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Mycoremediation of Direct Red Scarlet Dye Used by Malegaon Textile Industry Isolated from Sewage Water

[1]Yahyakhan A. Pathan, [2]Ansari Faiza Firdaus, [3]Momin Aisha Siddiqua

[1,2,3]Department of Botany, J.A.T. Arts, Science and Commerce College (For Women), Islampura, Malegaon, Dist-Nashik- 423203.

ABSTRACT:

Mycoremediation is a technique where in various fungi are used in remediation of hazardous contaminants like aromatic compounds, dyes, hazardous phenolics, heavy metals and several others. The Effluents of textile dye industries is a serious problem, the untreated effluent causes environmental pollution and affect human health. The aim of this research was to mycoremediate of textile dye (direct red scarlet 4BS) by fungi isolated from sewage water. The dye is consistently used by Malegaon textile industry to color the fabric, after usage that dye was directly discharge in the nearby places and in the sewerage lines.

The isolated fungal species were *Aspergillus niger, A. flavous, Fusarium sp.,* and *Cladosporium sp.,* from sewage water.

This study was conducted to investigate the ability of fungal species to decolorize and degradation of textile dye at different concentrations. The physicochemical properties of different concentrations of textile dye were assessed before and after fungal inoculation. Mycoremediation was confirmed by measuring optical density of treated and untreated different dye concentration solutions at 37ºC. The fungal treatment to different concentrations of dye decreases in optical density as day increases i.e., fifth day, seventh day, nineth day and eleventh day respectively, which leads to the positive result of experiment. This change in OD results considerable change in pH and effective. Decolorization percentage indicate that fungi have high degradation potential and this research will help us to make the dye less toxic and eco-friendly to environment in future. The Bioremediation of textile dye by fungi is cheapest method and it is the advanced biodegradable microbial technology for the treatment of textile dyes effluent.

Keywords- Mycoremediation, *Aspergillus niger, A. flavus Cladosporium*

1. Introduction:

Industrialization processes are considered as one of the main aspects for enhancing economic growth worldwide; however, the effluents released from these processes are Major contributors to pollution and eco-toxicity [1,2]. Colored effluents are produced because all industrial processes, such as textile, dye manufacturers, pharmaceuticals, foods, Plastics, cosmetics, leathers, rubbers, papers, and pulp, contain dyes [3,5]. The textile Industry involves a broad range of machinery, raw substances, and different processes. To manufacture end products with specific shapes and high qualities. This industry is considered the oldest and popular industry worldwide, starting from 3000 BC, and provides the largest number of employments [6]. The effluents released from the textile industry are considered as one of the highest liquid pollutants, based on the published literature. About 280,000 tons of textile dyes are discharged as wastes in textile effluents every year worldwide [7,8]. A massive quantity of water is consumed during the textile processing steps such as washing, dyeing, seizing, and others. Approximately, 10–20 L of pure water is consumed for dyeing 1 kg of fabric [9]. Annually, the environment and different water ways receive about 1000 tons of dyes besides other pollutants such as aromatic amines, flame retardants, heavy metals, phenols, and so on without any treatment [10]. As a result, these effluents destroy the soil and aquatic ecosystems by altering the microbial community and reducing the quality of surface and groundwater [11]. The problem related to textile effluents is not only attributed to the presence of dyes but also to the presence of highly stable and non-degradable pollutants [7]. Recently, textile wastewater treatment has captured more attention because of the strict legislation concerning the discharge of this effluent in the ecosystem[7]. Textile wastewater contains other pollutants, such as color residues, inorganic compounds, catalytic chemicals, dye waste, and cleaning solvents[13,14]. The negative impacts of the discharge of dyes into environment without any treatment can be related to adverse effects on the photosynthetic activity, which restricts the access of light and causes a shortage of oxygen, further decreasing the survival rates of flora and fauna [15,16]. Additionally, different dyes discharged from industries are highly toxic and considered a mutagenic agent. Therefore, the major challenge is to establish new and viable approaches to degrade these dyes because they are difficult to degrade by traditional methods. Importantly, these dyes exert carcinogenic and toxigenic effects on human and aquatic systems [16,17]. Effective biological treatment processes are of great value due to their eco-friendly, low cost, and minor sludge-giving properties [12,18,19]. Different metabolites produced by microbes have a high potentiality in various biological activities [12]. The potentiality of fungi in biodegradation is known as mycore mediation. Fungi are well known for their superior abilities to produce a well-built variety of extracellular proteins and other organic compounds. Textile industries play a vital role in the economic increases in India. Water is one of the major products of nature used enormously by human beings and it is not unnatural that any growing community generates enormous wastewater or sewage. The textile industries dispose the waste water during the process of dyeing. The textile waste water generates

