular formula	$C_{29}H_{19}N_5Na_2O_8S_2$
Molecular weight (g/mol)	675.60
Color index (C.I.) name	C.I. direct red 81
Water solubility	Soluble

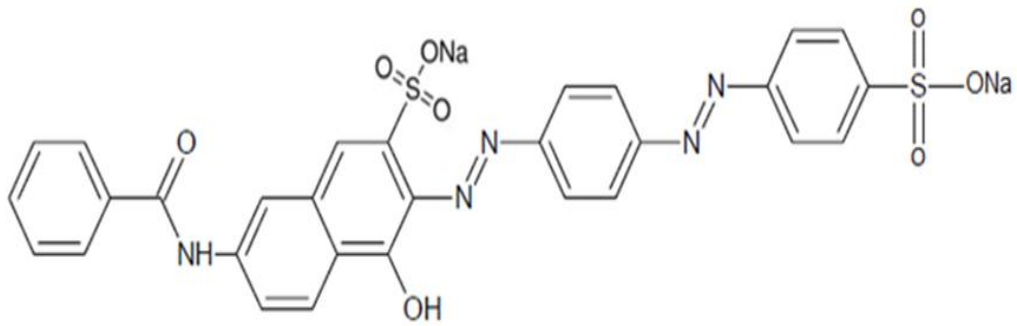

Fig. 1: The chemical structure of DR81 dye.

Determination of absorption maxima of DR81

The specific absorption spectrum of DR81 dye solution at different wavelengths (200–900 nm) was assayed using UV-VIS spectrophotometer (Jasco V-630, Japan). The maximum absorption wavelength (λmax) was usedfor assessment the decolorization of dye[13,14].

Samples collection

Six samples of dyes wastewater effluents with codes A, B, C, D, E, and F were collected from dye factory located in Shubra El-kheima, Qalyubia governorate, Egypt. Also, two samples with codes G and H were collected from textile industry located in 10th of Ramadan City, Sharqia governorate, Egypt. These samples were used for fungal isolation. According to Sulugambari[15], the microorganisms isolated from dye contaminated sites are highly tolerant to high concentrations of dyes and can be used effectively for the bioremediation of dyes. The wastewater samples were stored in plastic bottles instead of glass bottles where wastewater containing hazardous compounds react with sodium in the glass[16]. The collected samples were transported to laboratory and stored at 4ºC in the fridge until using for preventing non-indigenous microbes to contaminate it[17].

Isolation and identification of fungi

The 1% Glucose Czapek-Dox agar medium was used for isolation and purification of fungi from the samples of wastewater effluents. According to Saber et al.[18], with a slight modification, the composition is as follows: glucose, 10 g; $NaNO_3$, 1 g; KH_2PO_4, 0.5 g; $MgSO_4.7H_2O$, 0.5 g; KCl, 1 g; agar, 15 g, trace elements stock solution, 1 mL; distilled water, 1 L;. The medium was autoclaved at 1.5 atm. and 121°C for 20 minutes. One ml of each one of the samples was placed on the surface of sterile Czapek-Dox agar medium in sterilized petri dishes. The plates were kept for incubation at 30ºC for 7 days. After additional inoculation, the pure fungal cultures were obtained. Each single pure fungal culture was sub-cultured on Czapek-Dox agar slant and stored in the fridge at 4ºC.

Identification of the isolated fungi was carried out using the diagnostic morphological on the Czapek-Dox agar medium and the microscopic features for the genera and species of fungi by optical light microscope (10×90) Olympus CH40. These features are such as colony diameters, texture, degree of sporulation, color, the presence or absence of soluble pigments and exudates, colony reverse colors, the presence or absence of vesicles and their shape, the shape of conidia and phialides, the presence or absence of metulae, the branching patterns, the wall ornamentation of stipes and conidia, and dimensions of the conidiophores. Ainsworth[19] as a dictionary of the fungi, Barron[20] for the genera of Hyphomycetes, Booth[21,22] for *Fusarium* species, Christensen and Raper[23] for synoptic key to *Aspergillus nidulans* group and *Emericella* species, Ellis[24,25] for Dematiaceous Hyphomycetes, Klich and Pitt[26] for *Aspergillus* species, Ramírez[27], Pitt[28,29], and Samson and Pitt[30], for *Penicillium* species, and Zycha[31], for Mucorales group are used as references.

Screening of fungal isolates for decolorizing DR81 dye

According to Bankole et al.[32],with slight modifications, the capacity of the collected fungal isolates to decolorize DR81 dye was investigated. DR81 dye was added with a final concentration of 199.2 mg/L in 100 ml Erlenmeyer flasks containing 50 ml of Czapek-Dox broth media. Each experimental flask was inoculated with different fungal isolate. The experiment was carried out in shaking conditions at 120 rpm for 6 days at 30ºC. Control experiments were performed as described above but without fungi. The solution was filtered and the filtrate was centrifuged at 4000 rpm for 10 min by SIGMA 3-18 KS centrifuge.

The values of decolorization were calculated by measuring the absorbance of the filtrates by UV-VIS spectrophotometer (Jasco V-630, Japan). The decolorization percentages were calculated as percentage of changes of optical density in relation to control that contained the original concentration of dye and no fungal inoculant according to the following equation[33]:

$$\text{Decolorization (\%)} = \frac{\text{Optical density of control} - \text{Optical density of sample}}{\text{Optical density of control}} \text{ X } 100 \quad (1)$$

The decolorizing was considered high when percentage is above 80%, moderate when it is between 50% and 80% and low when it was less than 50%[34].

Optimization of the degradation process parameters

According to Al-Tohamy et al.[14] and Hemdan et al.[35]the process of dye decolorization were optimized for the highest decolorization percentage. The physiochemical parameters including pH, temperature, dye concentration, incubation period, agitation, dark and light were optimized in the selected fungal isolate. These experiments investigate the decolorization of dye using a single-factor optimization approach[14]. During the investigation of one factor, all other factors were kept constant. Spore suspension of the selected fungal isolate was prepared according to Teramura et al.[36] by adding sterile saline solution containing 0.85% NaCl and 0.1% Tween 80 on the sporulating plate of the selected fungal isolate. Then, gentle scraping of the fungal growth was performed with a sterile loop and the solution was collected and filtered to remove hyphal fragments. The suspension was subjected to spore count using a haemocytometer under a light microscope. The filtrate was kept in refrigerator as a stock spore solution.

