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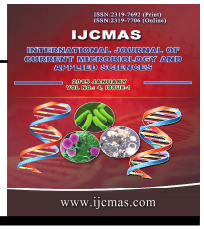

Original Research Article

Optimization of different parameters for decolorization of acid black 194 dye using the selected fungal species

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K e y w o r d s

Optimization, Acid Black 194, decolorization, *Aspergillus flavus* Link AUMC 9060, *Aspergillus tamarii* Kita AUMC 9061, *Aspergillus parasiticus* Speare AUMC 9062.

A B S T R A C T

Seven Egyptian fungal isolates were screened for decolorization of five acid textile dyes (red 399, black 194, blue 296, yellow 235 and yellow 218). Among the seven fungal isolates, only three showed the highest decolorization percent for acid red 399, acid blue 296 and acid black 194. *Aspergillus flavus* Link AUMC 9060, *Aspergillus tamarii* Kita AUMC 9061 and *Aspergillus parasiticus* Speare AUMC 9062 were the most efficient for decolorization of acid black 194 (85%, 91.15% and 85%, respectively). Optimum temperature, pH, incubation period and inoculums size for decolorization by *A.flavus*, *A.tamarii* and *A.parasiticus* was found to be 24 □ C, pH 7, 8 days and 1 disc of inoculums (1cm in diameter), respectively. However, other investigated factors (inoculums age, dye concentration and sucrose concentration) were variable among the tested species. The structural integrity of untreated acid black 194 dye and treated with the selected species was studied by FT-IR analysis. FT-IR analysis showed there was no difference between treated and untreated acid black 194

Introduction

Over 100,000 synthetic dyes are commercially available, with a total production of over 7×10^5 tons per year (Fu and Viraraghavan, 2001). Among these, azo dyes are the most important group of synthetic colorants. They represent the largest class of dyes, and more than half of the annually produced synthetic dyes (estimated for 1994 worldwide as 1 million tons) are azo dyes (Stolz, 2001).

Many physico-chemical methods, including adsorption, precipitation, chemical oxidation, photodegradation, or membrane filtration have been used for color removal from wastewaters (Yeh and Thomas, 1995; Gogate and Pandit, 2004). However, these methods have high operating costs and limited applicability. Further, they produce large quantities of sludge, which again creates a problem in its disposal. In recent

years, the research for biological decolorization method has been considered as effective, specific, less energy intensive and environmentally benign, since it results in partial or complete bioconversion of pollutants to stable non-toxic end products (Kuhad *et al*., 2004). Many fungi (especially white-rots), actinomycetes and bacteria are used for the development of biological processes for the treatment of textile effluents (Mielgo *et al*., 2001; Bhatt *et al*., 2005).

Moreover, textile effluents constitute one of the most problematic wastewaters to be treated not only for their high chemical and biological oxygen demands, suspended solids and toxic compounds but also for color. Thus, there is need to search other microorganisms for treating textile effluents, which are capable of growing at alkaline pH and having strong ligninolytic system. More recent researchers have demonstrated that decolorization studies carried out either the decolorization of various dyes by an individual fungal strain or the decolorization of a single dye by various fungal strains (Shahvali *et al*., 2000; Fu and Viraraghavan 2001; Alhassani *et al*., 2007). With the exception of white rot fungi that can decolorize dyes, reports on decolorization by yeast or other filamentous fungi through enzymatic processes are very limited (Yang *et al*., 2003).

Microbial decolorization can occur *via* two principal mechanisms: biosorption and enzymatic degradation, or a combination of both (Wu *et al*., 2012 and Phugare *et al*., 2010). FT-IR analysis has been studied in order to understand the decolorization mechanism of acid black 194. Among the commercial dyes, C.I. Acid Black 194 is one of the most popular in leather, wool, polyamide and silk dyeing (Burkinshaw and Lagonika (2006) and Koh *et al*. (2001)). The present study has been focused on the isolation of fungal species that can decolorize industrial wastewaters which encompasses different types of acid dyes (red 399, black 194, blue 296, yellow 235 and yellow 218) with high efficiency. Furthermore, conditions accelerating acid dye (C.I. Acid Black 194) decolorization were optimized by three non-basidiomycetes fungal species. Statistical analysis of data was carried out by using one way analysis of variance (ANOVA) followed by homogenous subsets (Duncan □) at confidence level of 5 % (0.05). Each experiment was conducted in triplicate and mean ±SE values were taken.

Materials and Methods

Dye stuff

Five acid dyes were used in this study: Lanasyn Red M-G sgr, Lanasyn yellow F-7GL sgr, Lanasyn yellow M-2GL p, Lanasyn Navy M-BL p and Lanasyn Black M-DL p 170. All dyes were kindly supplied by" Moket Mac" Company in industrial zone B1, **10**□□ of Ramadan City, El-Sharqyiah, Governerate, Egypt.

Isolation of fungi

Water samples were collected from wastewater of an Egyptian company for artificial carpet at 10□□ of Ramadan City, in sterile clean glass bottles then, stored at 4°C.

Wastewater samples were taken from the surface and at 50 cm depth from the surface. Fungi were isolated from water samples by using one ml of each sample, to which 9 ml of sterilized distilled water were added. The tubes content were mixed by shaking.

Serial decimal dilutions Benson (2002) were made from the original concentration to reach dilutions up to 1/1000. The media

used for fungal isolation were Potato-dextrose agar, Sabouraud □s glucose agar, Czapek -Dox agar and Malt extract agar. Three replicates were prepared for each dilution and incubated at 28˚C for 7 days.

Fungal identification and maintenance

Aspergillus sp1, *Aspergillus sp2*, *Aspergillus sp3***,** *Aspergillus niger*, *Aspergillus sp4* and *Aspergillus sp5*; were isolated from surface. *Aspergillus sp6* was isolated from water samples which collected at 50 cm depth. Identification was carried out according to the following references: John & Pitt (1979)and Gilman (1957)).

The identification of the most potent isolates (*Aspergillus sp1*, *Aspergillus sp2* and *Aspergillus sp3*) was confirmed by Mycological Center, Faculty of Science, Assiut University, Egypt, 71516 to be *A.flavus* Link, *A.tamarii* Kita and *A.parasiticus* Speare.

