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The Effect of *Aspergillus flavus* ES Isolate on the Decolourisation of Crystal Violet, Titan Yellow and Congo Red

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

In the textile and dyeing industries, chemical dyes are increasingly preferred over natural dyes. Chemical dyes are easier to use and more cost effective to synthesize. The firmness and colour variety of chemical dyes is also beneficial. Unfortunately, these chemical dyes are often found in industrial effluent. Decolourising these released dyes can reduce their environmental toxicity. In this study, an isolate of *Aspergillus flavus* ES was used to decolourise three commercial dyes. As dye concentrations increased, the percentage of decolourisation increased, even as growth of the fungal isolate decreased. Also, an increase in the dye incubation period led to an increase in the decolourisation percentage. Further analysis showed that the organic and fatty acid levels of the *Aspergillus flavus* ES isolate were affected by the dye. Finally, it was found that increasing dye concentration had a toxic effect on the fungal ultrastructure.

Keywords: Aspergillus flavus; decolourization; Crystal Violet, Titan Yellow and Congo Red.

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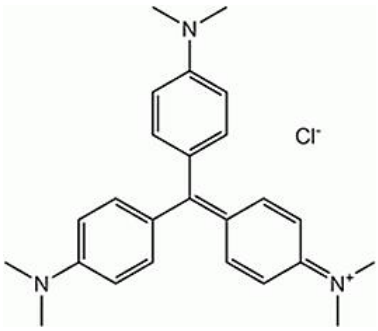
1. INTRODUCTION

Due to rapid industrialisation and urbanisation, many chemical dyes are used in day-to-day life. They are common substrates in food, cosmetics, paper, plastics and textiles. Dyes are synthetic, aromatic compounds that can be classified according to their dissociation in aqueous solution as: acid dyes, direct reactive dyes (anionic), basic dyes (cationic) and disperse dyes (non-ionic) [1]. Dyes are retained in solution by forming compounds with metals and salts through physical adsorption or covalent bonds.

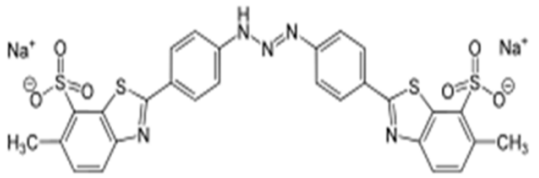
Crystal Violet (CV) or Gentian Violet (also known as methyl violet 10B or hexamethyl pararosaniline chloride) is a triarylmethane dye used as a histological stain and in Gram's method of classifying bacteria. Crystal Violet has antibacterial, antifungal and anthelmintic properties and was formerly important as a topical antiseptic. The medical use of the dye has been largely superseded by more modern drugs, although it is still listed by the World Health Organization [2].

The name gentian violet was originally used for a mixture of methyl pararosaniline dyes (methyl violet), but it is now often considered a synonym for crystal violet. The name refers to its colour, being like that of the petals of a gentian flower; it is not made from gentians or from violets [2].



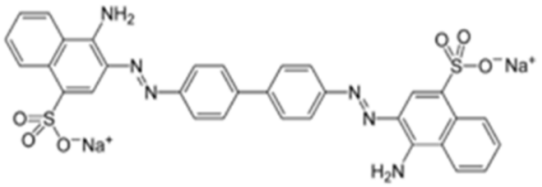
Crystal Violet (C.V) or Gentian Violet (G.V.)

Titan yellow (TY) is a compound with the formula $C_{28}H_{19}N_5Na_2O_6S_4$. It is a triazene dye used as a stain and a fluorescent indicator in microscopy. It is also used as a reagent for the detection of magnesium. As an acid-base indicator, it changes colour from yellow to red between pH 12 and pH 13 [3].



Titan Yellow (T.Y.)

Congo red (CR) is the sodium salt of 3,3'-([1,1'-biphenyl]-4,4'-diyl)bis(4-aminonaphthalene-1-sulfonic acid) (formula: $C_{32}H_{22}N_6Na_2O_6S_2$; molecular weight: 696.66 g/mol). It is a secondary diazo dye. Congo red is water-soluble, yielding a red colloidal solution, and its solubility is better in organic solvents such as ethanol. It has a strong, though apparently noncovalent, affinity to cellulose fibres. However, the use of CR in cellulose industries (cotton textile, wood pulp and paper) has long been abandoned, primarily due to its toxicity and tendency to run and change colour when touched by sweaty fingers [4].



Congo Red (C.R.)

Approximately 50 % of dyes are released into industrial effluents [5]. Discharge of dyes into aquatic environments causes serious problems, since dyes may affect the photosynthetic activity of hydrophytes by reducing the light penetration intensity [6]. Dyes are also toxic to aquatic fauna and flora due to their breakdown products, such as metals and chlorides [7,8].

Even at low concentrations, dyes reduce oxygen solubility and are toxic, carcinogenic or mutagenic for various organisms. Hence, it is necessary to remove these dyes from industrial effluents [9,10].

Many physical and chemical methods, like adsorption, photolysis, flocculation, chemical precipitation, chemical oxidation and reduction, electro-chemical treatment and ion-pair extraction have been used to remove dyes from effluent [11]. In addition, bioaccumulation and biosorption have potential as future dye-removal technologies [12].

Bacterial dye degradation has some limitations, so in recent years, fungal dye decolourisation has been pursued [13]. The importance of fungi and their enzymes in dye degradation and detoxification is globally appreciated [14], and many genera of fungi, either in living or dead form, have been used for dye decolourisation [15,16].

In addition [17] have reported that fungal isolates such as *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Fusarium oxysporum*, *Penicillium chrysogenum*, *Trichoderma viride* and *Mucor* sp. were good microbial sources for waste water treatment.