Influence of pH on the decolorization percentage

The pH of the medium was optimized according to Gul et al.[37] and Hemdan et al.[35]. In this study, the pH of the medium was adjusted to 5, 6, 7, 8, 9, and 10 before sterilization by employing 0.1 M NaOH or HCl using JENWAY 3510 pH meter. One ml of spore solution (6.25×10^4 spores) was added to each particular pH value in 100 ml sterilized Erlenmeyer flasks containing 50 ml Czapek-Dox broth medium and DR81 dye was added with a final concentration of 199.2 mg/L. The flasks were then incubated at 30ºC under static conditions. Control flasks were prepared without adding the fungal isolate. After incubation period the cultures were filtered and centrifuged for 10 min at 4000 rpm and the decolorization percentages were calculated as in equation (1) and the optimal pH value was determined.

Influence of different temperature degrees on the decolorization percentage

According to Ameen et al.[38] and Hemdan et al.[35], different temperature degrees were tested for determining the optimum degree. The pH of the Czapek-dox broth medium was adjusted to 5 and then it was sterilized. Then, one ml of fungal spore suspension was added along with DR81 dye with a final concentration of 199.2 mg/L. Control flasks were conducted without adding the fungal spore suspension. The flasks were incubated at 25ºC, 30ºC, 35ºC, and 40ºC for 7 days at static conditions. Then the flasks were filtered, centrifuged and the decolorization percentages were measured as in equation (1).

Influence of different dye concentrations on the decolorization percentage

Decolorization of different concentrations of the dye was tested according to Gul et al.[37] and Hemdan et al.[35] for optimization of the decolorization process. DR81 was added with final concentrations of 99.8, 199.2, 298.21, 396.83, and 495.05 mg/L to the sterilized flasks containing Czapek-Dox broth medium with pH 5.0. Then, one ml of the fungal spore suspension was added to the flasks. The control flasks were also conducted without addition of the fungal isolate. The flasks were incubated at 25ºC for 7 days in static conditions. After incubation, the flasks were filtered and centrifuged. According to equation (1) the decolorization percentages were calculated.

Influence of different incubation periods on the decolorization percentage

For optimizing the incubation time, different periods of incubation were tested according to Gul et al.[37]. In this study, the Erlenmeyer flasks containing sterilized Czapek-Dox broth medium with pH 5.0 were sterilized and DR81 with a final concentration 99.8 mg/L was added along with 1 ml of fungal spore suspension. The flasks were then incubated at 25ºC for different incubation periods 1, 3, 5, 6 and 7 days under static condition. Control flasks were also conducted without adding the fungal isolate. At the end of each incubation period, the flasks were filtered, centrifuged and the decolorization percentages were calculated as in equation (1). Then, the optimal incubation period was determined.

Influence of static and shaking conditions

The effect of the static and shaking conditions were tested to determine the dye decolorization efficiency as reported by Ekanayake and Manage[10]. The Erlenmeyer flasks containing Czapek-Dox broth medium were sterilized and DR81 with a final concentration 99.8 mg/L dye was added and inoculated with 1 ml fungal spore suspension of *P. mononematosum*. The flasks were then incubated for 6 days at 25ºC under static and shaking conditions. Control flasks were also conducted without adding the fungal isolate. The flasks were filtered and centrifuged at the end of the incubation period and the decolorization percentages were determined as in equation (1).

Influence of light and dark conditions

The light and dark conditions may have an effect on the fungal growth and metabolism as documented before by and Hill[39] and Velmurugan et al.[40]. In this study the effect of light and dark conditions on the decolorization process were tested. The Czapek-Dox broth medium was sterilized andDR81 was added with a final concentration of 99.8 mg/L. The flasks were inoculated with 1 ml fungal spore suspension of *P. mononematosum*. Then, the flasks were incubated at 25ºC for 6 days under shaking conditions at 120 rpm in the light and dark. Control flasks were also conducted without adding the fungal isolate. After the 6 days, the flasks were filtered and centrifuged. The decolorization percentages were calculated as in equation (1).

Spectrophotometer analysis of decolorized dyes

UV–VIS spectroscopy is a fundamental technique utilized to ascertain the degradation of dyes. A strong correlation exists between the absorption peaks observed and the degradation of the dye. The degradation of a dye can be inferred if its primary absorption peak disappears in the UV–VIS region or if new absorption peaks emerge. The DR81 dye was analyzed by UV-VIS spectrophotometer (Jasco V-630, Japan) before its degradation and was compared with the curve after its degradation at the optimum conditions[14].

Assessing of phytotoxicity

This experiment was performed to assess whether the decolorized dyes are toxic or not. In this study, *Trigonella foenum-graecum* (fenugreek), *Vicia faba* (faba bean), and *Lens culinaris* (lentil) are used. At room temperature 7, 7, and 5 seeds of fenugreek, faba bean and lentil, respectively, were placed separately into petri dishes which are embedded with cotton. Then, about 10 ml of the DR81 and its degraded solution are added separately. Control experiments of each crop were carried out with the addition of water. The plates were regularly irrigated by dye solution, decolorized dye, and water. This method is according to Gul et al.[37] with slight modification.

According to Gomare et al.[41] the determination of toxicity was assessed in terms of percentage germination, lengths of shoot and root after 7 days. The relative seed germination (SG%), relative shoot and root elongation (SE%) and (RE%), and germination index (GI%) were calculated through the following formula:

$$\text{Relative seed germination (SG\%)} = \frac{\text{No of seeds germinated in the treated sample}}{\text{No of seeds germinated in the control}} \text{X100} \quad (2)$$

$$\text{Relative shoot elongation (SE\%)} = \frac{\text{Mean shoot elongation in the treated sample}}{\text{Mean shoot elongation in the control}} \text{X 100} \quad (3)$$

$$\text{Relative root elongation (RE\%)} = \frac{\text{Mean root elongation in the treated sample}}{\text{Mean root elongation in the control}} \text{X100} \quad (4)$$

$$\text{Germinationindex(GI\%)} = \frac{\text{SG\% X RE\%}}{100} \quad (5)$$

Measuring of biological and chemical oxygen demand

DR81 dye solution with a concentration of 99.8 mg/L and the degradation metabolites of DR81 after decolorization by *P. mononematosum* in the optimum conditions were sent to the water pollution department, National Research Centre (NRC), Egypt, for determination the values of biological oxygen demand (BOD) and chemical oxygen demand (COD). The COD and BOD removal percentages were calculated using the following equations[42], respectively.

$$\text{COD removal } (\%) = \frac{\text{Initial}_{(\text{COD})} - \text{Final}_{(\text{COD})}}{\text{Initial}_{(\text{COD})}} \text{x}100 \quad (6)$$

$$BOD\ removal\ (\%) = \frac{Initial_{(BOD)} - Final_{(BOD)}}{Initial_{(BOD)}} x100 \quad (7)$$

Statistical analysis

The experiments were performed in triplicates and the results obtained are expressed as the mean $\pm$ standard deviation. Statistical analyses were performed using GraphPad Prism 8. One-way analysis of variance (ANOVA) and Tukey−Kramer multiple comparisons test along with independent samples t-test were used for data analysis. The results were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

In this study the remediation of the toxic azo dye DR81 was carried out by fungi where they have proved to be suitable and efficient in the treatment of dyes. The additive advantage of fungi over other microorganisms is solubilizing the insoluble substrates by extracellular enzymes that can tolerate high concentrations of toxicants. Also, fungi have a greater contact with the environment due to increased ratio of cell-to-surface[43].