the effluent system. Direct dyes represent about half of the dyes used in the textile industry and, as a consequence, a relevant problem of pollution related to the release of these products in the environment is taking place. Industrial wastewaters are the significant contributor to water pollution by polluting rivers, lakes and oceans. These wastewaters are released by different industries such as textile, paper, dyestuffs and pulp, distillery, tannery, oil mill and metal industries [20,21]. Textile wastewater frequently contains a large variety of chemicals additives and dyes expended in the dyeing procedure as soda ash, heavy metals, acetic acid and caustic soda. Effluence with these dyes signifies an important environmental confront to the industry of textile [22]. The wastes released from textile dyeing industry contains different hazard compounds difficult to degrade such as direct dye, which are the main source for environmental pollutions [23,24]. Textile industries are one of the largest generators of wastewater due to extensive volume of water is utilized in finishing and dyeing processes [25]. Other reports use technology to improve textile processes and reduce environmental pollutants [26,27]. It is predictable that, the liberalization of 10 -15% of dyes in the treated water [28], which affect the photosynthetic activities in aquatic life by reducing the intensity of light propagation that may also be lethal to some aquatic animals and plants due to the attendance of aromatic materials, metals, chlorides and because of low biodegradability [29,30]. Inspite of the dyes, textile effluent additionally contains variable pH and ionic strength, and high concentration of salts. Synthetic dyes, which are widely used in the industry of textile, act for a main problem in environment. Treatment of dyeing wastewater was very valuable before its safe clearing into environment. The spectrum of methods for treatment of textile wastewaters is extremely broad. Presently, numerous physicals, chemicals and biological treatment approaches are used. Numerous types of physio-chemical treatments like chemical and absorption treatments, such as chemical degradation, absorption and precipitation. Degradation by fungi is known as myco-remediation. Fungi are established for their superior talents to produce a well-built variety of extracellular proteins and other organic compounds, their capacities to adapt to severe environmental constraints, and they were easy to manipulate with different problems [31]. Dyes are eliminated by fungi and other microbes through biosorption, detoxification, bio-degradation, bio-accumulation. The current study aims to explore and compare the decolorization efficiency of two different textile azo dyes by *Aspergillus* spp. isolated from the effluent of textile wastes dyeing industry. In supplement, the effect of many parameters such as pH, dye concentration, inoculum sizes and different nitrogen and carbon sources on dyes decolorization was assessed. The augmentation of dyes decolorization was estimated by the optimization of these microbial and chemical parameters. Bioremediation is a process that removes xenobiotic compounds from the biosphere. The main aim of thebioremediation is to eliminate contaminants to undetectable concentration limit,which is established by regulatory agencies [32].Among all organisms, fungi are adverse group of organisms, which are ubiquitous in the environment. Their major contribution ranges from various industrial applications to remediation purposes [33]. Fungi can easily survive in most of the habitats and play dynamic role in ecosystem. They regulate the flow of energy and nutrients through their mycelial networks convert the organics and other metabolites into another form. Thus, these fungi are considered the natural and true ecosystem engineers [34]. Fungi microscopic and macroscopic eukaryotic organisms, can easily grow on different substrates and are capable of continuing their function almost indefinitely. Fungi include molds, yeasts, ascomycetes, and basidiomycetes fungi are highly tolerant to extreme conditions such as higher temperature, acidic or alkaline pH, higher concentration of metals, etc. Fungi are highly plastic bodies, and most of the fungal totipotent. Mycoremediation is the process wherein fungi degrade or cause to deteriorate the variety of materials and compounds [35].