Fungi from the Basidiomycetes group, known as white rot fungi, are a heterogenous group of microorganisms, but they have in common the capacity to degrade lignin as well as other wood components [18]. The white rot fungi are the most efficient ligninolytic microorganisms. They can degrade a wide variety of recalcitrant pollutants, including various types of dyes. Most of the data on the biodegradation of synthetic dyes by ligninolytic fungi has been obtained with *Phanerochaete chyrsosporium* [19].

The ability of fungi to decolourise textile industry wastewaters by bioaccumulation/biosorption or laccase production mechanisms represents an eco-friendly method with many advantages over traditional approaches. The potential of some fungal strains (*A. niger* ATCC 20107, *Fusarium oxysporum* MUCL 791, *Cunninghamella echinulata* and *Polyporus squamosus*) to decrease spectroscopic absorbance has been shown, with *A. niger* ATCC 20107 remarkably decreasing discoloration by more than 90% [20]. [21] has shown that *A. flavus* (F10) completely decolourised malachite green (150 mg/L) within 6–8 days under static aerobic conditions at pH 5.8.

In the present study, the biodegradation properties of a promising fungal species were evaluated. An isolate of *A. flavus* ES was tested for its ability to decolourise CV, TY and CR dyes.

2. MATERIALS AND METHODS

2.1 Materials

The following materials were used in this study.

Dye concentrations were prepared with Czapek's Dox broth medium containing 20 g/l sucrose; 2 g/l $NaNO_3$; 1 g/l K_2HPO_4; 0.5 g/l KCl; 0.5 g/l $MgSO_4.7H_2O$, and 0.002 g/l $FeSO_4.7H_2O$. The medium was prepared by dissolving the ingredients in one litre of distilled water and autoclaving the media at 1.5 atmospheric pressure and 121^{o}C for 20 minutes.

Potato dextrose agar medium was used to maintain and prepare samples for Transmission Electron Microscopy (TEM).

1 - Crystal Violet (CV)
2 - Titan Yellow (TY)
3 - Congo Red B12 (CR)

2.2 Methods

2.2.1 Isolation and identification of *Aspergillus* strains

Isolates of the fungal species of *Aspergillus* were obtained from soil samples. The most potent isolate was selected and identified as an *A. flavus* ES isolate by the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University, Cairo, Egypt, through biochemical, morphological and staining techniques. Pure culture of the isolate was maintained at 28°C in potato dextrose agar slants.

2.2.2 Detection of dye decolourisation by UV-absorbance

Adsorption capacity experiments were carried out with three dyes (CV, CR and TY). The experiments were performed by combining varying concentrations of dye solutions with isolated fungal cultures in a rotary shaker. The dye formulations were added to Czapek's Dox broth medium at the following concentrations: 250, 500, 1000 and 1500 µg/100 ml. Two control samples were prepared. Control-1 comprised Czapek's Dox broth medium with fungus, without the dye (to provide a comparison of normal fungal growth unaffected by dye toxicity). Control-2 comprised Czapek's Dox broth medium with the dye, without the fungus (to provide a comparison of dye intensity without the effects of fungal decolourisation).

Isolate cultures of *A. flavus* (1 ml, containing 1 x 10^8 spores) were inoculated into Czapek's Dox broth medium that had been supplemented with the dye concentrations specified above. Each sample was transferred into a 250 ml conical flask and incubated for seven days at 28°C in a shaker set to 150 rpm. At the end of the

incubation period, the samples were centrifuged at 10,000 x g for ten minutes. The supernatants were collected, and the amount of dye remaining in the supernatant was calculated using a UV-visible spectrophotometer at an absorbance of 521 nm. The absorbance values were used to analyse the per cent of decolourisation. The optical density values were taken as maximum absorbance values of each dye at days 2, 4, 6, 8 and 10.

2.2.3 Tolerance of dye concentrations by the *A. flavus* ES isolate

Initial dye concentration is an important variable that can affect the adsorption process. Adsorption of dyes at different initial concentrations leads to the determination of the adsorption capacity of the adsorbent. Different types of dyes were used in this respect: CV, TY and CR. For each dye, different concentrations were used (250, 500, 1000 and 1500 µg/100ml) and mixed in 250 ml conical flasks containing 100 ml of medium. The isolate was inoculated and incubated for seven days at 28°C in a shaker set to 150 rpm. Then the concentration of dye in the supernatant was measured with a UV spectrophotometer at an absorbance of 521 nm, and the fungal dry weight was measured.

2.2.4 Effect of the incubation period on adsorption and percentage of dye removal

The incubation period between adsorbate and adsorbent is an important design parameter that affects the performance of the adsorption processes. The effect of the incubation period on the adsorption of dyes was studied.

2.2.5 Measurement of fungal organic acids and fatty acids after dye incubation

A. flavus isolates were cultivated in test tubes containing Czapek's Dox broth medium that had been supplemented with different concentrations (250, 500, 1000 and 1500 µg / 100 ml) of three different dyes (CV, CR & TY). Then 5 gm of mycelial fresh weight was removed and ground up in 10 ml of a solution of chloroform : methanol (2:1, v / v). The material was filtered and concentrated to 1 ml and placed into gas chromatography auto sampler vials. The samples were analysed using a Shimadzu GCMS-QP 5050, software class 5000, search library Willy 229. The parameters were set at LIP column DP1 at 30 m 0.53 mm ID; 1.5 µm film carrier gas Helium (flow rate 1 ml / min); ionization mode: EL (70 ev); and temperature program: 70°C (static for two minutes), then gradually increased (at a rate of 2°C / min) up to 220°C (and held static for five minutes). The detector and injector temperatures were set at 250^{o}C. The chromatographs were analysed, and individual peaks were identified by comparison to library references of fatty acid mass spectra.