Determination of λ-max for DR81

The absorbance of DR81 dye was measured from 200 to 900 nm by using UV-VIS spectrophotometer (Jasco V-630, Japan) for determining the wavelength having the maximum absorbance. The maximum peak of DR81 dye was found to be 500 nm. This value of λ max was used to detect the decolorization percentages of the dye.

Isolation and identification of fungi

Thirteen fungal isolates were isolated from dyes industrial effluents of two industries. The codes 1A, 1B, 1C, 2C, 1F, 2F, 1G, 2G, 3G, 1H, 2H, 3H, and 4H were given for the isolated fungi. These fungi were purified for identification and screening their ability to decolorize DR81 dye. The genera of the isolated fungi with their diagnostic characters are in the **Table 2**.

The species of the isolated *Aspergillus* along with their diagnostic characters are in **Table 3.** The species of the isolated *Penicillium* and their diagnostic characters are in **Table 4.**

Microscopic observations such as spores and their arrangement, conidiophores, conidia, phialide, and chlamydospore and colony characteristics are used in the identification of the *Trichoderma* sp. The identified *Trichoderma* sp. is in the **Table 5.**

From these, the obtained fungal isolates were identified as: *A. flavus* (4 isolates), *P. roqueforti* (1 isolate), *A. nidulans* (1 isolate), *P. purpurogenum* (2 isolates), *T. viride* (1 isolate), *A. parasiticus* (1 isolate), *A. niger* (1 isolate), *P. mononematosum* (1 isolate), and *A. fumigatus* (1 isolate). The microscopic appearance of the fungi is in the **Fig. 2.**

Table 2: Genera of the isolated fungi with their diagnostic characters.

	Genus		
	Aspergillus	***Penicillium***	***Trichoderma***
Diagnostic Characters	- Distinct erect conidiophores arising from thick-walled foot cells and ending in vesicles.	- Branched and long thread-like filamentous hyphae.	- onidiophores irregularly branched.
	- Phialides arising from the vesicle or from metulae.	- They did not have a distinct vesicle. Penicilli is branched below the sterigmata.	- Phialides are short and arising from the conidiophores.
	- Conidia produced from phialides in basipetal succession.	- The conidia are produced in chains from the tips of the sterigmata.	- Fast growth in culture medium with green color of conidia.
Isolation Code	1A, 1C, 2C, 1G, 2G, 3G, 1H, and 3H	1B, 1F, 2H, and 4H	2F

Table 3: The identification of the species of the isolated *Aspergillus*.

***Aspergillus* Spp.**	**Diagnostic Characters**	**Isolation Code**
Aspergillusflavus var. columnaris	- The colonies on Cza medium after 7 days of incubation are 5 cm in diameter with dark green color and white thin margin. The exudates are lacking and the reverse is hyaline.	1A, 3G, and 1H
	- Under microscope, conidial heads are columnar and metulae absent. Conidiophores are long, hyaline and smooth.	
	- Sterigmata are uniseriate and ampuliform. Vesicles are subglobose, 33 µm in diameter and fertile over most of its surface.	
	- Conidia are 3.3-5.0 µm in diameter and globose to subglobose.	
Aspergillusflavus	- The colonies on Cza medium after 7 daysof incubation are 5-6 cm in diameter, yellow-green in color, flat, white with thin margin, exudate lacking and hyaline reverse.	1C
	- Under microscope, the conidial heads are radiate and metulae present. Conidiophores are long, hyaline, and coarsely roughened.	
	- Sterigmata are biseriate and ampuliform. Vesicles are globose to subglobose, 45 µm in diameter and fertile over most of its surface.	
	- Conidia are globose to subglobose and 3.5 – 5.0 µm in diameter.	
Aspergillusnidulans	- The colonies on Cza medium after 7 days of incubation are 5-7 cm in diameter with white margin and exudate lacking. The color is smoky green with reverse in purple red.	2C
	- Under microscope conidiophores are short, colored and smooth. Conidial heads radiate and metulae present.	
	- Sterigmata are biseriate and the vesicles are subglobose.	
	- Conidia are globose to subglobose and 3-3.5 µm in diameter. Cleistothecia are globose and solitary. The colored ascospores are present.	
Aspergillusparasiticus	- The colonies on Cza medium after 7 days are 5 cm in diameter with deep green color, wrinkled and white thin margin. The exudates are lacking and the color of reverse is slightly creamy.	1G
	- Under microscope, conidial heads are loosely radiate and metulae absent. Conidiophores are long, smooth below and rough above.	
	- Sterigmata are uniseriates. The vesicles are subglobose with 8-25 µm in diameter and fertile over most of their surface.	
	- Conidia are 4.0-5.0 µm in diameter and globose with coarsely echinulate.	
Aspergillusniger	- The colonies on Cza medium after 7 days of incubation are 5.5 cm in diameter, black in color with white to pale yellow margin, exudate lacking and hyaline reverse.	2G
	- Under microscope, conidial heads are black in color and metulae present. Conidiophores are smooth, hyaline at the base and brownish at the apex.	
	- Sterigmata are biseriate and the vesicles are globose to subglobose, 75 µm in diameter and fertile over the whole surface.	
	- Conidia are 3-4.2 µm in diameter and globose to subglobose.	
Aspergillusfumigatus	- The colonies on Cza medium after 7 days are 6.5 cm in diameter and smoky green in color with suede-like surface and white margin with hyaline reverse.	3H
	- Conidial heads are columnar, compact and metulae absent. The conidiophores are hyaline, short, and smooth.	
	- Sterigmata are uniseriate and ampuliform. Vesicles are closely packed with flask-shaped and 8-25 µm in wide. The surfaces of the vesicles are not completely fertile. Conidia are globose to subglobose and 2.5-4 µm in diameter.	