2. Materials and methods:

2.1 Materials:

The contaminated sewage water was used for fungal isolation were collected from the near by places of textile industry located in Islampura, Malegaon, Nashik, Maharashtra_ India. The final effluents from the same textile industry were obtained as a model to study the efficacy of fungal isolates in textile wastewaters treatment. Plastic bottles were used for because wastewaters containing hazardous substances react with sodium in the glass matrix. The sample was collected in 1L sterilized polypropylen bottle, kept at 4oC and transferred to laboratory during 24 hours.

2.2. Methods

Fungal Isolations:

The collected contaminated sewage water sample were screened for the isolation of the fungal species on Potato Dextrose Agar(PDA).

Preparation of PDA:

PDA plates were taken for fungal isolation by preparing serial dilution were made for the isolation of fungi.10 ml of sewage water was transferred to 100 ml of distilled water. Then 1 ml of that solution transferred to 9 ml of distilled water and serially diluted upto 10^{-9}.

About 3 ml of the sixth, seventh, eighth, and nineth dilution was inoculated onto the PDA plates for the fungal growth. The plates were incubated at 28+2 or 28-2 for 3-5 days. The fungal growth was checked for purity and the purified isolated was inoculated in the culture slant at 30°C.In total,4 purified fungal species were isolated.

Preparation of PDA

- **Fungal isolation and identification from sewage water:**

In this study, four fungal isolates were isolated from textile dye effluent on the basis of morphological and biochemical characteristics as identified as *Aspergillus niger, Aspergillus flavous, Fusarium sps, Cladosporium sps.*

❖ **Inoculation of fungal strain:**

0.1 to 0.5 concentration were in 100 ml of distilled water of dye (Direct Fast Scarlet 4BS/Direct Red 23)Isolated cultures were *Aspergillus niger, Aspergillus flavous, Fusarium sps, Cladosporium spp.* isolated into preinitiation and post initiation absorbance were taken with respective days. Fungal colonies were inoculated in sample using cork borer.

❖ **Screening for the Bioremediation Efficacy of Textile Waste water:**

The optical density of the textile effluent was observed at different wavelengths ranging between 500 to 600 nm using spectrophotometer UV.

The wavelength at which the textile effluent showed the maximum absorbance, (wavelength max at 560 nm), was used as a standard for all treatment. The all-inoculated samples were used for measuring the absorbance at wavelength max of 560 nm on consecutive days.

- **Decolorization percentage of non-adapted fungal strains:**

During the incubation period, samples were drawn at fifth, seventh, nineth and eleventh day intervals to measure their optical density (O.D). O.D.of solution treated with fungal isolates was measured by taking absorbance reading of cell free supernatant using spectrophotometer at 560 nm. The extent of decolorization was expressed as percentage of decolorization estimated as

$$\text{Decolorization Percentage} = \frac{(Ai\text{-}At)}{Ai} X100$$

Where initial absorbance of the dye solution and absorbance at the cultivation time denoted by **Ai** and **At** respectively. Initial absorbance reading (Ai) was obtained by taking O.D of untreated dye solutions.

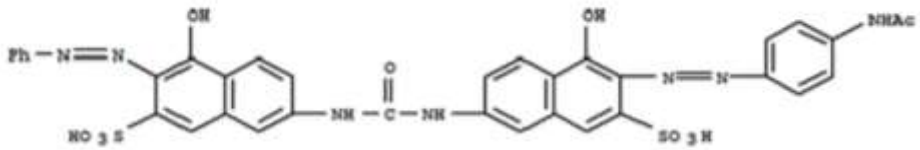
- **Red Dye Content:**

Name: Direct Red Scarlet 4BS (Direct Red 23)

Composition:2-Naphthalenesulfonic acid, 3-[2-[4-(acetylamino) phenyl] diazenyl] -4-hydroxy-7- [5-hydroxy-6- (2-phenyldiazenyl) -7-sulfo-2-naphthalenyl] amino] carbonyl] amino]-, sodium salt (1:2)

Density:1.606g/cm3**Formula:**C35H25N7Na2O10S2**Molecular Weight:**813.77

Molecular Structure:



- **Analysis of pH**

The H+ ion concentration of untreated dye solutions and solutions inoculated with fungal cultures were measured using pH paper and comparing with pH scale.