The selected fungal species were maintained and sub-cultured on potato dextrose agar (PDA) and Czapek □s Yeast (autolysate) extract Agar media (CYA).

Fungal cultures

Pure cultures of the selected fungi were grown in petri-dishes for 7 days using Czapek □s Yeast (autolysate) extract Agar media (CYA) Samson & Pitt (1985). This medium contains 30g/L sucrose, 1g/L KH_2PO_4, 0.5g/L KCL, 0.5g/L $MgSO_4$.7H2O, 3g/L $NaNO_3$, 10 mg/L $FeSO_4$, 5g/L Yeast extract, and 20g/L agar.

Decolorization assay

The experiments were carried out in 250 ml Erlenmeyer flasks containing 100 ml of broth Dox □s medium to which the dye was added at concentarion 0.05g/L. The pH was adjusted at 7±0.2 using phosphate buffer solution. The autoclaved flasks were left to cool then, separately inoculated with one disc (1cm in diameter) of each selected fungal spp. The flasks were then incubated for 7 days and at the end of this period, 10 ml of the culture medium were centrifuged at 5000 rpm for 15 min (Hettich Zentrifugen Mikro22 R D-78532 Tuttlingen) then measured spectrophotometrically at λ_{max} for each acid dyes using T60 UV VIS spectrophotometer to calculate decolorization percent.

$$\% \text{ Decolorization} = \frac{A\square - A_t}{A\square} \times 100$$

Where A□= the absorbance at zero time, while A_t = the absorbance after some time, t Olukanni *et al*. (2006). Decolorization percentage is representing the mean of three replicates.

Analytical methods

a. FT-IR Analysis: After decolorization, residual culture medium (before and after treatment with the selected fungi) was analyzed using FT-IR (JASCO/FT/IR-460) at Micro Analysis Center, Cairo, University, Egypt.

Result and Discussion

Screening the decolorization of acid dyes using the selected fungal isolates, two types of media and two inoculums diameter

The results in table 2 show that, *Aspergillus sp1*, *Aspergillus sp2*, *Aspergillus sp3* were effectively able to remove more than 79% of three acid dyes out of the tested 5 acid dyes (red 399, blue 296 and black 194) on Dox □s broth medium using one mycelial disc (1cm in diameter). Accordingly, they were

selected for further investigations. Elizabeth *et al*. (1999) found that, *Pleurotus ostreatus* (IE8) was able to decolorize 12 of 23 industrial dyes, while *Phanerochaete chrysosporium* (ATCC 24725) decolorized only 5 dyes. They stated that, these industrial dyes were selected on the basis of their stability to a range of pH (pH 3–11), thermostability and stability under culture conditions in non-inoculated flasks.

Determination of the highest decolorization rate among the tested acid dyes

According to the results of the previous experiment, three dyes (a.red 399, a.blue 296 and a.black 194) were chosen to investigate the decororization rate of the most efficient fungal spp. The results recorded in fig 2 show that, *Aspergillus flavus* Link, *Aspergillus tamarii* Kita and *Aspergillus parasiticus* Speare were able to decolorize acid black 194 by the following rates: 85%, 91.15% and 85.25%, respectively. Accordingly, Acid Black 194 has been chosen for further studies using *Aspergillus flavus* Link, *Aspergillus tamarii* Kita and *Aspergillus parasiticus* Speare. Park *et al.* (2004) studied decolorization of three acid dyes by *Trametes versicolor* ATCC 200801, *Trametes versicolor* KCTC 16781, and *Phanerochaete chrysosporium* KCCM 60256. They observed that, acid blue 350 was the most rapidly decolorized, while the other acid dyes were difficult to be decolorized. In order to obtain better results, the systematic study on the relationship between dye structure and fungal decolorization is necessary; however, this kind of study until now has been rather scarce (Chagas and Durrant (2001) and Knapp *et al*., 1995).

Effect of incubation temperature on decolorization of acid black 194 dye

The results in fig3 show that, the decolorization ability of *A.flavus* was less than 50% when incubated at 20 □C. The increase in incubation temperature resulted in a significant increase which reached to its maximum value (85.9%) at 28˚C. There was no a significant differences between 24˚C and 28˚C so, 24˚C was selected to be the incubation temperature for further studies with *A.flavus*. In the case of *A.tamarii* the level of decolorization showed no a significant difference under all tested temperatures, so 24˚C was chosen as the optimum for decolorization of acid black 194 by *A.tamarii*, while in the case of *A.parasiticus*, increase in temperature resulted in a significant decrease in decolorization percent. It was found there was no a significant difference between 20˚C and 24˚C, so 24˚C was chosen for decolorization of acid black 194 by *A.parasiticus.*

Accordingly 24˚C has been chosen to carry out the following experiments as the incubation temperature for all tested species. Ponraj *et al*. (2011) found that, *A.niger* and *Mucor sp* were the most effective decolorizer at 27 and 37 □C, respectively, whereas at 4 □C *A. niger* was the most effective decolorizer. Tamer *et al*. (2008) reported that, the biosorption capacity of acid black 40 onto *Thuja orientalis* was favored at lower temperatures and they stated that, an increase in the temperature from 20 to 40˚C leaded to a decrease in the biosorption capacity. Radha *et al*. (2005) reported that, at higher 35˚C or lower 35 □ C, the decolorization activity of the fungus reduced and they stated that, the fungus either unable to produce peroxidases for decolorization or peroxidases denatured.

Effect of initial pH values on decolorization of acid black 194 dye

The results recorded in fig 4 show that, the

decolorization percent of acid black 194 at pH 7 was 89%, 75.9% and 89.9%, respectively with *A.flavus, A.tamarii* and *A.parasiticus*.

According to the results pH 7 was selected to be the optimum for decolorization of acid black 194 using the selected fungal species. These results could be due to an electrostatic attraction between the dye and the biosorbent during the biosorption process. If there was a negative charge on the dye and a positive charge on the biosorbent, they would attract each other. But at acidic pH, when there was a large amount of H^+, that the attraction could be disrupted because of an attraction between the $H^+$and dye. A similar but opposite reaction could be occured at alkaline pH when there was a large amount of OH in the solution (Khalaf, 2008).