Table 4: The identified species of the isolated *Penicillum.*

Species	Diagnostic characters	Isolation Code
P. roqueforti	- The colonies on Cza medium after 7 days of incubation are 4-5 cm in diameter, velvety, green in color, and smooth with reverse dark green.	1B
	- Conidial heads are large, asymmetric with three-stage branching. Conidiophores are strictly mononematous.	
	- Stipes tuberculate, short, and rough-walled.	
	- Phialides are flask-shaped with a short wide neck. The branching pattern is asymmetric terverticillate.	
	- Conidia are relatively large, smooth, nearly globose, dark green in color, and 4-6 µm in diameter.	
P. purpurogenum	- The colonies on Cza medium after 7 days of incubation are 4-5 cm in diameter, velvety, greyish green in color with heavy sporing and red pigment on the back of the colony. The colonies did not produce cleistothecium or sclerotium.	1F and 4H
	- Conidial heads narrow and funnel-shaped.	
	- Conidiophores are long and smooth bearing narrow conidial heads with narrow cells supporting the conidiogenous cells.	
	- Stipes are smooth, hyaline, conspicuously encrusted and 50-300 µm in length.	
	- Phialides are uninucleated, acerose and there are 3 to 5 phialides per metula.	
	- The branching pattern is symmetrical biverticillate.	
	- Conidia are elliptical, globose, and rough with apiculate walls.	
P. mononematosum	- The colonies on Cza medium after 7 days of incubation are 3 cm in diameter, non-fasciculate colony texture with slow growth.	2H
	- Conidial heads are funnel-shaped.	
	- Conidiophores are mononematous and smooth.	
	- Phialides are small with broadly cylindrical base and a short narrowed neck.	
	- The branching pattern is terverticillate.	
	- Conidia are greyish green, subglobose and smooth.	

Table 5: The identification of the isolated *Trichoderma*sp.

Species	Diagnostic Characters	Isolation Code
T. viride	- The growth was fast and the colonies on Cza medium after 7 days of incubation are 7 cm in diameter with green color.	2F
	- Under microscope, conidiophores appear with central axis 100–150 µm in length.	
	- Branches pair and arise at nearly right angle with respect to its supporting branch.	
	- Phialides are 9 µm in length, cylindrical, swollen in the middle with elongated neck, straight, sinuous and singly arising.	
	- Pustules are 0.5–1.0 mm in diameter, hemispherical, and uniformly cottony.	

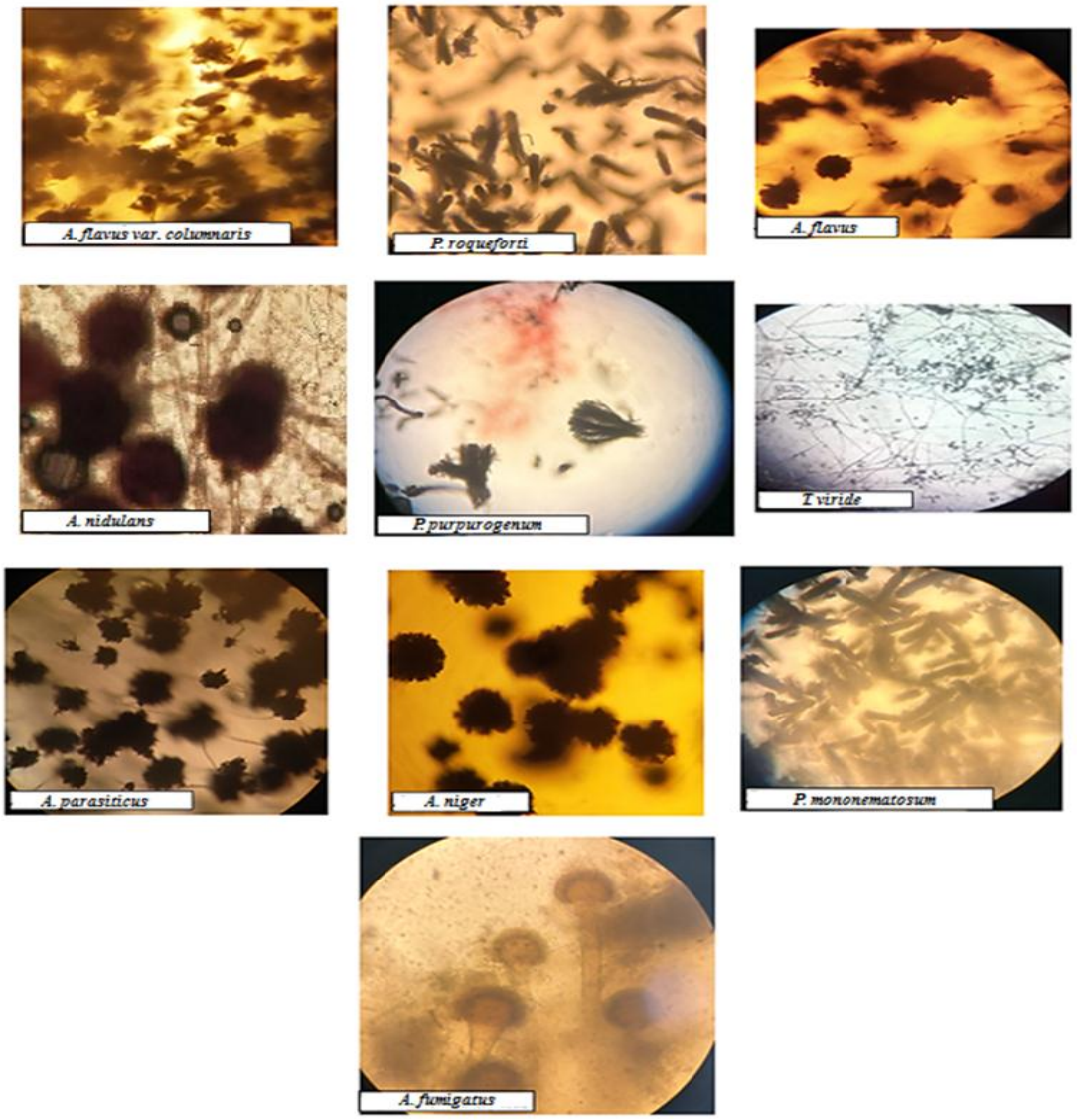

Fig. 2: The view of fungal isolates under the microscope.

Screening of fungal isolates for decolorizing of the DR81 dye

In recent years, dye decolorization studies involved cultivating fungal cells in liquid media containing dyes has been employed in several studies[44]. The azo dyes biodegradation by fungi varies depending on the mechanism which followed by different fungi for dyes decolorization[45]. The obtained fungal isolates in this study were screened for their ability to degrade DR81 dye. The decolorization percentages of DR81 dye are presented in **Table 6**.

The results in **Table 6** indicate that most of the obtained fungal isolates have ability to decolorize DR81, it also could be noticed that the fungal isolates revealed different decolorization percentage of the DR81 dye. The most potent fungal isolate in the decolorization of DR81 is *P. mononematosum* with 88.85% decolorization percentage. This fungus was selected for further investigation in the following experiments. In a previous study by Hefnawy et al.[3], different fungal isolates showed different decolorization percentages of direct blue dye. The fungi *A. flavus* exhibited the highest decolorization percentage followed by *P. canescens*,*P. crustosum*,*P.* sp., *Fusarium* sp., and *A. niger*.

