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Biological decolorization of Congo red from textile effluent and wastewater by *Aspergillus terreus and Penicillium funiculosum*

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Abstract

The goal of this study was to isolate several fungus from soil and polluted water samples that were taken from textile facilities in the Thi Qar province. The most prevalent and highly potential to remove Congo Red dye among the isolated fungi were *Aspergillus terreus* and *Penicillium funiculosum*, according to the data. In addition to ITS gene sequencing, morphological and cultural characteristics were used to identify these isolates. The azo dye concentration, pH, carbon source, and nitrogen source were all varied during the dye removal procedure. According to the findings, both fungus were able to break down Congo Red dye at concentrations of 50 and 150 ppm, however the fungi *Penicillium funiculosum* and Aspergillus terreus were nearly fatal at 250 ppm.. The maximum color removal efficiency of the dye at a concentration of 50 ppm was recorded as (94.33%, 93.33%) for the fungi *Penicillium funiculosum* and *Aspergillus terreus*, respectively, at pH=10. However, at pH=7, the proportion of color removed from *Penicillium funiculosum* and *Aspergillus terreus* respectively, was 88.21% and 79.62%). After seven days of shaking conditions the studies were carried out at 37°C using glucose and NH_4Cl present as suppliers of carbon and nitrogen. By analyzing the treated dye's ultraviolet-visible (UV-Vis) spectrum, the decolorization % was verified. The reactive dye reached its maximum peak at 489 nm at pH=7 and 490 nm at pH=10, indicating that the fungi's biological activity was the cause of the dye's decolorization. This confirms that pH=10 is the ideal value for decolorization, as indicated by the fungi's increased dry weight. For *Penicillium funiculosum* and *Aspergillus terreus*, the dry weight at pH=10 was 2.60 and 2.47 grams, respectively. Conversely, however,

Keywords: *Aspergillus terreus, Penicillium funiculosum*, textile wool factories, Azo dye, Decolorization, Congo red, (UV-Vis) spectrum

Introduction

Because wastewater contains a wide variety of pigments, some of which are carcinogenic and mutagenic, it is regarded as a hazardous source of pollutants for all living things [1]. Human kidney، liver، brain، reproductive, and central nervous system functioning are among the effects of dyes [2]. Heavy industries include textile paint, mining chemical dyestuff, and battery manufacture are common sources of wastewater. electroplating, and metal finishing, can release a significant amount of both organic and inorganic pollutants into the atmosphere [3].

In the paper paint culinary cosmetic textile and leather sectors among others dyes are a vital resource. Approximately twenty-five different types of dye groups are available depending on the chromophore's chemical structure [4]. Thousands of dyes have been identified as textile dyes, and they are used to color a wide range of textiles [5]. Precursors to dyes are called dye intermediates. With the help of certain chemical reactions, they can be extracted from unprocessed materials like naphthalene and benzene [6,7]. With two chromophoric groups (azo groups) in its structure Congo red dye (sodium salt of

benzidinediazo-bis-1-naphtylamine-4-sulfonic acid, C32H22N6Na2O6S2) is a classic diazo dye. It, is extremely soluble in water and enduring when released into an untreated natural area [8,9].

Pollution has a negative impact on the environment and can pose a direct or indirect health risk to all life forms on the planet [10,11].Based on their composition and use, dyes can be categorized. Because dyes are highly soluble in water, they are challenging to remove using conventional techniques [12,13]. Colors used in textile dye can harm artwork and prevent light from diffusing into the water, which lowers the amount of dissolved oxygen and slows down the pace at which aquatic life photosynthesizes [14]. The scientific world has been much more aware of biological approaches in the past few years. Compared to conventional procedures these methods have a number of advantages including reduced costs environmental friendliness, safe operation, and reduced sludge output. Nowadays, bioremediation is regarded as a potential therapy option for the elimination of dye under various circumstances. Because the bioremediation method's operating parameters and design are flexible it can destroy harmful compounds using both natural and recombinant microorganisms. The fact that they can be utilized ex situ, or off-site or in situ or even with plants, as in phytoremediation explains the technique's flexibility. The biologically aided breakdown of a dye molecule into many by-products through the action of different enzymes is known as biodegradation. It's an energy-dependent process [15]. Decolorization and the disintegration of the dye molecules into smaller pieces are the outcomes of dye biodegradation. Many microorganisms, including fungi, bacteria, and algae, are used to break down and decolorize manmade dyes. Numerous dyes can be decolored by microbes in different ways. When it comes to the biodegradation of synthetic colors, certain types of bacteria have distinct advantages over others. The efficiency of dye bioremediation depends on the activity and adaptability of the microorganisms [16]. The decolorization of dye waste water by fungi has been the subject of extensive investigation in the past few years. As a result, it is beginning to show promise as a replacement or substitute for current therapeutic procedures. The objective of this work is to isolate and characterize fungal strains that can effectively decolorize the textile dye, congo red (RD). The current study set out to find out how well *A. terreus* and *P. funiculosum* removed Congo red dye in different environments.