- **TABLE 1 : pH readings of fungal strains:**

Fungal strains	concentration	Ph
Aspergillus niger	0.1	5
	0.2	5
	0.3	5
	0.4	5
	0.5	5
Aspergillus flavous	0.1	5
	0.2	5
	0.3	5
	0.4	5
	0.5	5
Fusarium sps	0.1	5
	0.2	5
	0.3	3
	0.4	3
	0.5	3
Cladosporium sps	0.1	5
	0.2	5
	0.3	5
	0.4	5
	0.5	3

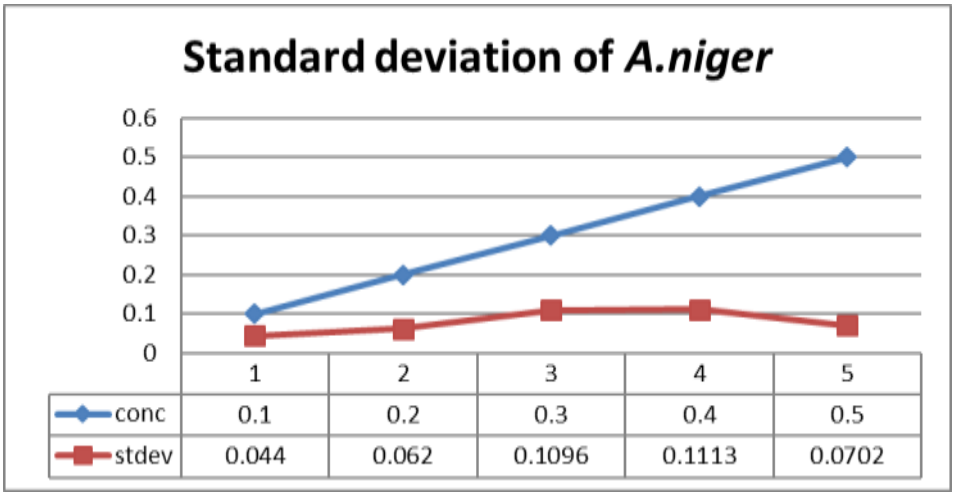
3. RESULTS AND DISCUSSION:

TABLE NO.2: STANDARD DEVIATION , MEAN AND CV (COEFFICIENT VARIATION) OF ABSORBANCE:

- ***Aspergillus niger:***

Concentration	Preinoculation O.D	post inoculation O.D	Mean	Standard deviation	CV (Coefficient variation)
0.1	1.585	_			
		1.465			
		1.523	1.46575	0.044642095	3.045682772
		1.461			
		1.414			
0.2	1.638	_			
		0.941			
		0.923	0.88125	0.062622547	7.10610457
		0.856			
		0.805			
0.3	2.041	_			

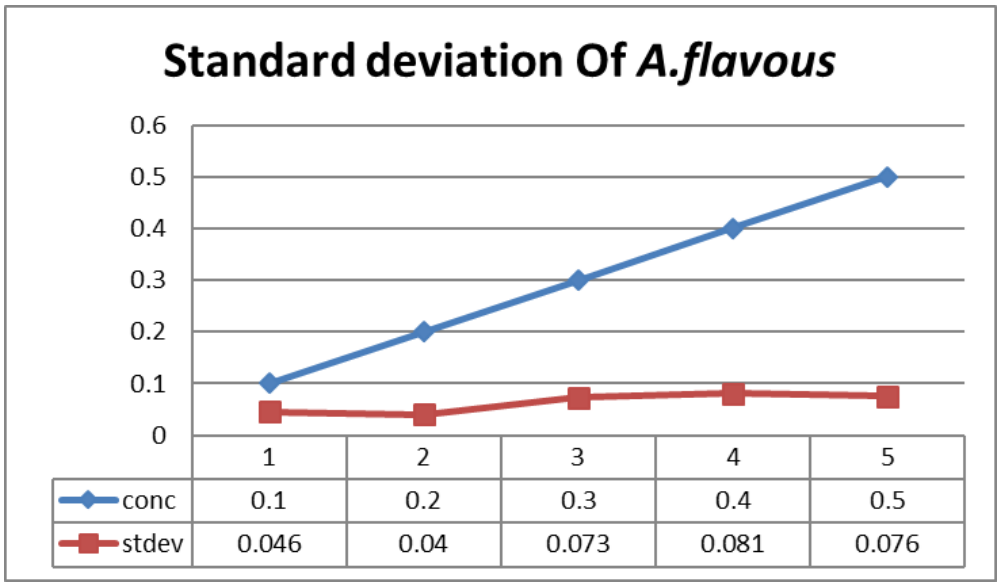
		1.419			
		1.463	1.3795	0.109691993	7.951576152
		1.418			
		1.218			
0.4	2.351	_			
		2.024			
		1.959	1.9075	0.111326846	5.836269783
		1.882			
		1.765			
0.5	2.448	_			
		2.135			
		2.13	2.0805	0.070211585	3.374745734
		2.073			
		1.984			