Table 6: The decolorization percentages of DR81 dye by the isolated fungi.

Isolate code	Scientific name	Decolorization percentage of DR81 dye (%)
1A	*A. flavus var. columnaris*	51.78±1.5
1B	*P. roqueforti*	83.44±2.1
1C	*A. flavus*	55.31±1.9
2C	*A. nidulans*	40.09±1.6
1F	*P. purpurogenum*	52.92±1.4
2F	*T. viride*	62.62±1.2
1G	*A. parasiticus*	33.01±1.7
2G	*A. niger*	16.30±1.0
3G	*A. flavus var. columnaris*	65.91±1.4
1H	*A. flavus var. columnaris*	60.67±1.5
2H	*P. mononematosum*	88.85±0.8
3H	*A. fumigatus*	76.46±1.3
4H	*P. purpurogenum*	44.90±1.6

Optimization of the degradation process parameters

To maximize the efficiency of dyes decolorization by fungi, the optimal conditions are essential[38]. The fungus growth, production of enzymes and dye decolorization rate and efficiency are influenced by various parameters such as temperature, pH, initial dye concentration, and static and shaking conditions. In this study, the efficiency of the *P. mononematosum* to decolorize DR81 dye under different conditions was investigated for determining the optimal ones. These parameters include (pH, temperatures, dye concentration, incubation period, static and shaking, light and dark). The results of these experiments are showed in the following.

Influence of pH on the decolorization process

One of the most important factors in dye decolorization process is the pH which influences on the properties of dyes and the charge of the biosorbent surface[46]. At lower pH, the charge of the fungal biomass is positive and thus the charged sites become available for binding anionic groups of dyes[47]. The optimum pH value varies according to the fungal species and the type of dye. It was found that the growth of the fungi is affected largely by the pH and they are usually found to grow at low pH. In addition, efficient dye decolorization mostly observed at low pH[44].

In this study, the results of the effect of different pH (5, 6, 7, 8, 9, and 10) on the decolorization of DR81 dye by *P. mononematosum a*reshown in the **Fig. 3**.

From **Fig. 3**, the optimum pH for the degradation process of DR81 by *P. mononematosum* is 5 with a decolorization percentage of 70.62%. As the pH increasing, the decolorization percentage decreases. At the alkaline pH values 9 and 10, the decolorization decreasing obviously. The decolorization percentage in the pH 5.0 is significantly different from the others pH values. These results are in agreement with that by Hefnawy et al.[3] who found that the optimum pH for decolorization of direct blue dye by *P. canescens* is 5. Also Yan et al.[46] found that the optimum pH for decolorization of congo red by *P. janthinellum* is 6.0. In addition Namdhari et al.[48] reported that acidic condition facilitates better dyes removal by fungi. However, in astudy bySalem et al.[13] the optimum pH of the decolorization of two azo dyes by *Aspergillus niger* was 9.

Influence of temperature on the decolorization process

Temperature is another important factor in the process of dye decolorization. It can influence indirectly on the removal of dyes by fungi and directly on the growth and morphology of mycelia and enzyme activities[47]. Different fungal isolates have different optimum temperatures. It was found that the optimum temperatures is around 25-37ºC for most of the fungi[45]. Beyond the optimum temperature, denaturation of enzymes can occur

which resulting from the breakdown of the weak hydrogen and ionic bonding that stabilize the active site of the enzyme[3,49]. In **Fig. 4** the result of effect of different temperature degrees on the degradation of DR81 dye by *P. mononematosum* is shown.

Fig. 4 shows that the optimum temperature degree for the decolorization of DR81 is 25ºC with 72.89% decolorization percentage and the further increase in temperature resulted in decrease in decolorization activity. This decrease could be as a result of decrease of growth or due to the denaturation of some enzymes. The decolorization percentage in the 25ºC is significantly different from 35ºC and 40ºC. In a study by Akdogan et al.[50],the optimum temperature for decolorization of the azo dye reactive blue 19 by *Coprinus plicatilis* was 26ºC.Yan et al.[46] found that *Penicillium janthinellum* decolorize Congo red more efficient in 30ºC. However the optimum temperature for *Penicillium canescens* was 35ºC in the decolorization of direct blue dye[3].

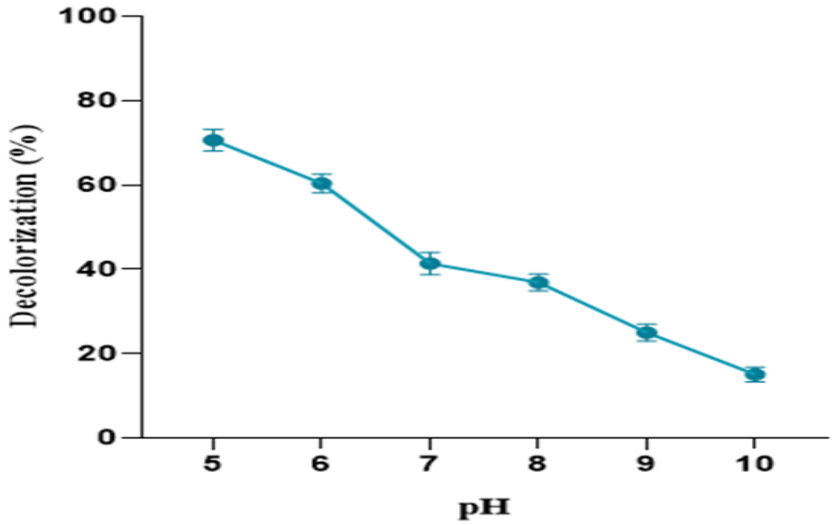

Fig. 3: The influence of pH on decolorization of DR81 dye.

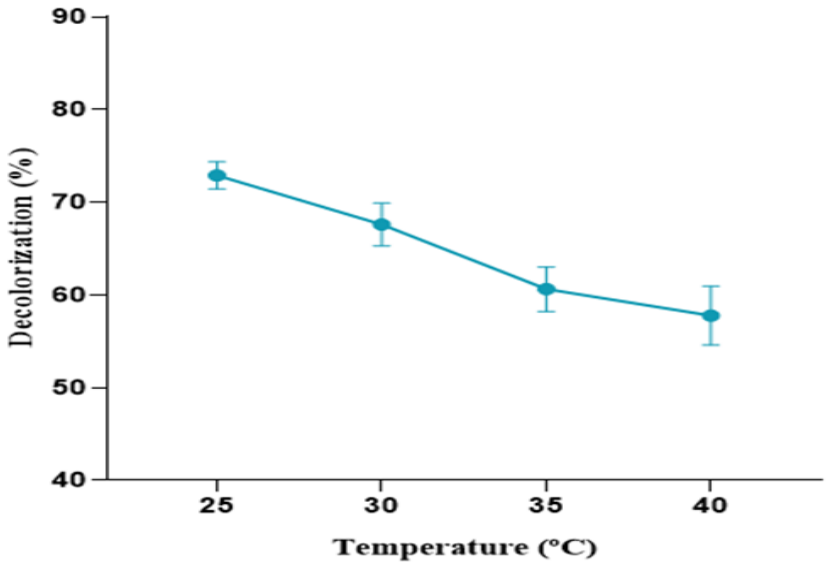

Fig. 4: The influence of temperature on decolorization of DR81 dye.