Mycelium extract was also placed into 1 ml amber High-Performance Liquid Chromatography (HPLC) vials (Fisher) and stored at -20°C until they could be processed. Organic acids were measured using an HPLC Waters 600E system controller with a Waters 470 fluorescence detector and a Waters 712 WISP autosampler. The analysis took place at RCMB.

2.2.6 Examination of fungal samples with TEM

Specimens were prepared for TEM using the following procedure. *A. flavus* isolates were cultivated on dextrose potato agar plates supplemented with different dye concentrations (250, 500, 1000, and 1500 µg / 100 ml). The plates were incubated for four days (before sporulation), stained and examined by a JEOL 1010 Transmission Electron Microscope at RCMB.

2.2.7 Statistical analysis

Three replicates were done to all data used. Data was analysed using a Sigmaplot statistical package ver. 12.5 and SPSS ver. 23. Descriptive statistics of all data were achieved using the SPSS ver. 23, while comparisons were done using Sigmaplot ver. 12.5. Two-way analysis of variance was used for the growth comparisons, while three-way analysis of variance was used for the decolourisation comparisons. Some data points failed the normality test. Therefore, the log transformation method was applied to all data. The normality test results were $P < 0.050$ before transformation and 0.198 after transformation for fungal growth, while the decolourisation normality test results were $P < 0.050$ before transformation and 0.134 after transformation. For all pairwise comparisons, the Holm-Sidak method was applied. The powers of all experiments were evaluated to be 1 using Sigmaplot.

3. RESULTS AND DISCUSSION

In the textile and dyeing industries, chemical dyes are increasingly preferred over natural

dyes. Chemical dyes are easier to use and more cost effective to synthesize. The firmness and colour variety of chemical dyes is also beneficial. About 100,000 commercial dyes are manufactured in several varieties such as acidic, basic, reactive, azo, diazo and anthraquinone-based meta complex dyes. Over 10,000 dyes with an annual production of over 7 x 10^5 metric tons are commercially available [1].

A wide variety of microorganisms are capable of decolourising dyes [22-26]. In addition, [27] have reported that adsorption of dyes to the microbial cell surface is the primary mechanism of dye decolourisation.

In the present investigation, adsorption capacity experiments were performed by incubating varying concentrations of dye solutions with isolated fungal cultures. Absorbance values were used to analyse the percent of decolourisation.

The decolourisation performance of an *A. flavus* isolate on three dyes (CV, TY, and CR) was studied at increasing dye concentrations. Acceptable colour removal was achieved by the *A. flavus* isolate in an extensive range of dye concentrations.

The results, as shown in Fig. 1 and Plates 1, 2 and 3, reveal that an increase in the dye (CV, TY and CR) concentration from 250 to 1500 µg / 100 ml caused a decrease in the spectrophotometer absorbance and, consequently, a decrease in the percentage of dye decolourisation from 50 to 14%, 66.5 to 28.5% and 88 to 50%, respectively.

Manikandan [28] reported on a decolourisation study that was carried out by treating textile dye effluent with a variety of fungal strains (*A. niger, Penicillium* spp., and *Rhizopus* spp. isolated from spent mushroom substrate). Of these three fungal strains, *A. niger* showed the highest efficiency of decolourisation. The study indicated that eco-friendly fungal dye decolourisation was preferred over conventional methods due to its viability and low cost.

The current study evaluated the ability of an *A. flavus* isolate to tolerate different dye concentrations. The concentration of dye in the supernatant was measured using a UV spectrophotometer at an absorbance of 521 nm. The fungal dry weight was also measured. The effect of dye concentration on the adsorptive removal by the *A. flavus* isolate was evaluated by incubating different concentrations of dyes with fungal cells.

Table 1 and Fig. 2 show that for all three dyes, the growth of the *A. flavus* isolate decreased as the dye concentration increased.

3.1 Two Way Analysis of Variance

There was a statistically significant relationship between dye and concentration (P = < 0.001) in all pairwise multiple comparison procedures (Holm-Sidak method): The overall significance level = 0.05.

The results of the two-way analysis of variance indicate that there was no statistically significant effect on the fungal growth for the three dyes used at the concentration of 1500 µg / 100 ml.

The effect of the incubation period on the adsorption of dyes was studied to determine the time taken for the fungus to degrade the different dyes. In this study, the higher the concentration of the dye, the longer the degradation time to decolourise.

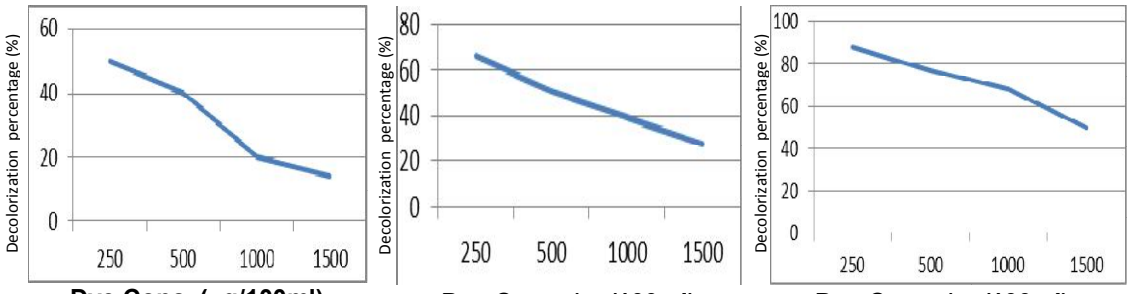


Fig. 1. Decolorization of Crystal Violet (CV), Titan Yellow (TY) and Congo Red (CR) with *Aspergillus flavus* ES isolate

Plate 1. Decolorization of Crystal Violet (CV) with *Aspergillus flavus* ES isolate