- ***Aspergillus flavous:***

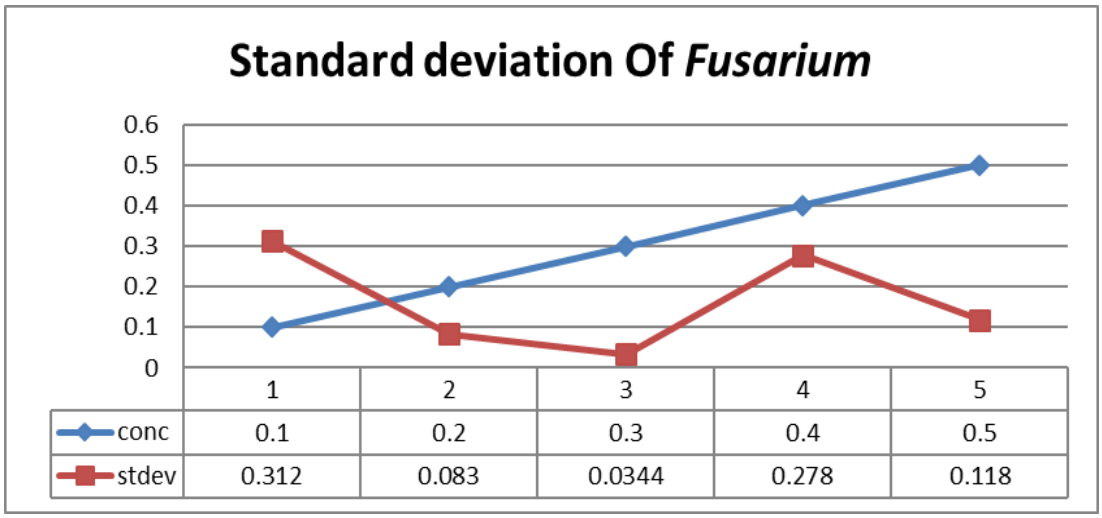
Concentration	**Preinoculation O.D**	**post inocualtion O.D**	**Mean**	**Standard Deviation**	**CV (Coefficient Variation)**
0.1	1.568	_			
		0.703			
		0.619	0.638	0.046065171	7.220246274
		0.634			
		0.596			
0.2	1.638	_			
		1.576			
		1.566	1.53775	0.040631474	2.642267849
		1.52			
		1.489			
0.3	2.041	_			
		1.551			
		1.527	1.481	0.073157365	4.939727524
		1.457			
		1.389			
0.4	2.351	_			
		1.736			
		1.827	1.72775	0.081883556	4.739317351
		1.721			
		1.627			

0.5	2.448	_			
		2.099			
		2.054	2.0235	0.076943702	3.802505676
		2.023			
		1.918			