Influence of different dye concentrations on the decolorization process

It was found that dye concentration have a pivotal role in the decolorization process where high dye concentration may influence on growth of the fungi. In addition, blocking of the active sites of enzymes can occur by the dye molecules[44]. The effect of different concentrations of DR81 with a final concentration 99.8, 199.2, 298.21, 396.83, and 495.05 mg/L on the decolorization percentages by *P. mononematosum* was observed. The results are presented in the **Fig. 5**.

From **Fig. 5**, it can notice that when the dye concentration increases the decolorization percentage decreases. The optimum concentration for the decolorization of DR81 is 99.8 mg/L with 78.26% decolorization percentage. The concentration of 99.8 mg/L is significantly different from the other tested concentrations in its effect on the decolorization percentage. It was reported by Kamal et al.[8] that sulphonated azo dyes such as DR81 can inhibit microbial growth which causes decreasing in the ability for dye decolorization. In a study by Sahasrabudhe et al.[51] on the decolorization of DR81, more than 80% decolorization percentage was at 500 mg/L dye concentration and lower decolorization was at 600–700 mg/L. In another study by Junnarkar et al.[52] the rate of decolorization of DR81 by bacterial consortium increased with increase in dye concentration up to 200 ppm. Then the additional increase in dye concentration decreased the decolorization rate.

Influence of different incubation periods on the decolorization process

Fig. 6 represents the effect of different incubation periods 1, 3, 5, 6, and 7 days on the decolorization percentages of DR81 by *P. mononematosum*. The results proved that the decolorization activity reached the maximum value after 6 days of incubation with 78.38% decolorization percentage and then reaching a steady state. The percentage of the DR81 decolorization after 6 days of incubation is significantly different from 1, 3, and 5 days. In a study by Pundalik et al.[53], authors showed that the optimum time is 6 days for the decolorization of four azo dyes (acid violet 49, acid orange 7, basic yellow 3, and basic blue 3) by different fungal isolates.In another study byHefnawy et al.[3],decolorization of direct blue dye by *P. canescens* reached its maximum percentage after 7 days of incubation. Gul et al.[37] studied the decolorization of the azo dye Congo red to be optimum after 4 days by the fungal isolate *A. flavus.*

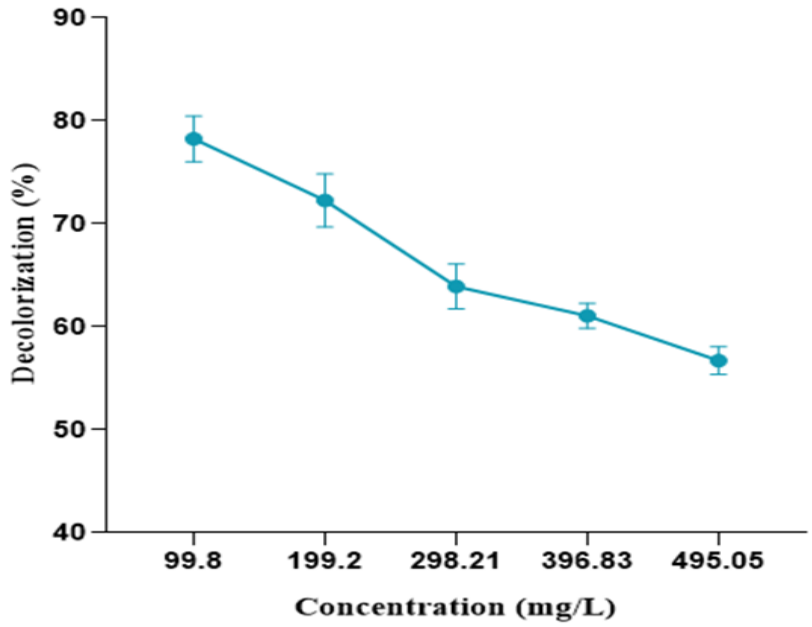

Fig. 5: The influence of different concentrations on decolorization of DR81 dye.

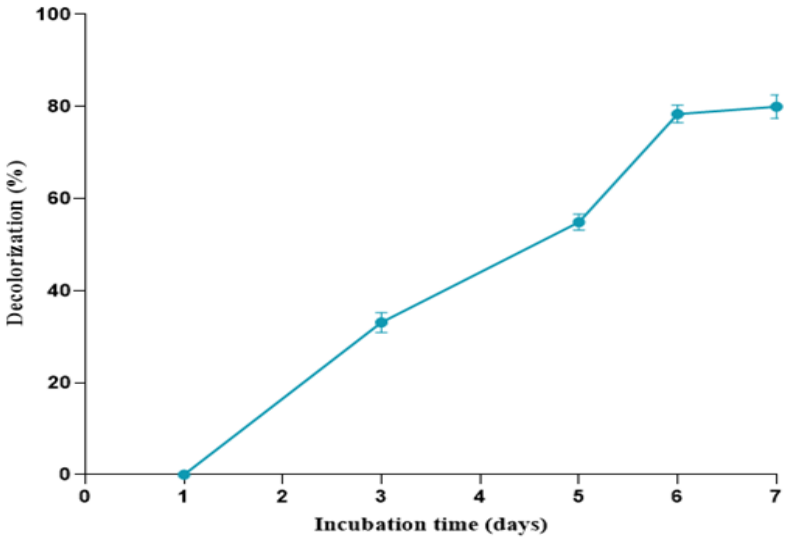

Fig. 6: The influence of different incubation time on decolorization of DR81 dye.

Influence of static and shaking conditions on decolorization percentage

The efficiency of *P. mononematosum* to decolorize DR81 dye under both static and shaking conditions is in the **Fig 7**.

Fig. 7 shows that the shaking condition is optimum for decolorization of DR81 and significantly different from static condition with 88.77% decolorization percentage. The previous studies showed that most of the decolorization experiments by fungi give higher removal percentage in shaking than in the static conditions where the better transfer of oxygen improves the contact between the biomass and dyes and also increase the distribution of nutrients[47,54]. According to Revankar and Lele[55], *Ganoderma* sp. decolorized amaranth dye with 75% decolorization percentage in agitated cultures while only 27% was in static condition. Also many authors showed that shaking enhanced the effectiveness of the decolorization[45,54,56]. On the contrary Rani et al.[57]found that shaking condition inhibited the decolorization of Amaranth by *Daedalea flavida*.