2-Material and Methods

2-1 Azo dyes and the gathering of samples

In the Thi Qar province, I gathered sediments and wastewater samples from textile and wool enterprises. Three laboratory stations were used to isolate fungi and three replicate samples were taken from each station. The same facility provided the congo red (CR) azo dye type that was utilized.

2-2 Fungi isolation

The fungi isolated from the sediment and water samples using the dilution method were subjected to one of the techniques after the samples gathered for fungal isolation were inspected. In particular, 9 ml of distilled water and 1 gram of dirt were successively added to 1 ml of waste water or 1 ml of water that had been serially diluted up to 10-4 [17]. Following homogenization, 1 milliliter of each prior dilution was taken out and placed onto sterile petri plates using the pour-plate method. Next, the antibacterial agent chloramphenicol (250 mg)

was added, and then the culture medium potato dextrose agar, or PDA was added. For seven days, the plates were incubated at 25°C.

2 -3 Molecular identification of fungi that degrade azo dyes

The internal transcribed spacer (ITS) region was amplified and sequenced in order to perform molecular identification. Using primers (ITS1 and ITS4), the internal transcription space region (ITS1-5.8S-ITS2) was amplified using polymerase chain reaction (PCR) technology. The source of these primers is Macrogen, Korea. Using the genomic DNA as a template and the ITS primers of ITS1 (5 π - TCCGTAGGTGAACCTGCGG -3 R) and ITS4 (5 R - TCCTCCGCTTATTGAT ATGC- 3 R), the ITS region was amplified using polymerase chain reaction (PCR).One microliter of isolated fungal genomic DNA, 0.5 micrograms of each primer and 50 microliters of Maxima Hot Start PCR Master Mix (Thermo) made up the PCR mixture. AL Ameen Fundation For Study used a DNA Engine Thermal Cycler to do the PCR. & Research (Najaf, Iraq) with a hot start that lasted for four minutes at 94ºC, thirty cycles of 94ºC, 56ºC, and 72ºC, and a final extension that lasted for seven minutes at 72ºC. At Macrogen Company (Korea), a DNA sequencer was used for the commercial sequencing. The NCBI BLAST tool was used to match the ITS sequence against the Gen Bank database. Then, using BLASTN, sequences were matched with ITS sequences in the Gen Bank database.

2-4 The capacity of separated fungus to proliferate on solid media enhanced with Congo red dye

In fifteen 250 ml conical flasks, potato dextrose agar (PDA) was used as the medium. The flasks were autoclaved for 20 minutes at 121 °C and 15 pounds per square inch of pressure. The culture medium was then heated to the proper temperature and three concentrations of the aromatic dye (congo red) were added for each component. (50, 150, 250) ppm each dye in three flasks. In order to provide a control for comparison, one flask was left empty. Subsequently, the 8.5 cm-diameter sanitized Petri dishes were filled with the medium, and they were allowed to dry for 30minutes.

A 4 mm-diameter disc of pure Aspergillus terreus and penicillium funiculosum, both at 7 days old, was used to pierce a sterile cork into center of each plate to introduce the fungal inoculum into the dishes. After that, the dishes were incubated for seven days at 25 °C. Three replications of each treatment were used in the experiment, and the colony diameter was used to calculate the fungi's growth rates[18].

2-5 The capacity of isolated fungus to proliferate in a medium containing mineral salts and added Congo red dye.

The solution of mineral salts was made ready for the growth of fungus. The following chemicals make up one liter of this medium: 1.71 g of K2HPO4; 1.32 g of KH2PO4; 0.42 g of NaNO3; 0.42 g of MgSO4.7H2O and 0.02 g of $CaCl_2$. After setting up fifteen(250) ml conical flasks, sterilize the medium in an autoclave for 20 minutes at 121 °C and 15 pounds per square inch of pressure. once the outside temperature has dropped .Next incorporate the

chosen aromatic dyes into the culture medium while lowering the temperature. Methylene blue is the chosen dye, and it is introduced to the medium at concentrations of 50 ppm, 150 ppm, and 250 ppm. Using different pH values (7, 10), repeat the experiment while adding sources of carbon and nitrogen. Once the carbon and nitrogen sources (glucose and NH_4CL) were added, add 0.1 g per liter for each addition. The flasks were then left empty of the material for comparison, and a disc was transferred to inoculate them. Using a sterile Cork Borer, 4 mm of 7-day-old fungal cultures for *Aspergillus terreus* and *Penicillium funiculosum* were examined. Three replications of each treatment were used in this experiment, which was conducted with the flasks incubated for seven days at a temperature of 25°C. The mycelium was weighed using a sensitive scale after being dried on filter paper for 30 minutes at 50°C in the oven[18].