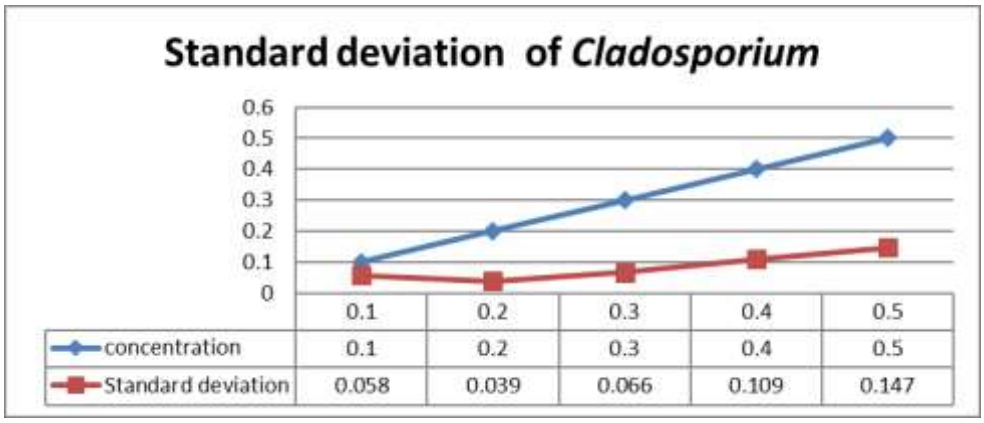
- ***Fusarium spp:***

Concentration	**Preinoculation O.D**	**Post inoculation O.D**	**Mean**	**Standard deviation**	**CV (Coefficient variation)**
0.1	1.568	_			
		1.184			
		1.158	0.90075	0.312878438	34.73532473
		0.655			
		0.606			
0.2	1.638	_			
		1.128			
		1.173	1.0805	0.083180527	7.698336563
		1.019			
		1.002			
0.3	2.041	_			
		1.972			
		1.919	1.92225	0.034490337	1.79426905
		1.898			
		1.9			
0.4	2.351	_			
		1.586			
		1.733	1.76675	0.278826559	15.78189099
		2.171			
		1.577			
0.5	2.448	_			
		1.927			
		2.049	1.9105	0.11891033	6.224042416
		1.759			
		1.907			



- ***Cladosporium spp:***

Concentration	Preinoculation O.D	Post inocultion O.D	Mean	Standard deviation	CV (Coefficient variation)
0.1	1.568	_			
		0.578			
		0.546	0.51475	0.058099771	11.28698796
		0.449			
		0.486			
0.2	1.638	_			
		0.993			
		1.009	1.03475	0.039567453	3.823865954
		1.071			
		1.066			
0.3	2.041	_			
		1.305			
		1.213	1.2125	0.066138743	5.454741696
		1.177			
		1.155			
0.4	2.351	_			
		2.288			
		2.279	2.18875	0.109715921	5.012720551
		2.085			
		2.103			
0.5	2.448	_			
		2.398			
		2.354	2.25225	0.14723762	6.537356854
		2.091			
		2.166			



3.1) Decolorization Percentage:

The fungal strains isolated from sewage water was found to have significant potential to decolorize dye solutions and their ability measured in terms of absorbance and decolorization percentage is mentioned in tables and graphs below. In the present study, the fungal strain showed efficient decrease in optical density and efficient decolorization percentage ranging from 40% to 75% with maximum decolorization % of 71% by ***Cladosporium*** after 72 hours of inoculation.

Treated solution sample by different fungi at different concentration

Untreated solution of different concentration

Red dye (Direct Red 23)

Serial Dilution of Sewage Water

Plating method

3.3) UV- Visible Spectrum for Textile Dye before and after optimization:

According to UV-visible spectroscopic analysis of textile effluent before and after treatment with *A.niger,A.flavous, Fusariumand Cladosporium*, we noted that the absorbance peaks in the visible region decreased without any shift in wavelength max,there by indicating the potentiality of fungal strain Decolorize the dye solutions .The treated direct red dye with fungal strains of *A.niger,A.flavous, Fusarium and Cladosporium* shows different results.*A.flavous* and *Cladosporium* show maximumdecrease in optical density at different concentrations of direct red dye and it is shown in the result table.

Aspergillus flavous showed maximum degredation with preinoculating absorbance 1.586, post inoculation absorbance is 0.596 with percent decolourization 0.1 concentration of dye is 62% of the 11th day of inoculation at 560nm.