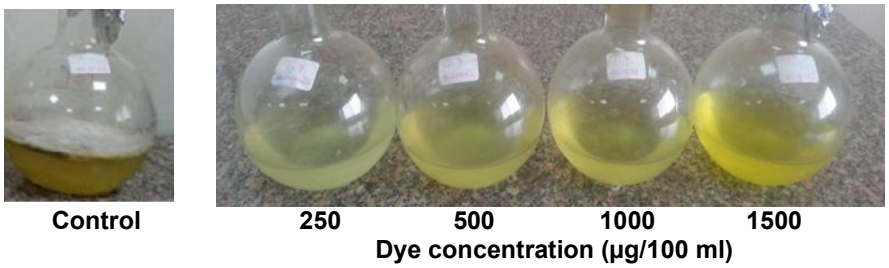


Plate 2. Decolorization of Titan Yellow (TY) with *Aspergillus flavus* ES isolate

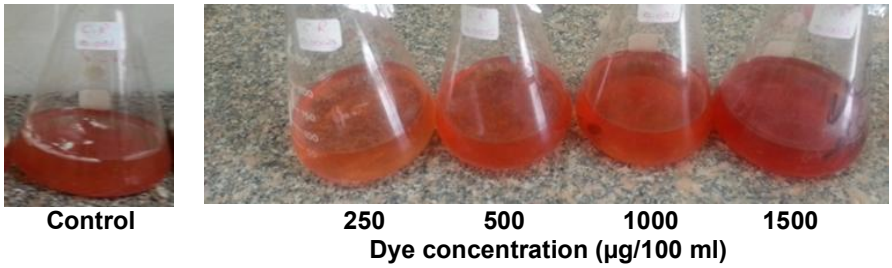


Plate 3. Decolourisation of Congo Red (CR) with *Aspergillus flavus* ES isolate

Table 1. The ability of *Aspergillus flavus* ES isolate to tolerate different concentrations of the different dyes (Crystal Violet (CV), Titan Yellow (TY) and Congo Red (CR)

Conc. of dye sample (µg/100ml)	Fungal Dry wt. (g/100ml)		
	(CR)	(CV)	(TY)
250	0.95 ± 0.07	2.37 ± 0.04	1.63 ± 0.03
500	0.8 ± 0.01	1.77 ± 0.03	1.24 ± 0.06
1000	0.67 ± 0.03	1.74 ± 0.03	1.06 ± 0.14
1500	0.4 ± 0.01	0.36 ± 0.03	0.36 ± 0.02

Where C.R.=Congo Red, C.V.=Crystal Violet and T.Y.=Titan Yellow
Dry Weight of Aspergillus flavus,E.S.isolate on blank (without dye) = 2.5 g/100 ml

3.2 Three Way Interaction Term Analysis

There was a statistically significant interaction between the dye, the incubation period and the concentration (P = < 0.001). This indicates that the effect of one factor was not consistent at all combinations of the two other factors. Therefore, an unambiguous interpretation of the main effect was not possible. Sigma Stat was used to evaluate the significance of the interactions.

3.3 Evaluation of Dye Incubation Periods across Different Levels of Concentration

The effect of the dye incubation period depended on the level of dye concentration. There was a

significant relationship between the dye incubation period and the dye concentrations of 1500, 1000, 500 and 250 µg / 100 ml (P = < 0.001). The difference in the mean values among the different incubation periods for the CV, TY and CR dye at concentrations of 1500, 1000, 500 and 250 µg / 100 ml was greater than would have been expected by chance. There was a statistically significant difference (P = < 0.001). In all pairwise multiple comparison procedures (Holm-Sidak method), the overall significance level = 0.05.

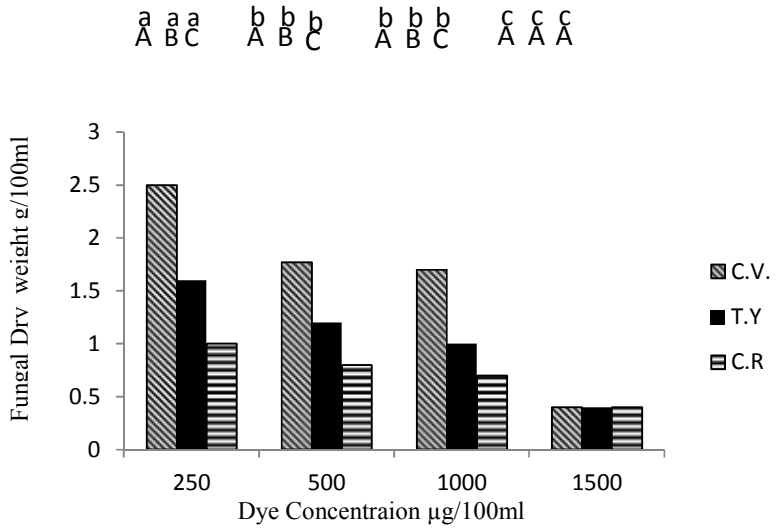


Fig. 2. The ability of *Aspergillus flavus* ES isolate to tolerate different concentrations of the different dyes (Crystal Violet (CV), Titan Yellow (TY) and Congo Red (CR)

The letters are expressing pairwise comparisons among different concentrations and dyes. Different letters are expressing significant differences, where capital letters represent comparison within the same concentration of different dyes, while small letters represent comparison among different concentrations of the same dye.