Influence of light and dark conditions on the decolorization percentage

The influence of light and dark conditions on degradation ability of *P. mononematosum* on DR81dye was studied. The results are illustrated in the **Fig 8**.

Fig. 8 shows that the dark condition for decolorization of DR81 is significantly higher than the light condition with 95.48% decolorization percentage with compared to 86.50% in static condition.There are some studies which includes the effect of the light and dark on the fungal growth. In a study by Velmurugan et al.[40] found that incubation in total darkness increased the biomass in the five tested fungal isolates *Monascus purpureus*, *Isaria farinosa*, *Emericella nidulans*, *Fusarium verticillioides* and *Penicillium purpurogenum*. However Hill[50] showed that the production of conidia in *Aspergillus ornatus*occur when grown in light while in the darkness the production is few.

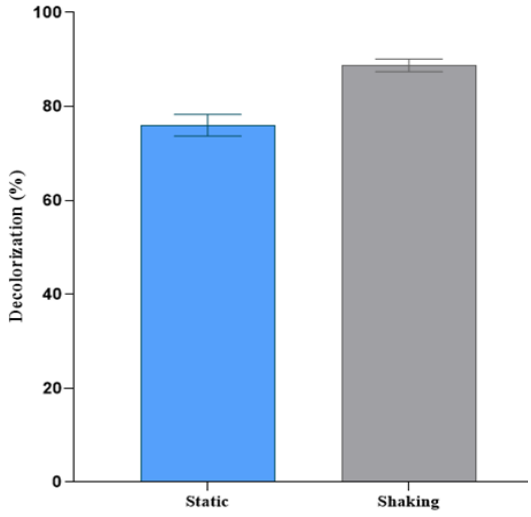

Fig. 7: The influence of static and shaking conditions on decolorization of DR81 dye.

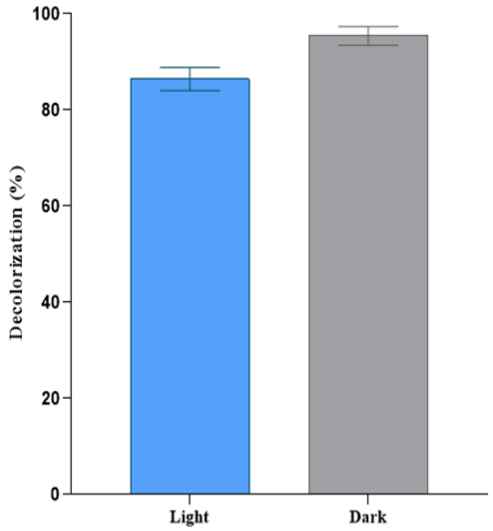

Fig. 8: The influence of light and dark conditions on decolorization of DR81 dye.

Spectrophotometer analysis of decolorized dye

The DR81 dye was analyzed by UV-VIS spectrophotometer (Jasco V-630, Japan) before and after its degradation. The curve is showed in the **Fig. 9**.

This figure presents that the maximum peak of the curve disappeared after the decolorization of the DR81 by *P. mononematosum* which indicate that the dye is degraded under optimized conditions (pH 5.0, temperature 25ºC, 99.8 mg/L dye concentration, 6 days incubation, shaking in darkness). In a study by Amin et al.[58], the peak of the absorption maximum of DR81 dye in the UV-VIS spectra disappeared after its biodegradation which indicates the decolorization of the dye.

Assessing of phytotoxicity

The dye decolorization is usually checked by observing the dye concentration after the degradation. However, the dye decolorization is not always causes reduced toxicity. Incomplete degradation may form more toxic intermediary metabolites than the parent dye[47]. Therefore, it is required to study the phytotoxicity of the degraded metabolites of the dyes. In this study, the effect of DR81 dye and its degradation metabolites on the germination percentages of fenugreek, faba bean and lentilwas investigated in comparison to a distilled water (control) treatment. The germination index of fenugreek is in the **Table 7**.

Table 7 indicates that the germination percentage and the GI in the case of the degraded dye is 76.32%and is significantly higher than in the case of the non-degraded dye which is 38,05% which means that the toxicity of the dye is reduced due to the degradation of DR81 by *P. mononematosum*.

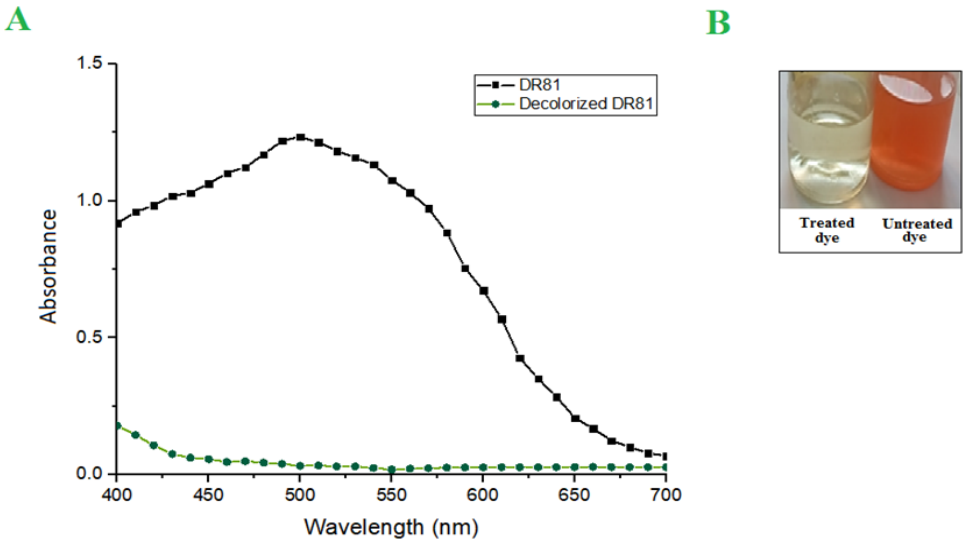

Fig. 9: (A) The difference in UV spectra between DR81 dye and decolorized DR81 after treatment under the optimal conditions; (B) The appearance of the untreated and treated dye**.**

Table 7: The germination index of fenugreek with DR81 dye and its degradation metabolites.