2-6 **Maximum detection λ for every dye**

Congo red (CR) dye's absorption maxima (λ max) were found using a UV-visible spectrometer (MD 1105 PG instrument Ltd., UK). Every dye solution's optical density in water was measured at various wavelengths between the visible ranges (300–800 nm).

2-7 Using fungal isolate, decolorization(%) of congo red in a liquid media

They were tested for their capacity to decolorize azo dyes in MSM using the procedure outlined in [19]. After the fungal isolates were activated, 900 milliliters of mineral salt medium were ready. After adjusting, the pH was (7,10). Carbon and nitrogen supplies were provided in the form of glucose and NH_4CL. Each of the 250 mL flasks that were created was filled with 90 mL of the prepared mineral salt medium that had been combined with the previously indicated ingredients. Three doses of the aromatic dye Congo Red were applied to each flask (50, 150, 250) ppm. Each flask was supplemented with the dye-specific fungal isolates. To achieve decolorization, control flasks with MSM medium and Congo Red dye were made without the presence of fungal isolates. In order to zero the UV-visible spectrometer, blank flasks were made using the same growth media but without fungal isolates and dye. The flasks were kept in an incubator set at 25 °C for seven days. Following the incubation time, 10 mL from each flask were collected and put into a tube. centrifugation for ten minutes at 5000 rpm, and following the aforementioned additions, the supernatant was scanned in a UV/VIS spectrophotometer at particular wavelengths identified by scanning the dye samples. The following formula was used to calculate the percent decolorization in accordance with [19].

$$\textbf{Decolorization (\%) = [Dy (i) – Dy (1) / Dy (i)] X 100}$$

Where D, decolorization percentage %; Dy (i), initial absorbance;

Dy (1), Final absorbanc

Statistical analysis:

The present study conducted an Anova (analysis of variance) which was performed on all the treatments and done using the spss (version 23.0) package to determine whether or not significance difference.

RESULT

Fungal isolation and identification

In the current study, a number of fungi were isolated from water and soil samples of textile wool factories. There were two types of fungi that appeared most prominent among the isolated fungi that were used in the decolorization. The taxonomic status of fungal isolate was defined by sequencing of ITS genes. The morphological culture characteristic as well molecular identification based on ITS sequencing analysis for isolates was similar to *Aspergillus terreus* and *Penicillium funiculosum* as showed in Fig (1,2)

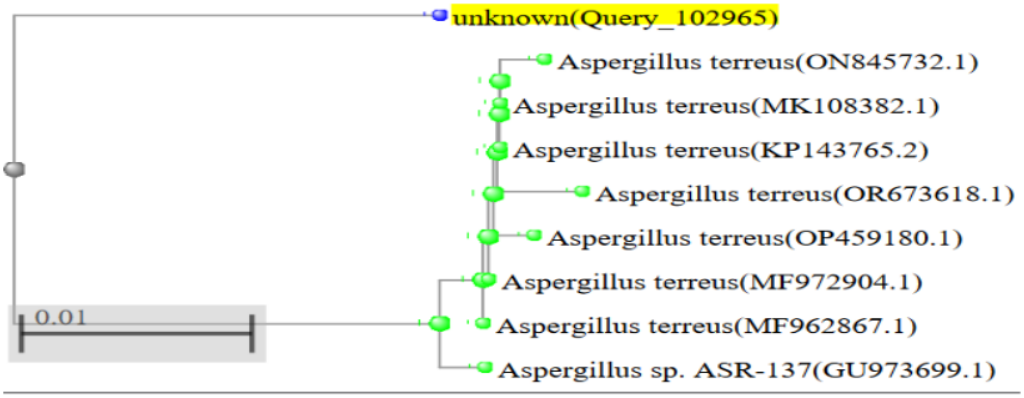


Fig. 1. Phylogenetic tree of ITS sequences of the fungal isolate with the sequences from NCBI and designated as *Aspergillus terreus*