Table 2. Effect of incubation period on decolourisation percentage of the three dyes using *Aspergillus flavus* ES isolate at different dye concentrations

Dye	Dye conc. (µg/100 ml)	Decolourisation percentage (%)				
		Incubation period (day)				
		2	4	6	8	10
CR	250	14.8 ± 0.16	31.6 ± 1.66	58.3 ± 3.33	88.3 ± 1.66	91.6 ± 1.66
	500	11.8 ± 0.16	28.3 ± 1.66	50 ± 2.88	81.6 ± 1.66	83.3 ± 1.66
	1000	10.1 ± 0.16	19.3 ± 0.44	43.3 ± 1.66	68.3 ± 4.40	75 ± 2.88
	1500	9.66 ± 0.16	14.5 ± 0.28	30 ± 2.88	51.6 ± 1.66	56.6 ± 3.33
CV	250	10.5 ± 0.28	24 ± 0.57	38.6 ± 1.85	47.6 ± 1.45	52.6 ± 1.45
	500	10 ± 0.57	20 ± 0.57	29.6 ± 1.20	38 ± 1.52	41.6 ± 1.66
	1000	9.9 ± 0.05	15.0 ± 0.24	17.9 ± 0.03	20 ± 0.57	24 ± 0.57
	1500	9 ± 0.28	11.9 ± 0.05	14.0 ± 0.21	15 ± 0.11	15.1 ± 0.16
TY	250	11.5 ± 0.28	24.5 ± 0.28	43.3 ± 1.66	68.3 ± 4.40	75 ± 2.88
	500	10.6 ± 0.16	20.1 ± 0.44	34.6 ± 0.33	53.3 ± 1.66	60 ± 2.88
	1000	9.5 ± 0.28	19.3 ± 0.33	31.6 ± 1.66	39.5 ± 0.28	41 ± 2.08
	1500	9 ± 0.57	14.0 ± 0.29	24.1 ± 0.44	31.6 ± 1.66	35 ± 2.88

Where CR=Congo Red, CV=Crystal Violet and TY=Titan Yellow

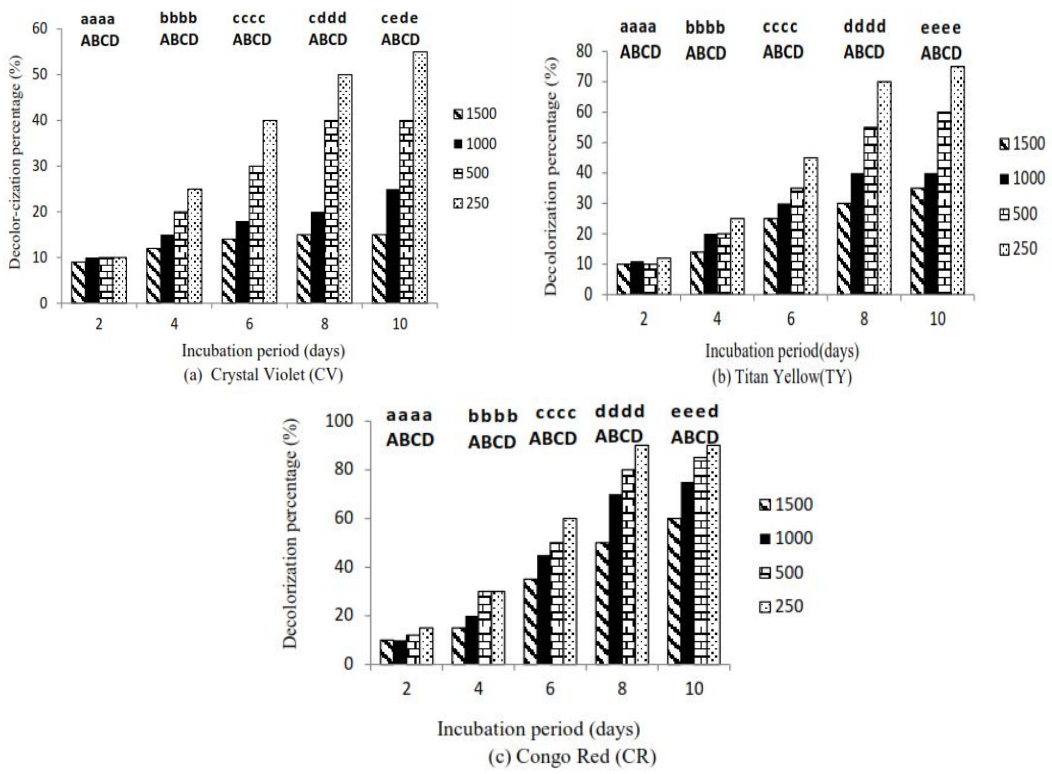


Fig. 3. Effect of incubation period on decolourisation percentage (%) of Crystal Violet (CV), Titan Yellow (TY) and Congo Red (CR) using *Aspergillus flavus* ES isolate at different dye concentrations

The letters are expressing pairwise comparisons among different concentrations, Different letters are expressing significant differences where capital letters represent comparison within the same Incubation period of different dye concentrations, while small letters represent comparison among different Incubation period for the same concentration

Table 2 and Fig. 3-a, 3-b and 3-c show the decolourisation percentages of CV, TY and CR at different dye concentrations, as achieved by the *A. flavus* isolate. The decolourisation percentages decreased as dye concentration increased, but the percentages increased with increasing incubation periods.

The difference in the mean values among the different dye concentrations evaluated at days 2, 4, 6, 8 and 10 of the incubation period and the 250 µg / 100 ml dye concentration was greater than would have been expected by chance. There was a statistically significant difference (P = < 0.001).

The difference in the mean values among the different levels of dye evaluated at 2 days of incubation and the 500 µg / 100 ml dye concentration was not great enough to exclude the possibility that the difference was due to random sampling variability. There was not a statistically significant difference (P = 0.008). Meanwhile, the difference in the mean values among the different levels of dye evaluated at days 4, 6, 8 and 10 of the incubation period and the 500 µg / 100 ml dye concentration was greater than would have been expected by chance. There was a statistically significant difference (P = < 0.001).

The difference in the mean values among the different levels of dye evaluated at day 2 of the incubation period and the 1000 µg / 100 ml dye concentration was not great enough to exclude the possibility that the difference was due to random sampling variability. There was not a statistically significant difference (P= 0.448). On the other hand, the difference in the mean values among the different levels of dye evaluated at days 4, 6, 8 and 10 of the incubation period and the 1000 µg / 100 ml dye concentration was

greater than would have been expected by chance. There was a statistically significant difference (P = < 0.001).