Frida (2009) mentioned that, the highest decolorization rates were obtained between pH 4 and 10. In contrast to our results Yuyi *et al*. (2011) reported that, acidic conditions could be favorable for the biosorption between the two dyes (acid black 172 and congo red) and the fungal biomass, because a significant high electrostatic attraction could be existed between the positively charged surface of the adsorbent under acidic conditions and the anionic dyes (AB and CR are anionic dyes in solution for $^-SO3$ group in their structure). At pH 10.0, the biosorption values were 22.56 and 21.67 mg g^{-1} for AB and CR, which were 44% and 48% of maximum values, respectively.

Effect of inoculum size on decolorization of acid black 194 dye

The results in fig 5 indicate that, the highest decolorization percent achieved using two discs of *Aspergillus flavus* Link (87.4%). In the case of *Aspergillus tamarii* Kita the highest decolorization percent achieved using ten discs (85%). There was no a significant difference between the use of one disc and the other used inocula for *A.flavus* and *A.tamarii*. While in the case of *Aspergillus parasiticus* Speare the highest decolorization percent took place using one disc (95.2%). The increase in inoculum size exhibited no a significant increase in the decolorization percent of *A.flavus* and *A.tamarii*, so the maximum decolorization percent has been achieved using one disc(1cm in diameter) but, in the case of *Aspergillus parasiticus* exhibited slight a significant decrease in the decolorization percent, so one disc(1cm in diameter) was the most effective. Kumar and Sumangala (2012) observed that, the ideal volume of inoculum was found to be 2% for *Penicillium chrysogenum* and 10% for *Aspergillus niger*. Radha *et al*. (2005) reported that, the maximum decolorization of synthetic dyes using *Phanerochaete chrysosporium* occurred at an inoculum size of 2 ml (approximately 3.2 x 10^5 cell/ml). Shahvali *et al*. (2000) reported that, an inoculum size of 10% was sufficient for the decolorization of the textile wastewater, above which there was no change in decolorization percent.

Effect of inoculum age on decolorization of acid black 194 dye

The results recorded in fig 6 show that, the optimum decolorization percent for *A.flavus* Link was 96.2% using 4 days old culture, while in the case of *A.parasiticus* Speare there was no a significant difference between 8 day old culture and 4 day old culture, so four days old culture (92.4%) was chosen to be used as the optimum inoculum age, but in the case of *A.tamarii* Kita the optimum decolorization percent was achieved using two days old culture (90.4%). Older inoculums age resulted in a significant decrease in the decolorization

percent especially in case of *A.flavus* and *A.tamarii*, but in case of *A.parasiticus* exhibited no a significant increase in the decolorization percent. Inoculums age vary from a fungal species to another and this might due to variation in molecular structure of fungal cell wall. Also, young inoculums age characterized with enormous activity and viability

Effect of incubation period on decolorization of acid black 194 dye

The results in fig7 show that, the decolorization percent has been increased by increasing the incubation period until reaching the optimum decoloriztion at the 8□□ day of incubation. Longer incubation periods revealed no a significant difference between decolorization percents of 8□□, 10□□ and 12□□ day. According to results, 8 days of incubation were taken as the best incubation period for the maximum decolorization abilities of *A. flavus* Link, *A. tamarii* Kita and *A. parasiticus* Speare, with the following percents: 88.8%, 98.3% and 97.4%, respectively. In contrast to our results, Belsare and Prasad (1988) studied effect of incubation period on color removal using *Shizophyllum conmune* and they observed that, 80% color reduction was achieved within one day incubation and 82% in two days, but no increase was observed, if the incubation period increased more than two days. So, they suggested that, two days of incubation were sufficient for the process of decolorization.

Other studies, for example, Husseiny (2008) reported that, the maximum reduction% was recorded for *Aspergillus niger*, after 4 days of incubation period for both reactive red 120 and direct red 81 dyes. Also, Assadi & Jahangiri (2001) and Mcmullan *et al.* (2001) reported that, the maximum color reduction% was achieved by *Penicillium spp* after 4 days of incubation period at temperature 35˚C for both reactive red 120 and direct red 81 dyes.

Effect of acid black 194 initial concentration on dye decolorization

The results in fig8 show that, the decolorization percent for *Aspergillus flavus* Link reached its maximum value (87.3%) at dye concentration 25 mg/L, while in the case of *Aspergillus tamarii* Kita and *Aspergillus parasiticus* Speare the maximum decolorization values (92.5% & 96.4% , respectively) were observed by the addition of 50 mg/L of the used dye. Then, increase in the dye concentration resulted in a significant decrease in the decolorization percent.

In case of *A.parasiticus* Speare, the fungal cells kept their ability to decolorize the dye up to 62.6% even when dye applied at high concentration (150 mg/L). On the other hand, the decolorization percent of *A.flavus* Link and *A.tamarii* Kita decreased significantly (21.9% and 42.7%, respectively) when dye added at concentration (150 mg/L). The present results are in agreement with those of Youssef *et al*. (2008) who studied the decolorization of malachite green by *Acremonium kiliense* and observed that, 95.4% of malachite green was decolorized, when the concentration of the dye was 5 mg L^{-1} but decolorization was only 35.48%, when the dye concentration was doubled. They have attributed this trend to the inhibition of fungal growth at high dye concentration.

Also, Zhang *et al*. (1999) observed that, color removal efficiency decreased with an increased in the concentration of the cotton bleaching effluent. Mou *et al*. (1991) reported that, high dye concentration result

in low color removal. Young and Yu (1997) also reported that, high dye concentration decreased decolorization rates. Other studies carried out by Radha *et al*. (2005) showed that, decolorization of synthetic dyes using *Phanerochaete chrysosporium* reached up to 90% for an initial dye concentration (0.02 g/L). However, at higher concentrations, *Phanerochaete chrysosporium* proved to be more effective for decolorization of Congo red than acid red 114. Hu and Wu (2001) reported that, desorption of the dyes from the fungal cells especially at higher dye concentrations may be due to higher molecular mass and structural complexity of the dyes.