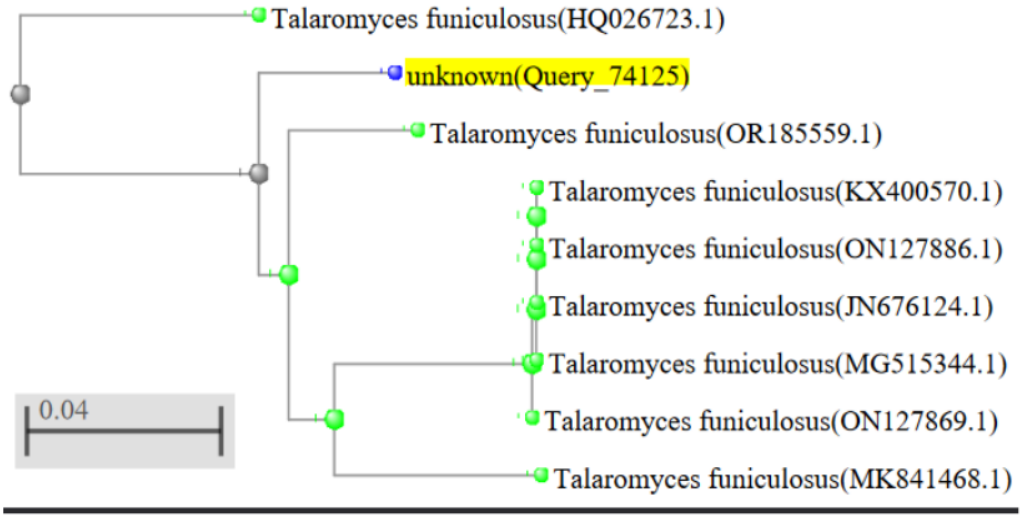


Fig. 2. Phylogenetic tree of ITS sequences of the fungal isolate with the sequences from NCBI and designated as *Penicillium funiculosum*

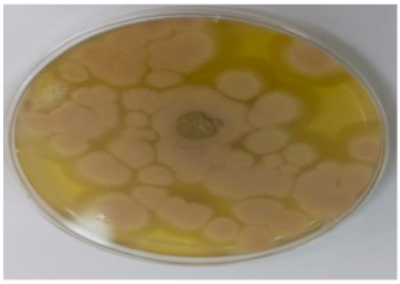
The capacity of separated fungus to proliferate on solid media enhanced with congo red dye

The development of fungi in PDA (Potato Dextrose Agar) medium treated with Congo Red dye is demonstrated by the data shown in (Table 1). The colony diameters of *Penicillium funiculosum a*nd *Aspergillus terreus* fungi are found to be growing every day. The findings showed that A. terreus and P. funiculosum were clearly resistant to all of the Congo Red dye doses that were employed. At concentrations of 250, 150, and 50 ppm, respectively, the colony diameter of *A.terreus* reached (8.83, 6.16, 7.00) cm, compared to the control's (9.00) cm. However, *P. funiculosum* shown a higher degree of resistance to the dye, as seen by colony diameters of (8.66, 7.33, and 6.50) cm for the identical concentrations employed, as opposed to 9.00 cm for the control.

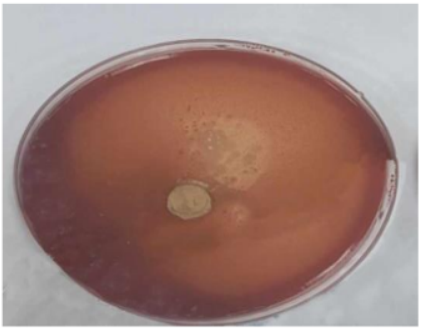
The data clearly show that, with the exception of the 250 ppm concentration, which had a minor impact on colony growth, none of the concentrations to which the fungus were exposed caused a substantial change in colony diameter when compared to the control. The fungi *P. funiculosum* and *A. terreus* had colony diameters of 6.50 and 6.16 cm, respectively. The statistical analysis, which revealed no significant variations between fungus and concentrations Table (1) fig (3,4), supported this.

Table(1): Ability the growth of *Aspergillus terreus and penicillium funiculosum in* solid medium with Congo red

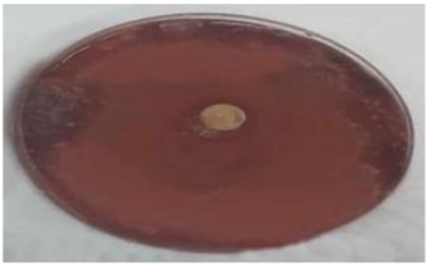
Fungi	Concentration(ppm)				Mean
	control	50	150	250	
Aspergillus terreus	9.0 ±0.0	8.83 ±0.28	7.0 ±0.50	6.16 ± 0.28	7.75
Penicillium funiculosum	9.0 ±0.0	8.66 ± .028	7.33 ±0.28	6.50 ± 0.00	7.87
L.S.D ((P<0.05)				L.S.D fungi = 0.345	