Parameters studied	Distilled water (control)	Decolorized DR81 dye	DR81 dye
SG%	100	100	85.71*
Mean of root elongation (cm)	1.58±0.03	1.2±0.02*	0.7±0.01*
Mean of shoot elongation (cm)	4.8±0.02	4.3±0.05*	1.58±0.03*
RE%	100±1.60	76.32±1.0*	44.40±0.63*
SE%	100±0.32	88.4±1.04*	32.8±0.52*
GI%	100±1.60	76.32±1.0*	38.05±0.54*

'*' indicate that the value is significantly different ($P < 0.05$) from the control

In the **Table 8**, the germination index of faba bean which was irrigated by DR81 dye and its degradation metabolites along with control is presented.

Table 8 shows that the percentage of germination, root and shoot elongation and GI of faba bean in the case of the degraded dye are higher than in the case of the non-degraded dye. This indicates that the toxicity of the DR81 dye is decreased after degradation.

The germination index of lentil which was irrigated by DR81 dye and its degradation metabolites along with distilled water as control is showed in **Table 9.**

Table 9 presents that the percentage of germination inhibition is 20% in the case of DR81 also the elongation of the root and shoot and GI of lentil are lower with compared to the degraded metabolites which indicates that the toxicity of the dye is reduced by fungal degradation of DR81. In a study by Yan et al.[46],authors found that the germination rate of *Triticum aestivum* L. that were irrigated by Congo red dye and its degraded solution was significantly affected when treated with the dye with compared to its degraded solution.

Table 8: The germination index of faba bean with DR81 dye and its degradation metabolites**.**

Parameters Studied	Distilled water (control)	Decolorized DR81 dye	DR81 Dye
SG%	100	100	71.43*
Mean of root elongation (cm)	6.53±0.25	5.8±0.1*	1.3±0.15*
Mean of shoot elongation (cm)	4.93±0.15	3.6±0.15*	1.17±0.15*
RE%	100±3.9	88.78±1.5*	20.41±2.3*
SE%	100±3.1	73.65±3.1*	23.65±3.1*
GI%	99.97±3.8	88.78±1.5*	14.58±1.7*

'*' indicate that the value is significantly different ($P < 0.05$) from the control.

Table 9: The germination index of lentil with DR81 dye and its degradation metabolites.

Parameter Studied	Distilled water (control)	Decolorized DR81 dye	DR81 Dye
SG%	100	100	80*
Mean of root elongation (cm)	1.9±0.1	1.6±0.09	0.5±0.1*
Mean of shoot elongation (cm)	4.1±0.1	3.6±0.1*	1.3±0.2*
RE%	100±5.3	84.21±5.3*	26.32±5.3*
SE%	100±2.4	87.8±2.4*	31.71±4.9*
GI%	100±5.3	84.21±5.3*	21.05±4.2*

'*' indicate that the value is significantly different ($P < 0.05$) from the control.

Measuring of biological and chemical oxygen demand

To determining the degree of pollution, the COD and BOD values are important parameters and their decreasing indicates degradation[8]. In this study, the COD and BOD were measured before and after dye degradation for determining the effect of the treatment on their percentages of removal. The values of BOD and COD removal percentages are in **Table 10**.

This Table indicates that the percentages of removal of COD and BOD of DR81 are high and it 79.94% and 77.76%, respectively. In a study by Selim et al.[16], the treatment of the crude textile wastewater with *A. flavus*, *Fusarium oxysp*orum, or their consortium reduces the COD, BOD with varying percentages which indicates the toxicity is reduced.

Table 10: The COD and BOD removal percentages of DR81 dye and its degradation metabolites.

Parameter	Percentage (%)
COD removal	79.94 ± 0.08
BOD removal	77.76 ± 0.25

Conclusion

The current study was carried out to investigate the efficient decolorization and degradation of the toxic azo dye DR81 by fungal isolate from dyes industrial effluents. Along the tested fungal isolates, *P. mononematosum* showed the highest DR81 decolorization ability. The parameters were optimized for achieving maximum dye decolorization. The selected fungal isolate decolorize DR81 optimally at pH 5.0, 25ºC under shaking and dark conditions in 6 days and a dye concentration of 99.8 mg/L. The UV-VIS spectra of DR81 and its degraded metabolites by *P. mononematosum* under optimized conditions showed disappearance of essential peak of the DR81 dye after decolorization. The phytotoxicity study was carried out to assess the phytotoxicity of DR81 before and after decolorization on germination of *Trigonella foenum-graecum, Vicia faba and Lens culinaris.* Furthermore, 79.94% and 77.76% reduction percentages of COD and BOD, respectively, of the DR81 were achieved.The results showed that the toxicity of the DR81 has been reduced after decolorization by *P. mononematosum*. The present study proved the efficiency of fungal isolate from samples of dyes industrial wastewater to degrade and detoxify DR81 dye.

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التكسير الحيوى للصبغة الحمراء المباشرة ٨١ ثنائية مجموعة الأزو بواسطة الفطريات المعزولة من مياه الصرف الصناعى المحتوية على الصبغات

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إن مياه الصرف الصناعى المحتوية على صبغات الأزو تكون سامة ومن الممكن أن تسبب العديد من التأثيرات الخطيرة على البيئة لذلك يجب معالجة هذه المياه قبل تصريفها إلى البيئة. و الصبغة الحمراء المباشرة ٨١ هى من صبغات الأزو التى من الممكن أن تكون سامة للانسان والحيوان والبيئة المائية. معالجة هذه الصبغات الصناعية بالفطريات يعتبر طريقة فعالة ومناسبة. الهدف من هذه الدراسة هو عزل فطريات لها القدرة على إزالة لون وتكسير الصبغة الحمراء المباشرة ٨١. تم اختبار قدرة ١٣ فطر ينتمون إلى *Trichoderma* sp. ، *Penicillium* spp. ، *Aspergillus* spp. على تكسير الصبغة الحمراء المباشرة ٨١. قد وجد أن الظروف المثلى لإزالة لون الصبغة بالفطر الذى لديه أعلى قدرة هى عند درجة الحموضة ٥.٠ ودرجة حرارة ٢٥ درجة مئوية فى ظروف الاهتزاز والظلام بعد ٦ أيام من التحضين و ٩٨.٨٥مجم/لتر تركيز للصبغة. كانت نسب إزالة اللون وإزالة الاحتياج الأكسجينى الكيميائى والحيوى هى ٩٥.٤٨٪، ٧٩.٩٤٪، ٧٧.٧٦ ٪ على التوالى. تم تأكيد أن أعلى قمة فى التحليل الطيفى للأشعة فوق البنفسجية والمرئية قد اختفت بعد إزالة لون الصبغة الحمراء المباشرة ٨١ فى الظروف المثلى. وقد دلت اختبارات السمية النباتية على الحلبة والفول والعدس أن سمية الصبغة قلت بعد إزالة لونها بالفطر الأكثر فعالية. أظهرت النتائج كفاءة العزلة المختارة فى معالجة الصبغة الحمراء المباشرة ٨١.