Effect of sucrose concentration on decolorization of acid black 194 dye

Results in fig 9 reveal that, 10 g/L was the best sucrose concentration for *Aspergillus flavus* Link with decolorization percent reached to 94.7%. In the case of *Aspergillus tamarii* Kita, the decolorization was 86.1% if sucrose added at 20 g/L. However, in the case of *Aspergillus parasiticus* Speare, 2.5 g/L sucrose was enough to obtain 95.4% decolorization, also all tested sucrose concentrations revealed no a significant increase in the decolorization percent. So, 2.5 g/L was the optimum sucrose concentration which used for *Aspergillus parasiticus* Speare.

In case of *A.tamarii* the results revealed that sucrose concentration up to 10 g/L was not effective in decolorization level. However, the decolorization level significantly increased to 86.1% by the addition of 20 g/L. The sucrose concentration vary from fungal species to another due to each fungal species has specific requirement of sucrose for its growth and decolorization ability. The effect of sucrose concentration on decolorization rate has been rarely reported. A general tendency observed is that, Mou *et al*. (1991) studied the effect of glucose concentration on decolorization of dyes by *Myrothecium verrucaria* and observed that, the glucose concentration did not influence on the bio-decolorization process. Also, observed that, rapid growth of the fungus in C-limited medium with dye indicated that, the fungus utilized the dye as the sole source of carbon and produced enzymes to degrade the dyes.

FT-IR analysis: The bands in the case of *A.flavus* Link at 3396.03 cm^{-1} that referred to OH group, 2938.02 cm^{-1} that referred to CH group, 1639.2 cm^{-1} that referred to C=C group and 1240 cm^{-1} that referred to C-N group. These peaks presented in spectrum of control. In the case of *A.tamarii* Kita observed after treatment bands appeared at 3386.39 cm^{-1} referred to OH group, 2936.09 cm^{-1} referred to CH group, 1638.23 referred to C=C group and 1247.72 cm^{-1} referred to C-N group all these groups presented in the spectrum of control. In the case of *A.parasiticus* Speare gave bands at 3408.57 cm^{-1} referred to OH group, 2939.95 cm^{-1} referred to CH group, 2119.39 cm^{-1} referred to C≡C group, 1638.23 cm^{-1} referred to C=C group, 1413.57 cm^{-1} referred to CH group and 1251.58 cm^{-1} referred to C-N group all peaks presented in control spectrum. The results showed that, there was no variation between peaks of treated and untreated dye (control).

Husseiny (2008) found that, treatment of direct red 81 with *Aspergillus niger* gave an IR band at 3549.1 cm^{-1} referring to NH_2 group and band at 333.6 cm-1 referring to OH group. These peaks did not present in spectrum of original dye. This meant that, the dye degraded by *Aspergillus niger*, while treatment of that dye with *Penicillium spp* gave the same peaks of the original dye, this meant that dye might adsorb by *penicillium*

spp.

Table.1 Summary data on acid dyes studied

Acid dye	λ max (nm)	Type of dyes	Product Name
Red 39	500	Metal complex	Lanasyn Red M-G Sgr
Yellow 235	436	Metal complex	Lanasyn Yellow M- 2GL P
Yellow 218	400	Disulphonated	Lanasyn Yellow F- 7GL sgr
Blue 296	614	Metal complex	Lanasyn Navy M-BL P
Black 194	570	Metal complex	Lanasyn Black M-DL P 170

Note. Determination of the maximum wavelength of each acid dye has been carried out at the central lab of Faculty of Science, Helwan University, Egypt using UV/Vis spectrophotometrically (Jasco-V-530).

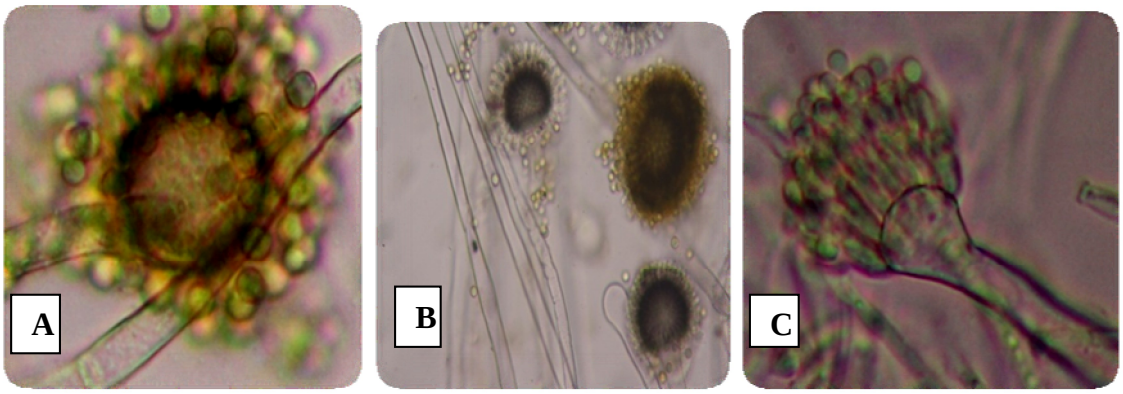


Figure1. Microscopic observation of the selected fungal species.
A. ***Aspergillus flavus*** **Link**
B. ***Aspergillus niger*** **Van Tieghem**
C. ***Aspergillus parasiticus*** **Speare.**

Table2. Screening the decolorization of acid dyes using the selected fungal isolates, two types of media and two inoculums diameter