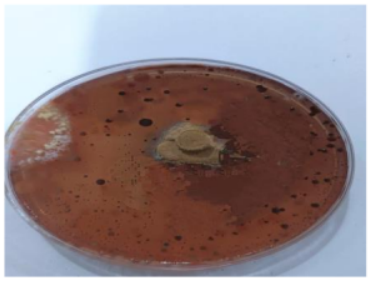
Control



50 ppm



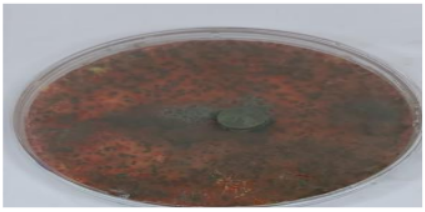
150ppm



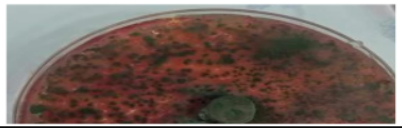
250 ppm

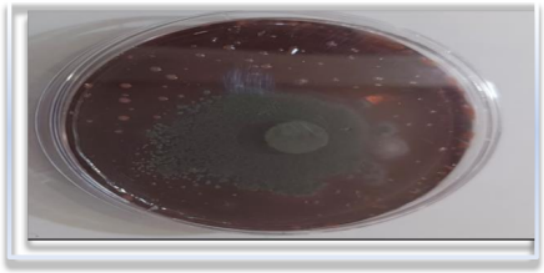
Figur 3: Decolrization of Congo red by *Aspergillus terreus*
On solid medium

Control



50ppm





150ppm

250ppm

Figure 4: Decolrization of Congo red by *Penicillium funiculosum* on solid medium

The capacity of isolated fungus to proliferate in a solution containing mineral salts and congo red

The current study's findings show that robust fungal growth can occur in the liquid metal medium SMS at a pH of 7, supplemented with glucose as a carbon source, congo red dye, and NH_4Cl as a nitrogen source. This implies that certain fungi, among the isolated fungal species, are capable of growing in a liquid media with the dye present, at different concentrations and to different degrees. The statistical analysis results, which demonstrated no discernible difference between the fungi and concentration levels. Table (2), made this clear.

It was discovered that when the fungus's dry weight concentration rose. In contrast to the control, where the dry weight was 1.96 g, the dry weight of the fungus *A. terreus* reached 1.65, 1.82, and 2.10 grammes, respectively, at concentrations of (50, 150, 250) ppm. The fungus *Penicillium funiculosum* was shown to have a higher dry weight increase in comparison to A. terreus. At 50, 150, and 250 ppm concentrations, the dry weight was 2.25, .2.30, and 2.53 grams, respectively, while the control had a dry weight of 2.62 grams

Table (2): Ability the growth of *Aspergillus terreu* and *Penicillium funiculosum* in mineral salts medium with congo red, pH = 7

Fung	Concentration (ppm)				Mean
	Control	50ppm	150 ppm	250 ppm	

Aspergillus terreus	**1.96 ±0.00**	**1.65 ±0.03**	**1.82 ±0.02**	**2.10 ±0.01**	**1.88**
Penicillium funiculosum	**2.62±0.00**	**2.25 ± 0.04**	**0.02±2.30**	**0.02 ±2.53**	**8.10**
L.S.D (P<0.05)		**L.S.D concentration= 0.041**			

Our investigation revealed that, in comparison to fungus at pH = 7, the dry weight of fungi at pH = 10 increased. At 50, 150, and 250 ppm concentrations, respectively, the dry weight of *A. terreus* was (2.21, 2,23, and 2.47 grams), while the control weight was (1.96 grams). Regarding *Penicillium funiculosum*, the dry weights at 50, 150, and 250 ppm concentrations were 2.11, 2.33, and 2.60 grams, respectively, in contrast to the 2.62-gram control weight. The findings show that there are statistically significant variations between the concentrations and the fungus Table (3).

Table (3): Ability the growth of *Aspergillus terreu* and *Penicillium funiculosum* mineral salts medium with congo red, PH=10

Fungi	**Concentration(ppm)**				**Mean**
	Control	**50ppm**	**150 ppm**	**250 ppm**	
Aspergillus terreus	**1.96±0.00**	**2.21 ±0.02**	**2.23 ±0.03**	**2.47 ±0.03**	**2.21**
Penicillium funiculosum	**2.62±0.00**	**2.11 ± 0.03**	**2.33 ±0.02**	**2.60 ±0.05**	**8.10**
L.S.D (P<0.05)		**L.S.D fungi =0.036**			

Determination of absorption maxima (λmax) of congo red dye when pH= 7 and pH=10.

Using a UV-visible spectrometer (U.K. / MD 1105 PG instrument Ltd.), the maximum absorption for the azo dye employed was found. Each dye's optical density was measured at various wavelengths between 200 and 900 nm when it was dissolved individually in water. The maximum absorbance of the Congo red dye was seen at 489 nm and 490 nm at pH values of 7 and 10 respectively. Consequently, the subsequent optimization procedure employed the identified λ max to determine the percentage of decolorization. Figures (5, 6).