The difference in the mean values among the different levels of dye evaluated at day 2 of the incubation period and the 1500 µg / 100 ml dye concentration was not great enough to exclude the possibility that the difference was due to random sampling variability. There was not a statistically significant difference (P = 0.296). Meanwhile, the difference in the mean values among the different levels of dye evaluated at days 4, 6, 8 and 10 of the incubation period and the 1500 µg / 100 ml dye concentration was greater than would have been expected by chance. There was a statistically significant difference (P = < 0.001).

In all pairwise multiple comparison procedures (Holm-Sidak method), the overall significance level = 0.05.

CV at a concentration of 5 ppm produced only slight inhibition of Deuteromycetes strains. GV at a concentration of 500 ppm in potato dextrose agar inhibited the growth of *A. flavus*, *A. fumigatus*, *Candida albicans*, *Fusarium moniliformis* and *Penicillium camemberti*. In another case, a final concentration of 75 ppm was enough to inhibit all fungal strains except *A. flavus*, which required 1,000 ppm [29].

Organic acids, particularly citric acid, are metabolites commonly produced by fungi. Organic acid production is associated with the solubilization of insoluble metal-containing compounds. A variety of organic molecules are excreted by fungal cells to chelate metal ions [30].

As shown in Table 3, the addition of dye to the medium suppressed the synthesis of fumaric acid. In contrast, citric acid levels increased with the addition of dye at 250 µg / 100 ml. Citric acid levels increased four-fold with the addition of CV and two-fold with the addition of TY, while in the case of CR, citric acid levels increased 1.6 fold. A further increase in dye concentration resulted in a decrease in citric acid production, while oxalic and acetic acids were not present at the higher dye concentrations.

According to [31] organic acid analysis has shown that some fungal organic acid levels increased in the presence of safranin dye compared to a control. Other organic acids, such as gallic acid, acetic acid and citric acid synthesized in *M. hiemalis*, decreased in the presence of safranin.

Table 4 reports the fatty acid biosynthesis of the *A. flavus* isolate used in this study. While the addition of dye to the medium suppressed the synthesis of most fatty acids, the levels of a few fatty acids, including palmetic and oleic acids, increased after the addition of the dye. The increase in these acids may indicate that the dyes induced a specific mechanism in the *A. flavus* isolate to control the presence of such dyes. This mechanism may play a role in protecting fungal cells. As for the other fatty acids that disappeared from the fungal mycelium, their biosynthesis may be inhibited by the presence of the dyes.

Table 3. Effect of different dyes (CV), (TY) and (CR) concentrations on the organic acids percentage produced by the fungal mycelium of *Aspergillus flavus* ES isolate

Dye type	Organic acid type	Organic acid percentage (%)				
		Dye concentration (µg/100ml)				
		Control	250	500	1000	1500
(CV)	Fumaric acid	15.00	0.00	0.00	0.00	0.00
	Citric acid	25.00	100.00	20.00	0.00	0.00
	Oxalic acid	0.00	0.00	0.00	0.00	0.00
	Acetic acid	0.00	0.00	0.00	0.00	0.00
(TY)	Fumaric acid	15.00	0.00	0.00	0.00	0.00
	Citric acid	25.00	50.00	8.00	0.00	0.00
	Oxalic acid	0.00	0.00	0.00	0.00	0.00
	Acetic acid	0.00	0.00	0.00	0.00	0.00
(CR)	Fumaric acid	15.00	0.00	0.00	0.00	0.00
	Citric acid	25.00	40.00	0.00	0.00	0.00
	Oxalic acid	0.00	0.00	0.00	0.00	0.00
	Acetic acid	0.00	0.00	0.00	0.00	0.00

Control is the medium inoculated with Aspergillus flavus,E.S.isolate without dye

Table 4. Effect of different dyes (CV), (TY) and (CR) concentrations on the fatty acids percentage produced by the fungal mycelium of *Aspergillus flavus* ES isolate

Dye type	Fatty acid type	Fatty acid percentage (%)				
		Dye concentration (µg/100ml)				
		Control	250	500	1000	1500
(CV)	Caprilic C8	3.00	0.00	0.00	0.00	0.00
	Capric C10	2.50	0.00	0.00	0.00	0.00
	Lauric C12	2.00	0.00	0.00	0.00	0.00
	Tridecanoic C13	8.00	0.00	0.00	0.00	0.00
	Myristic C14	6.00	6.00	0.00	0.00	0.00
	Pentadecanoic C15	2.00	0.00	0.00	0.00	0.00
	Palmitic C16	30.00	45.00	60.00	80.00	80.00
	Heptadecanoic C17	5.00	0.00	0.00	0.00	0.00
	Stearic C18	10.00	5.00	4.00	0.00	0.00
	Oleic C18:1	20.00	30.00	40.00	45.00	50.00
	Linoleic C18:2	5.00	0.00	0.00	0.00	0.00
(TY)	Caprilic C8	3.00	0.00	0.00	0.00	0.00
	Capric C10	2.50	0.00	0.00	0.00	0.00
	Lauric C12	2.00	0.00	0.00	0.00	0.00
	Tridecanoic C13	8.00	0.00	0.00	0.00	0.00
	Myristic C14	6.00	4.00	0.00	0.00	0.00
	Pentadecanoic C15	2.00	0.00	0.00	0.00	0.00
	Palmitic C16	30.00	45.00	50.00	60.00	60.00
	Heptadecanoic C17	5.00	0.00	0.00	0.00	0.00
	Stearic C18	10.00	5.00	0.00	0.00	0.00
	Oleic C18:1	20.00	25.00	35.00	40.00	50.00
	Linoleic C18:2	5.00	0.00	0.00	0.00	0.00
(CR)	Caprilic C8	3.00	0.00	0.00	0.00	0.00
	Capric C10	2.50	0.00	0.00	0.00	0.00
	Lauric C12	2.00	0.00	0.00	0.00	0.00
	Tridecanoic C13	8.00	0.00	0.00	0.00	0.00
	Myristic C14	6.00	3.00	0.00	0.00	0.00
	Pentadecanoic C15	2.00	0.00	0.00	0.00	0.00
	Palmitic C16	30.00	40.00	55.00	60.00	60.00
	Heptadecanoic C17	5.00	0.00	0.00	0.00	0.00
	Stearic C18	10.00	5.00	0.00	0.00	0.00
	Oleic C18:1	20.00	25.00	30.0	35.00	40.00
	Linoleic C18:2	5.00	0.00	0.00	0.00	0.00