Dyes C.I	Media / Fungal Isolates	PDB (%D) ± SE		Dox□s.b (% D) ± SE	
		Inoculum diameter		Inoculum diameter	
		0.5cm	1cm	0.5 cm	1cm
A.Red 399	*Aspergillus sp1*	72± 2	80.3± 1.5	85.6± 2	87.6± 2.4
	Aspergillus sp2	63± 3.7	72.3± 2.5	78.6± 1.5	89.8± 0.7
	Aspergillus sp3	71.6± 1.5	77.3± 2	87.3± 2	88.3± 1.5
	Aspergillus niger	62± 1	61± 2.6	73±2	78± 2.6
	Aspergillus sp4	52± 2	63.6± 3.2	66.3± 3.2	73.6± 3.2
	Aspergillus sp5	58.6± 3.2	64.3±2	62.6± 2	63.6±3.2
	Aspergillus sp6	54.6± 4.5	64± 3.6	55±2	75± 4.3
A.Yellow 218	*Aspergillus sp1*	51±1	61.6± 1.5	54.7± 0.5	65.3± 1.1
	Aspergillus sp2	48± 2.6	52.3± 2.5	50.3± 0.5	60.3± 0.5
	Aspergillus sp3	42±2	42.3±2	44.6± 0.5	50.3± 0.5
	Aspergillus niger	23± 2.6	37.6± 2.6	25.6± 1.1	40.6± 1
	Aspergillus sp4	32± 2	32.3± 2.5	38.3± 1.1	40.3± 0.5
	Aspergillus sp5	38.6± 3.2	37.6± 2	30.3± 0.5	39.6± 1
	Aspergillus sp6	28.6± 4.1	35.3± 2	32.6± 1.1	35.3± 0.5
A.Blue 296	*Aspergillus sp1*	81.6± 2	80.6± 0.6	79.6± 0.5	84.6± 0.5
	Aspergillus sp2	70.3± 0.5	74.3± 2.5	74.6± 0.5	79.3± 0.5
	Aspergillus sp3	76± 1.7	79.6±0.5	76.3± 1.1	82.6± 1.1
	Aspergillus niger	53.3± 2.8	63± 2.6	50.3± 5	51.6± 2.8
	Aspergillus sp4	64.6± 0.7	75± 1	61.3± 0.5	70.6± 1.1
	Aspergillus sp5	60.3±0.5	63.3± 2.8	62.6± 1.1	72.6± 2.3
	Aspergillus sp6	62.6± 2.3	70.7± 0.5	53.7± 0.5	66.3± 1
A.yellow 235	*Aspergillus sp1*	40.3± 0.5	42.6± 2.3	49.3± 0.5	50.3± 0.5
	Aspergillus sp2	41.3± 1.1	43± 1	43.3± 2.8	48.3± 5.7
	Aspergillus sp3	43.7± 0.5	40.3± 0.5	43.6± 0.5	40.6± 1.1
	Aspergillus niger	27± 2.6	30.6± 1.1	34.3± 7.5	26.3± 2.3
	Aspergillus sp4	32.6± 2.3	34.6± 0.5	42.3± 0.5	39.3± 1
	Aspergillus sp5	26.6± 2.8	33.3± 2.8	33.3± 2.8	37.6± 1
	Aspergillus sp6	31± 2.6	33.3± 0.5	32± 2	30.6± 2
A.Black 194	*Aspergillus sp1*	75.3± 0.5	75.6± 0.5	87.6± 0.5	90.3± 0.5
	Aspergillus sp2	70.3± 0.5	64.6± 0.5	80.3± 0.5	91.3± 0.5
	Aspergillus sp3	64.6± 0.5	70.3± 0.5	89± 0.5	92.6± 0.5
	Aspergillus niger	57.3±8.7	52.3± 2.3	76.3± 1.1	63± 2.6
	Aspergillus sp4	60.3± 0.6	64.3± 0.5	60.3± 0.5	76.6± 0.5
	Aspergillus sp5	63.3± 2.8	64± 3.4	59.3± 0.5	65± 0.5
	Aspergillus sp6	51.6± 2.8	55.3± 0.5	70.3± 0.5	62± 3.4