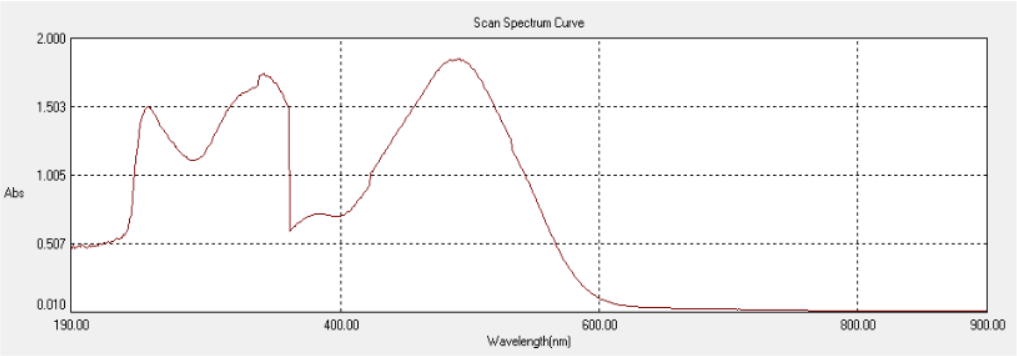


Fig 5. The maximum absorption of Congo red when pH= 7 by using UV- Visible spectrometer (U.K / MD 1105 PG instrument Ltd)

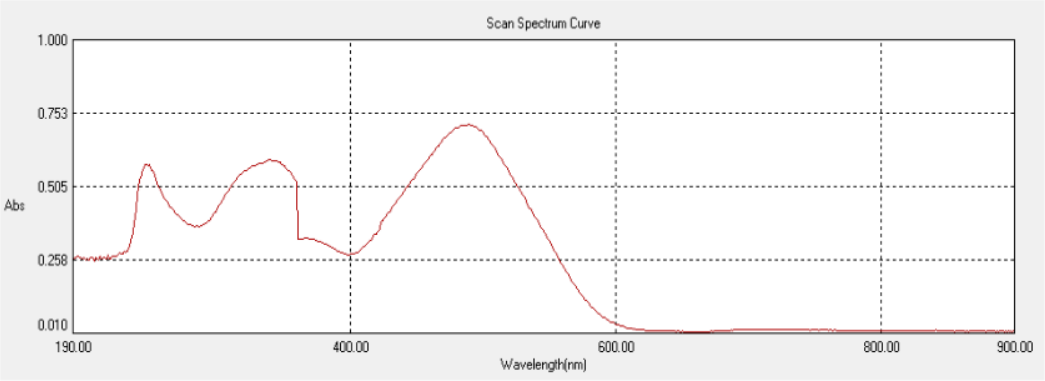


Fig 6. The maximum absorption of Congo red when pH= 10 by using UV- Visible spectrometer (U.K / MD 1105 PG instrument Ltd)

Decolorization (%)of congo red in liquid medium using fungal isolate

I have investigated the degrading capacities of *Aspergillus terreus* and *Penicillium funiculosum* fungi at several pH levels and quantities of Congo red dye. The degradation ability of both fungi was shown to diminish in this study when a dye concentration was increased from 50 ppm to 250 ppm and introduced to an SMS medium containing glucose and NH_4Cl as carbon and nitrogen sources. The percentage of *A. terreus* biodegradation at pH = 10 was found to drop to (93.33, 80.93, 67.81)% at dye doses of (50, 150 250) ppm, respectively. *P. funiculosum* showed biodegradation percentages of 94.33%, 82.90%, and 76.83%, respectively, at dye concentrations of (50, 150, 250) ppm.

In comparison to pH = 10, it was observed that at pH = 7, the removal capacity of *P. funiculosum* and *A. terreus* fungus was decreasing, reaching (79.62, 64.46, 43.95)% at

concentrations of (50, 150, 250) ppm. Regarding *P. funiculosum*, decolorization was attained at the same prior concentrations (88.21, 73.6, 53.92)%. fig (7,8).

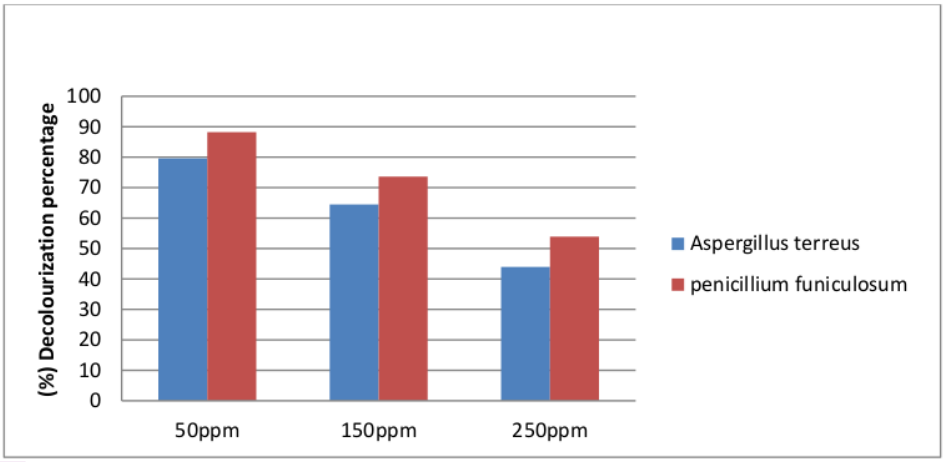


Fig.7: Effect of PH =7 on the decolorization percentages treated by *Aspergillus terreus* and *Penicillium funiculosum* through different concentration