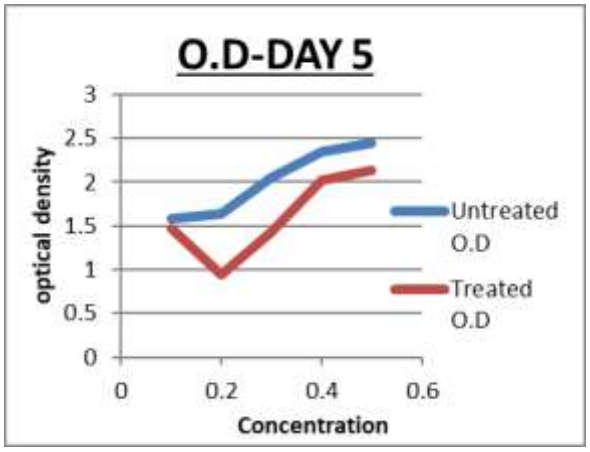
In addition to *A.flavous*, *A.niger* also showed significant degradation as 11th day with 0.805. In comparison with *A.niger* and *A.flavous*, *Fusarium* also showed significant degradation with 1.568 to 0.606 on 11th day after inoculation.

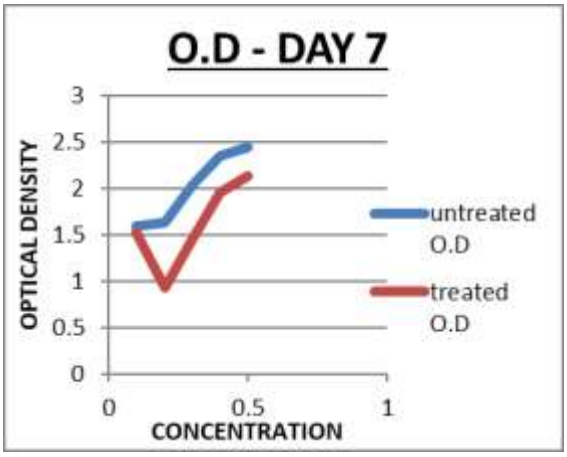
Again *Cladsoporium* sps showed significant degredation ranges from 1.586 to 0.449 on 9th day after inoculation at 0.1 concentration of red dye. All the fungal species showed maximum degredation at 0.1 concentration at 11th day of inoculation.

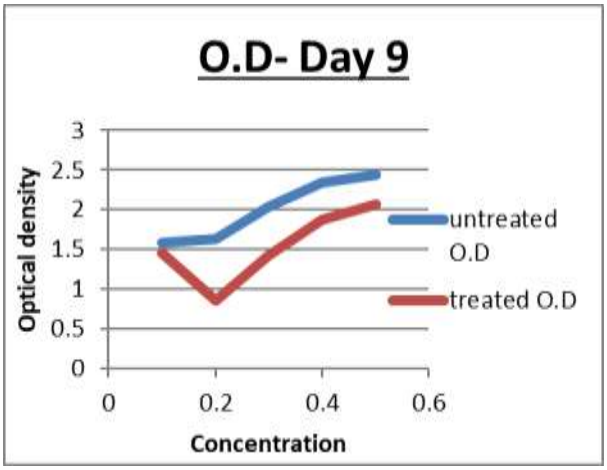
❖ **Table 3 : Result of O.D and Decolorization Percentage:**

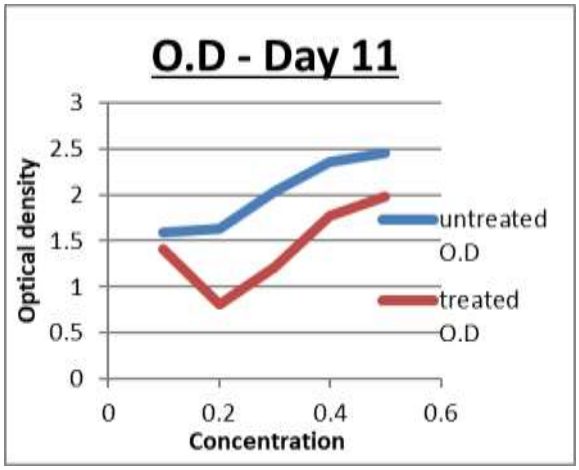
1. *Aspergillusniger* :