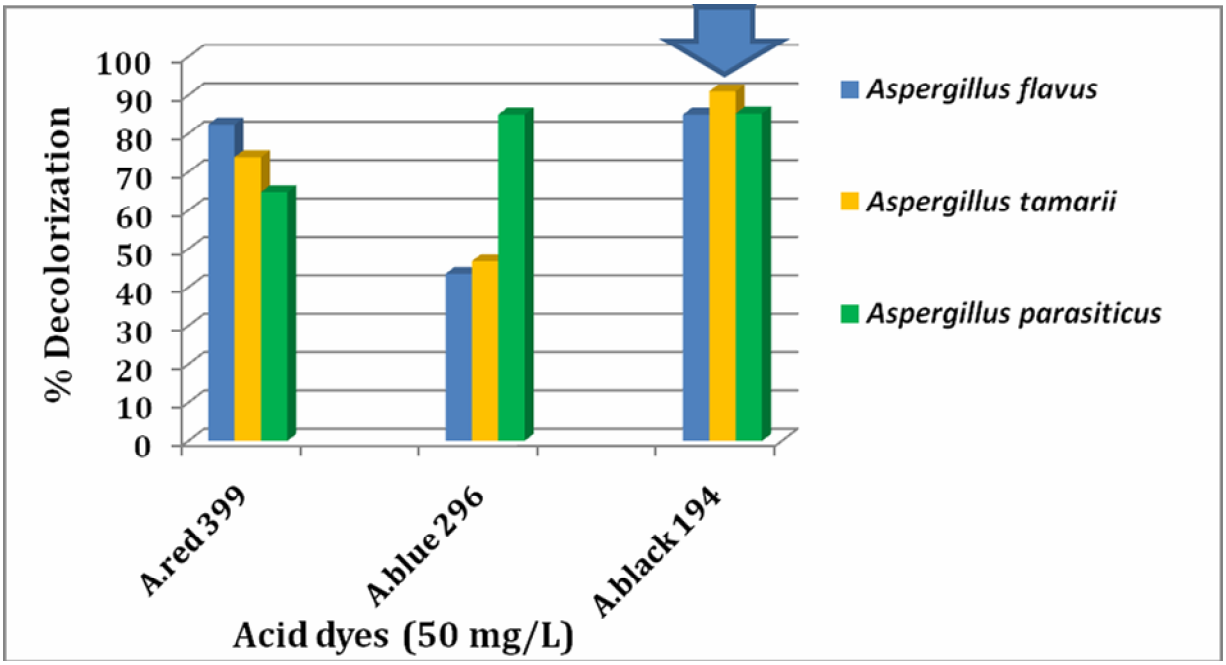
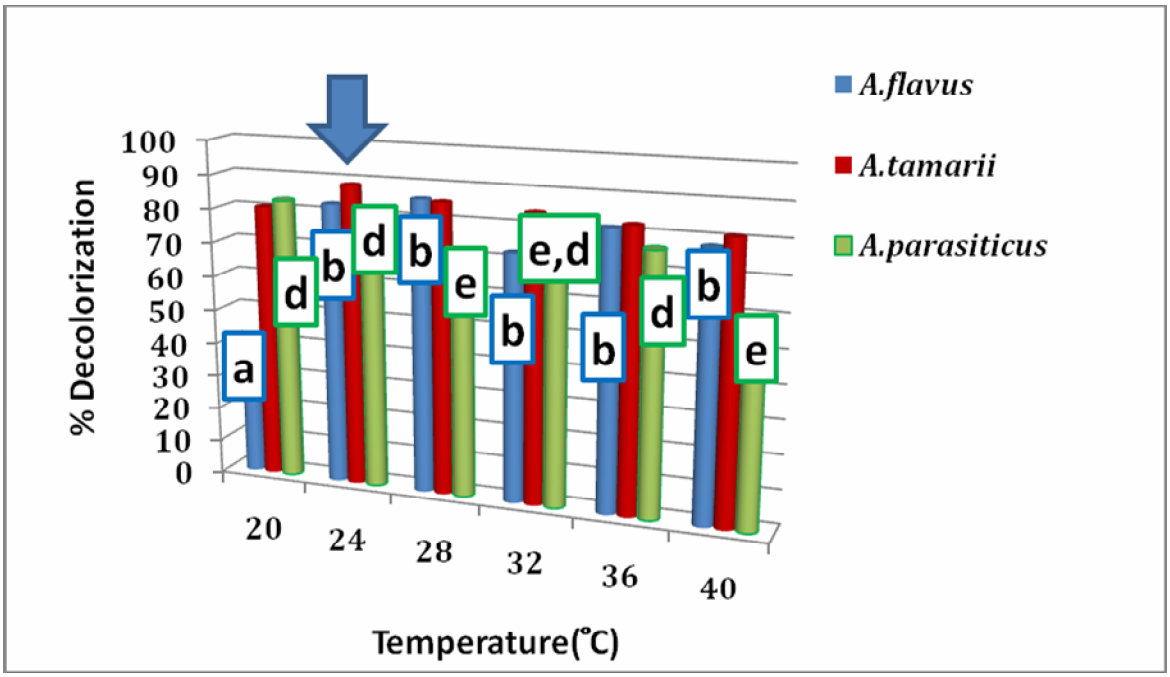
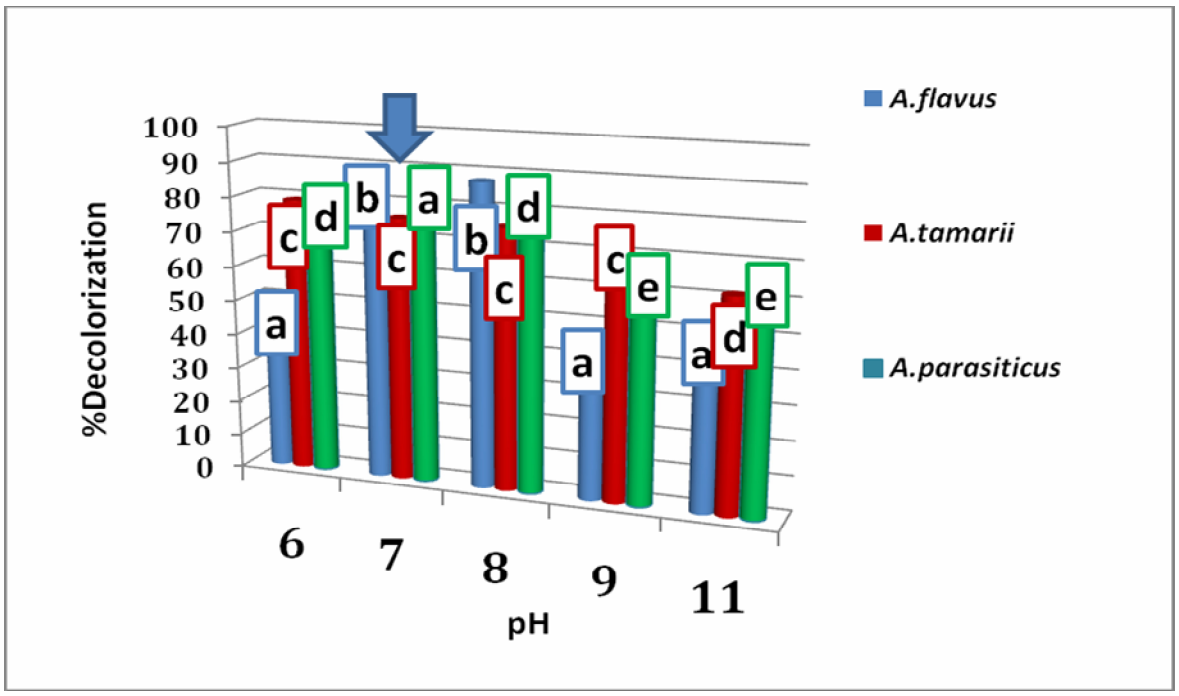


Fig2. Determination of the highest decolorization rate among the tested acid dyes