Control is the medium inoculated with Aspergillus flavus ES isolate without dye

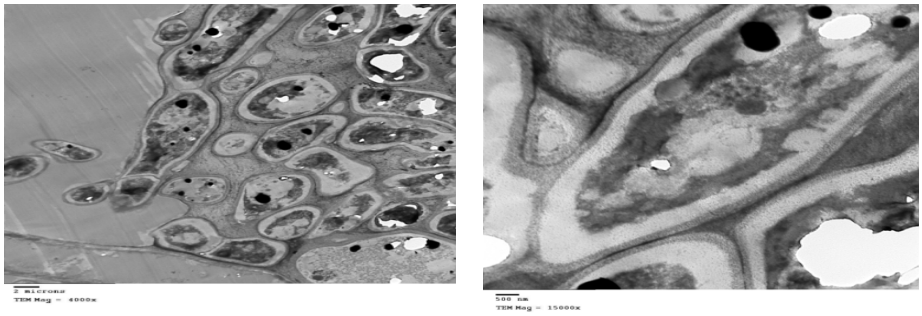
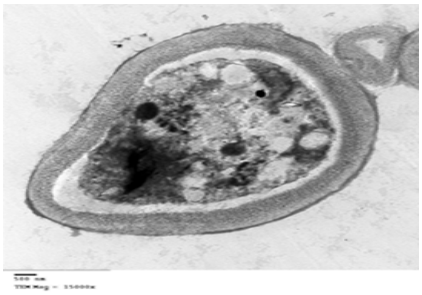


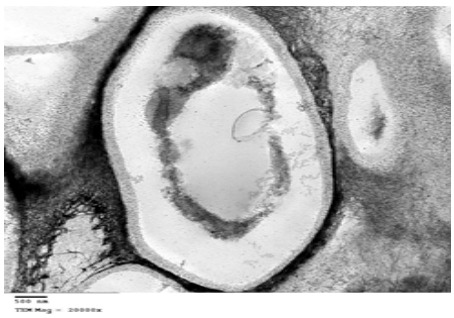
Plate 4. Control- The ultrastructure of the fungal mycelium of Aspergillus flavus ES isolate under TEM

Control is the growth of Aspergillus flavus ES isolate without dye

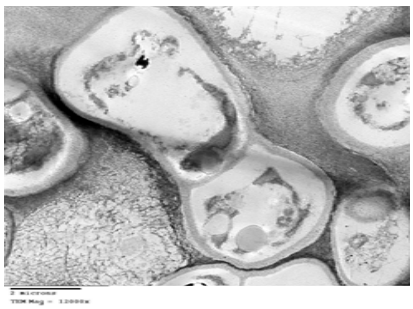
Conc.(250 µg/100 ml)



Conc. (500 µg/100 ml)

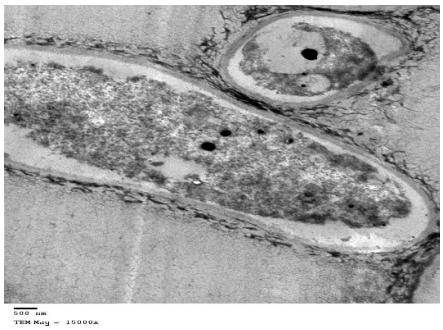


Conc. (1000 µg/100 ml)

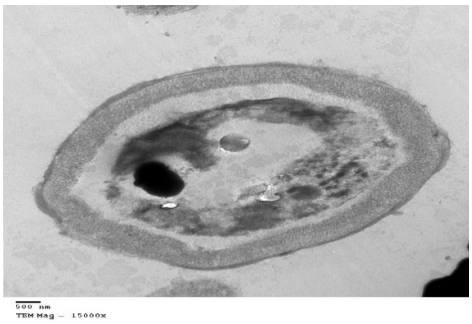


Conc. (1500 µg/100 ml)

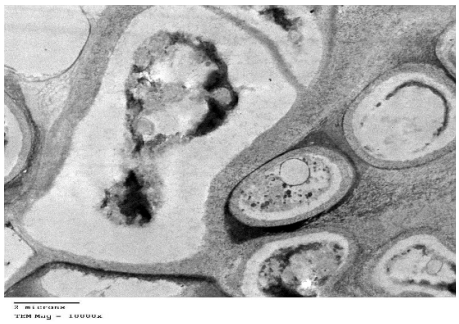
Plate 5. Effect of different concentrations of Crystal Violet (CV) on the ultrastructure of the fungal mycelium of *Aspergillus flavus* ES isolate under TEM



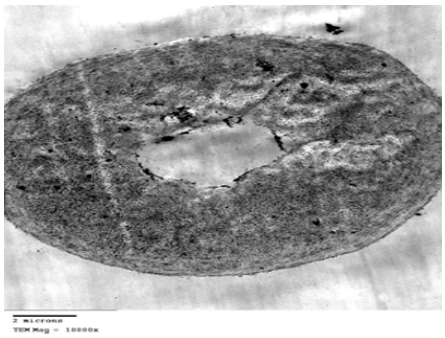
Conc. (250 µg/100 ml)