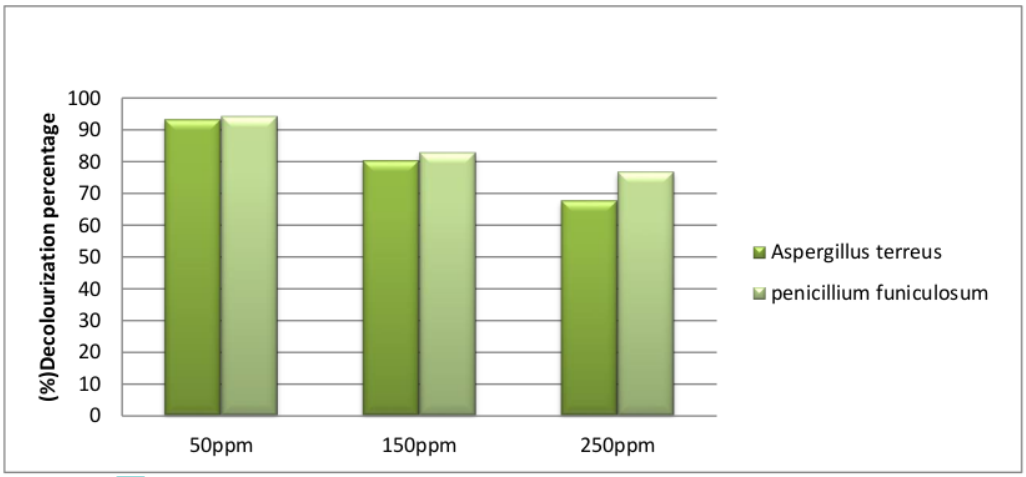


Fig.8: Effect of pH =10 on the decolorization percentages treated by *Aspergillus terreus* and *Penicillium funiculosum* through different concentration

DISCUSSION

The dye's aromatic structure resists deterioration from light, ozone, and other environmental factors. As a result, traditional methods of treating textile sector wastewater are still

ineffectual [20]. The development of a single, cost-effective technique for treating dyes in textile wastewater has been attempted by scientists up to this point, but it continues to be a significant difficulty [21]. Most research is focused on biological treatment since it is more environmentally friendly, produces less sludge, and yields consistent results [22]. This study involved the isolation of a number of fungus from soil and polluted water samples that were taken from textile wool companies in the governorate of Thi Qar. The outcomes showed that the most prevalent and highly capable of eliminating Congo Red dye among the isolated fungi were Aspergillus terreus and Penicillium funiculosum. The azo dye concentration, pH, carbon source, and nitrogen source were all varied during the dye removal procedure. The findings demonstrated that both fungi could break down Congo Red dye at amounts of (50 , 150,250) ppm . As evidenced by the daily increase in colony diameter in solid media, it was found that fungi of the Ascomycetes genus, specifically A. terreus and P. funiculosum, were clearly growing on all concentrations of red Congo dye. This suggests that the fungi can use the dye as a source of nutrients. These outcomes are in line with those of [18], which demonstrated that P. funigulosum had a stronger decolorization activity on solid medium containing CR than other fungi. that dyes might be broken down by fungus into other metabolites. P. funigulosum's dry weight (biomass) was recorded as 1.02 in CR-containing mineral salts medium (MSM).The decolorization of Congo red (CR) dye was investigated in this study using Aspergillus terreus GS28 and Aspergillus flavus CR500 that were isolated from industrial waste sludge. Because of the ideal pH, temperature, carbon, nitrogen, and heavy metal concentrations, the rate of CR decolorization increased. According to the study, after 120 hours under optimal conditions, *A. terreus* has a greater ability (95%) than *A. flavus* to decolorize CR (≈100 mg L-1) [23]. Regarding the mineral salts medium at pH=7, which is supplemented with glucose as a carbon source, NH_4Cl as a nitrogen source, and congo red dye. The addition of glucose and NH_4CL increased the dry weight of the fungal mycelium, according to the data. The reason for this is that the most efficient and easily accessible carbon source for microbial metabolism is glucose. A further supply of carbon and nitrogen is also necessary for many bacteria in order to promote growth, cellular development, primary metabolite creation, and enzyme secretion for the process of biodegradation. Azo dyes cannot be the exclusive source of carbon and energy for microorganisms. As a result, for the breakdown of azo dyes, microorganisms typically depend on the kind and presence of a carbon source [24-26].