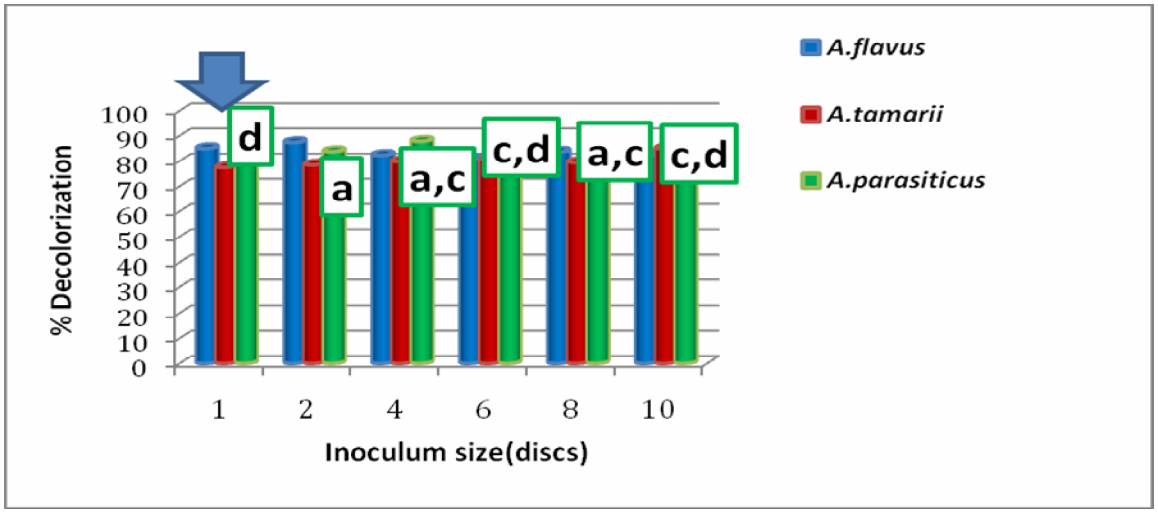
Mean in the blue, red and green columns with different letters have a significant difference between each other.

Fig3. Effect of incubation temperature on decolorization of acid black 194 dye



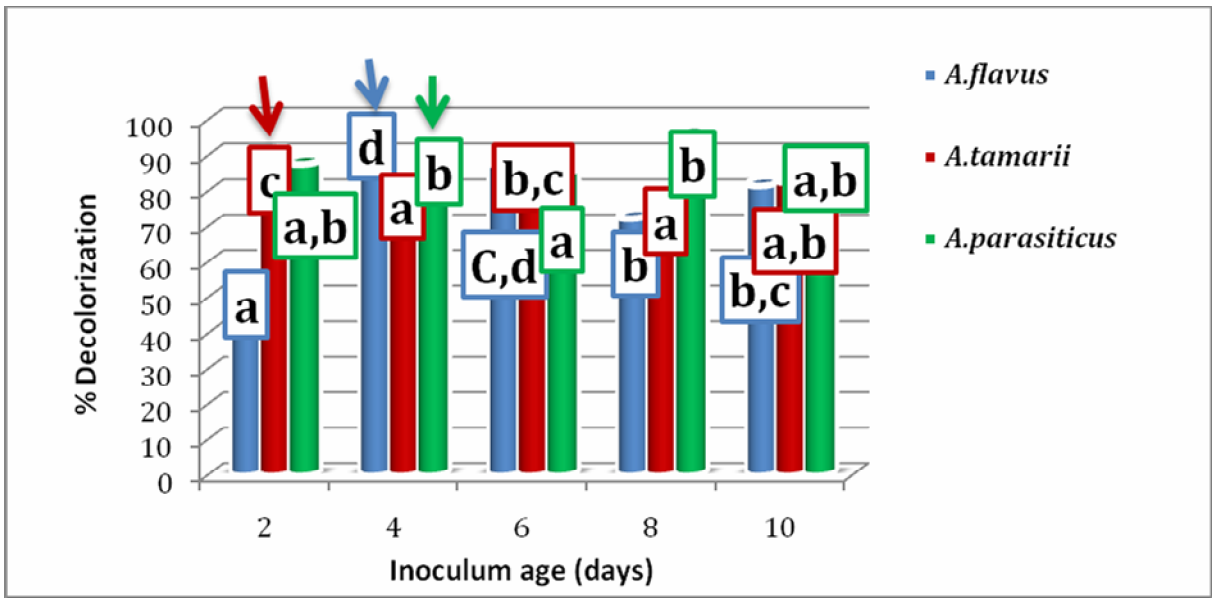
Mean in the blue, red and green columns with different letters have a significant difference between each other.

Fig4. Effect of initial pH values on decolorization of acid black 194 dye



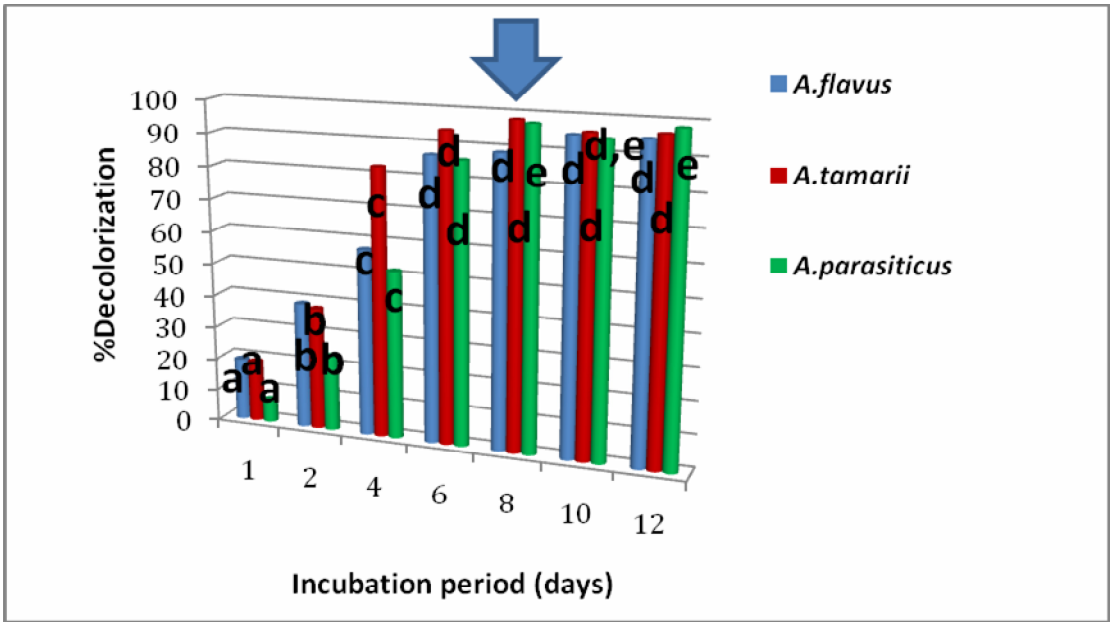
Mean in the blue, red and green columns with different letters have a significant difference between each other.