Conc. (500 µg/100 ml)

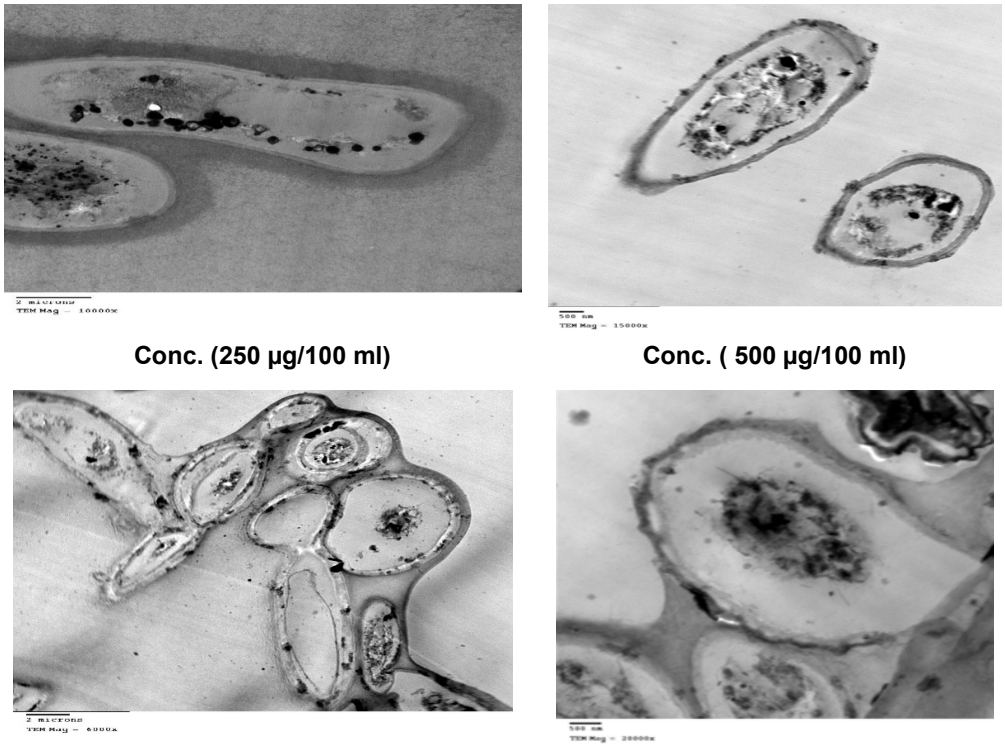


Conc. (1000 µg/100 ml)



Conc. (1500 µg/100 ml)

Plate 6. Effect of different concentrations of Congo Red (CR) on the ultrastructure of the fungal mycelium of *Aspergillus flavus* ES isolate under TEM



Conc. (250 µg/100 ml) **Conc. (500 µg/100 ml)**

Conc. (1000 µg/100 ml) **Conc. (1500 µg/100 ml)**

Plate 7. Effect of different concentrations of Titan Yellow (TY) on the ultrastructure of the fungal mycelium of *Aspergillus flavus* ES isolate under TEM

In accordance with the results of the current study, other research has found that the presence of safranin dye suppressed fungal synthesis of certain fatty acids such as caprylic, linoleic, archidic, and behenic acids, especially at high dye concentrations (500 ug / 100 ml). Dye degradation may interfere with the metabolic pathways of these fatty acids. A few fatty acids (palmitic and oleic) were shown to increase in concentration in the presence of safranin. These fatty acids provided a protective effect for the cell membrane [31].

The plates depict the TEM of the fungal ultrastructure of the *A. flavus* ES isolate at different dye concentrations for the three tested dyes (CR, TY and CV). As shown in the plates, increasing dye concentration had a toxic effect on the cells. There was tremendous damage to the cell ultrastructure. In contrast, the cells of the untreated fungus (control) in Plate 4 show that the cell wall is rigid and intact, the cellular material is well distributed, and the cells appear to be in their normal sizes and shapes. As dye concentration increased, many cells became deformed with undulating cell walls outlining the hydrolysed cell contents.

In a related study, *Saprolegnia monoica* were treated with CR, which prevents glycan microfibril assembly. In the presence of the dye, the fungus developed wall thickenings and exhibited aberrant hyphal tips that expanded into spherical swellings in the thickened cell walls. These morphological anomalies were accompanied by changes in the cell wall's glucose localization that was not limited to hyphal tips. CR uncoupled the cellulose into normal microfibrils [32].

4. CONCLUSIONS

Textile industries release large quantities of water and chemicals. This effluent often contains dyes that can cause ecological problems for aquatic plants and other living organisms by reducing light transmission and photosynthetic activity.

Dyes are also toxic, mutagenic and carcinogenic when they accumulate in aquatic organisms. Many microorganisms have been identified that

are capable of removing dyes and metal ions from wastewater.

The decolourisation percentage of CV, TT and CR using an isolate of *A. flavus* decreased with increasing dye concentration. An increase in the incubation period led to an increase in the dye decolourisation percentage and a decrease in the biomass growth of the fungal isolate. Also, statistical analysis showed that the type of dye had no statistically significant effect on fungal growth, while the concentration of dyes almost have a little effect. Most of the organic acids and fatty acids produced by the fungus were suppressed upon the addition of dyes to the growth medium. Increasing dye concentration had a toxic effect on the cells as shown by the tremendous damage to the cell ultrastructure revealed in the TEM photos.

Among the techniques and methods used for wastewater treatment and dye removal, adsorption, particularly biosorption, is now the most preferred. The advantages of using fungal cell walls for biosorption include good binding sites for toxic chemicals, simplicity of application, effectiveness, and lower cost than conventional methods.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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