Based on our research, we found that at pH = 10, the dry weight of fungi increased in comparison to that of fungi at pH = 7. This is due to the fact that pH has a significant impact on microbial cells because germs lack the ability to control their internal acidity. Every microbe has a pH range, and research has shown that the ideal range is typically between (6 and 10) for the biodegradation of azo dyes [27]. High alkaline conditions cause reactive azo dyes to lose hydrogen ions, which ionizes the dye and affects its consistency as well as makes it easier to remove from solutions [28]. On the other hand, it was noted in [29] That decolorization of dyes at higher concentrations was accomplished in an acidic environment, which further enables their better removal by enzymatic or fungal cell wall adsorption. Lastly, because most textile wastewater had alkaline pH values and industrial treatments have typically preferred decolorization under alkaline states due to the functionality of reactive azo

dye procedures, Our findings are more useful for extensive decolorization procedures. The addition of glucose and NH_4CL increased the dry weight of the fungal mycelium, according to the data. The reason for this is that the most effective and easily accessible carbon source for microbial metabolism is glucose. This is because many microbes need an extra source of nitrogen and carbon in order to thrive, produce more cells, synthesise primary metabolites, and secrete enzymes for biodegradation. Furthermore, microbes cannot use azo dyes as their only energy and carbon source because they don't usually act as a source of carbon. Thus, to break down azo dyes in general, microbes depend on the kind and presence of a carbon source[25,26]. The current study's findings were consistent with those of [30]. After three to four days of incubation at 30°C, the ability of Coprinus comatus was examined on potato dextrose agar with dyes at a concentration of 100 ppm. The dye that decolorized the fastest was aniline blue, followed by methyl red and Congo red. According to our findings, *Trichoderma harzianum* can partially and completely decolorize textile dyes (Blue, Yellow, and Red) at low concentrations (50 ppm).

Regarding the investigation on the capacity of *Aspergillus terreus* and *Penicillium funiculosum* fungi to break down Congo red dye at various pH levels and concentrations. The degradation ability of both fungi was shown to diminish in this study when a dye concentration was increased from 50 ppm to 250 ppm and introduced to an SMS medium containing glucose and NH4Cl as carbon and nitrogen sources. The data shown in Figures (7, 8) demonstrated that a dye concentration of 250 parts per million was below the lethal dose, hence impeding the azo dye's ability to remove dye. In addition to the detrimental effects of high dye concentration on the growth of fungi. There is an instance where the initial dye concentration intensified, leading to a reduction in decolorization [32]. Several reports demonstrated the extreme toxicity

The removal process is found to diminish at increasing hydrogen ion concentrations, notably at pH = 10, in relation to the effect of hydrogen ion concentration on the removal process. Since microorganisms lack a way to control internal acidity, this indicates that hydrogen ion concentration plays a significant role in influencing microbial cells. Every microorganism has a pH range within which it can grow, metabolize, and influence how quickly it degrades. At the ideal pH level, the biodegradation rate rises; at lower or higher pH values, it falls. Generally speaking, the ideal pH range for azo dye biodegradation is between (6 -10) The process of dye molecule transport across the microorganism's cell membrane, a stage that restricts the dye's rate of biodegradation, is similarly influenced by hydrogen ion concentration. Furthermore, through altering the structure of dye molecules, hydrogen ion concentration influences the chemistry of the dye in the medium[27,34,35]. The current study's findings were consistent with those of [28], who stated that pH 9 was the greatest decolorization of Remazol black. While the ideal pH for the largest azo-reductase enzyme is (7) Maximum decolorization and optimal pH within a range of pH 8 to 9 were demonstrated by some alkali-thermostable azo-reductase [28,36]. These findings are consistent with [37], who stated that at pH 5, *Aspergillus ochraceus* NCIM-1146 completely decolorized Reactive Blue-25 (100 ppm). On the other hand, decolorization was achieved at pH 3. 7, 9, and 87%, 81%, and 70%, respectively. This is also consistent with findings from

[38], who discovered that f[5] *Aspergillus niger* and *Penicillium sp*. using Reactive Red and Direct Red dyes, the largest percentage of decolorization occurred at pH (4–4.5).

CONCLUSION

After incubating for seven days, *Aspergillus terreus* and *Penicillium funiculosum* were able to remove the greatest percentage of dye color from Congo red cotton through fungal decolorization. According to the study's findings, the bioremediation method is the best way to lessen the toxicity of dyes in an economical and environmentally responsible way.

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