Fig 5. Effect of inoculum size on decolorization of acid black 194 dye



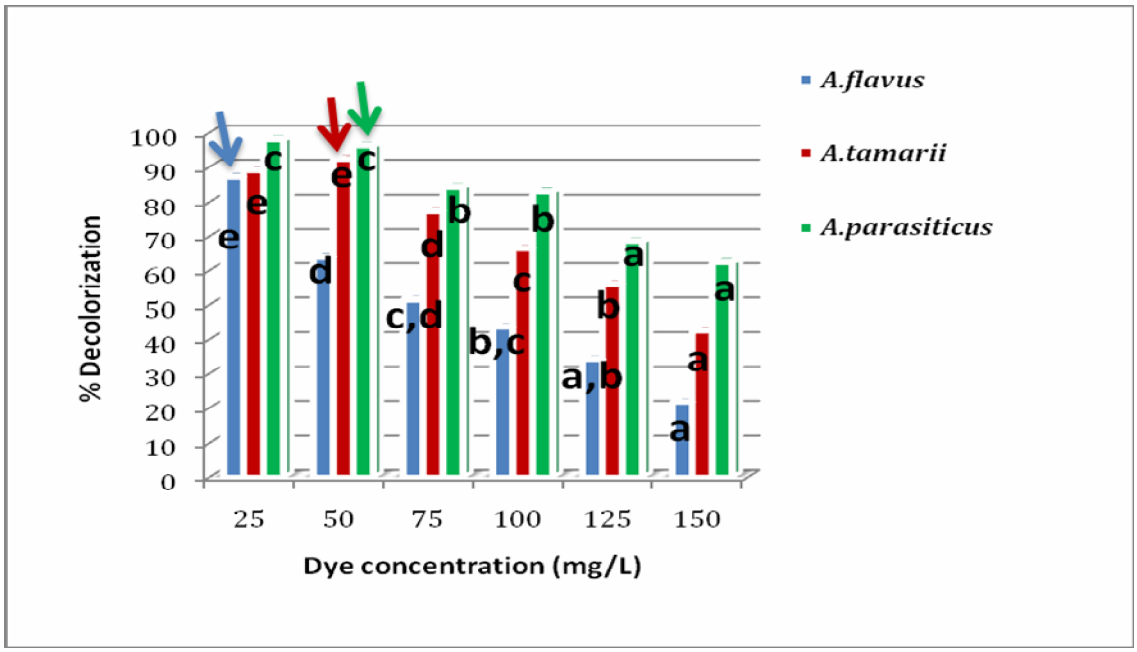
Mean in the blue, red and green columns with different letters have a significant difference between each other.

Fig6. Effect of inoculum age on decolorization of acid black 194 dye



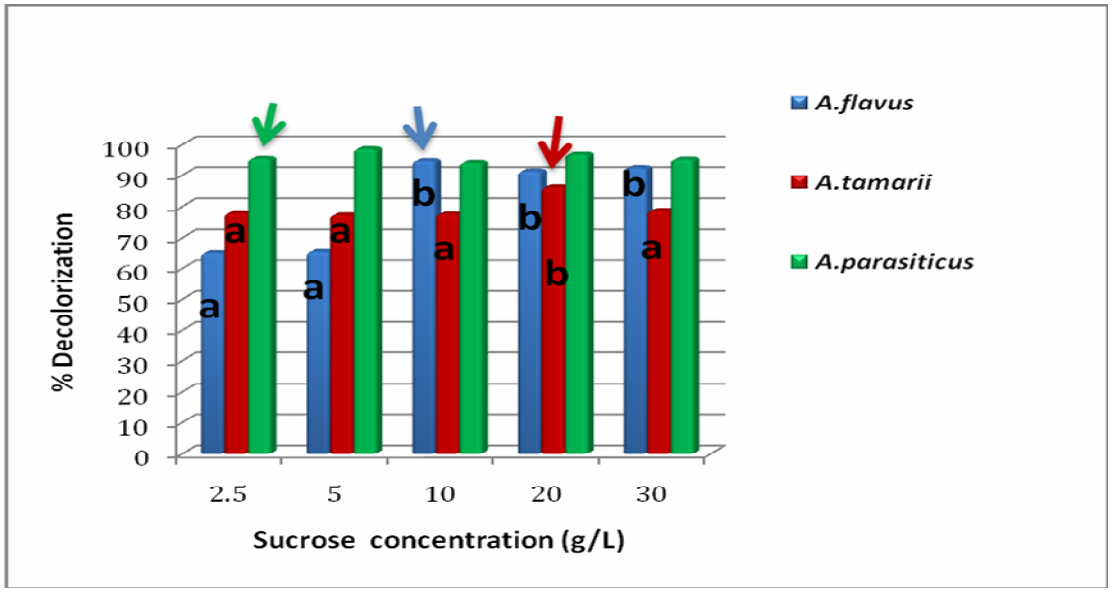
Mean in the blue, red and green columns with different letters have a significant difference between each other.

Fig7. Effect of incubation period on decolorization of acid black 194 dye



Mean in the blue, red and green columns with different letters have a significant difference between each other.

Fig 8. Effect of acid black 194 initial concentration on dye decolorization dye



Mean in the blue, red and green columns with different letters have a significant difference between each other.

Fig 9.Effect of sucrose concentration on decolorization of acid black 194 dye

Table.3: The FTIR spectral characteristics of residual culture medium (before and after treatment with the tested fungi).

Suggested assignment	Band positions (cm^{-1})			
	Unloaded Biomass	**acid black-loaded *A.flavus***	**acid black-loaded *A.tamarii***	**acid black-loaded *A.parasiticus***
-OH	3400.85	3396.03	3386.39	3408.57
-CH	2937.06	2938.02	2936.06	2939.95
-C=C	1646.91	1639.2	1638.23	1646.91
C-N	1259.29	1240	1247.72	1251.58

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