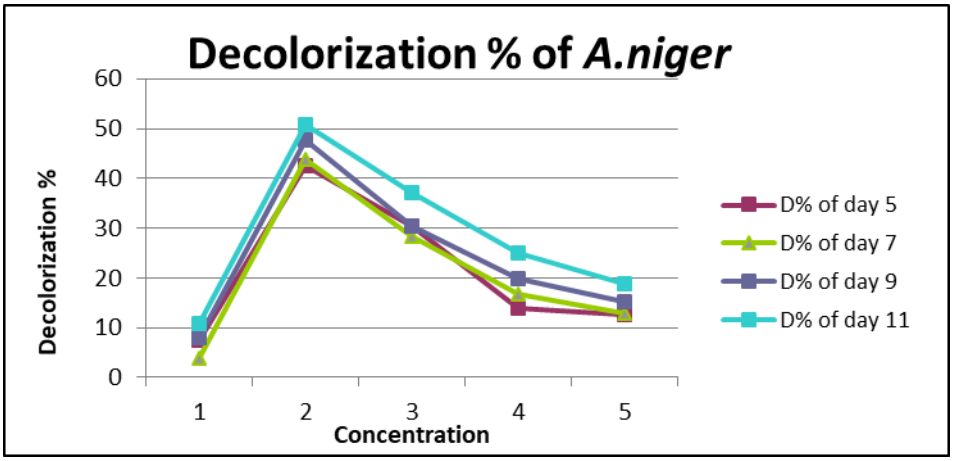
DAY	CONCENTRATION	PRE INOCULATION ABSORBANCE	POST INICULATION ABSORBANCE	DECOLORIZATION %
5	0.1	1.586	1.465	7.6
	0.2	1.638	0.941	42.5
	0.3	2.041	1.419	30.4
	0.4	2.351	2.024	13.9
	0.5	2.448	2.135	12.7
7	0.1	1.586	1.523	3.9
	0.2	1.638	0.923	**43.7**
	0.3	2.041	1.463	28.3
	0.4	2.351	1.959	16.7
	0.5	2.448	2.13	12.9
9	0.1	1.586	1.461	7.9
	0.2	1.638	0.856	**47.7**
	0.3	2.041	1.418	30.5
	0.4	2.351	1.882	19.9
	0.5	2.448	2.073	15.3
11	0.1	1.586	1.414	10.8
	0.2	1.638	0.805	**50.9**
	0.3	2.041	1.218	37.2
	0.4	2.351	1.765	24.9
	0.5	2.448	1.984	18.9





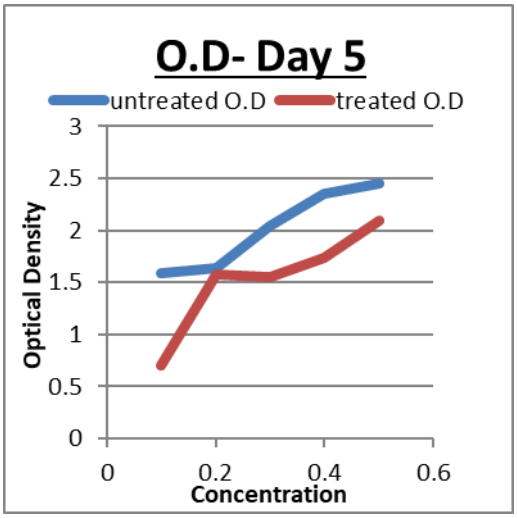


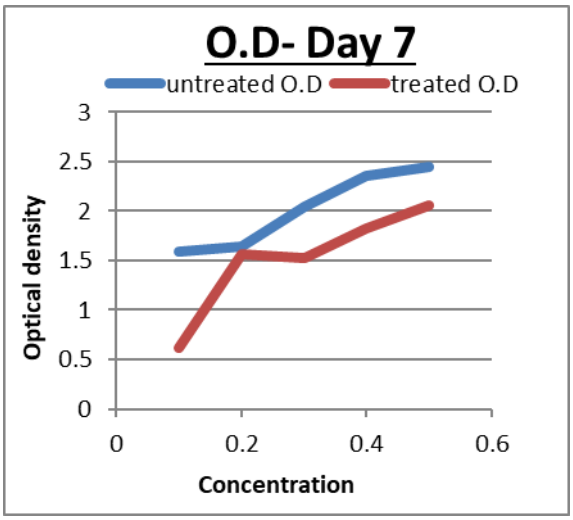


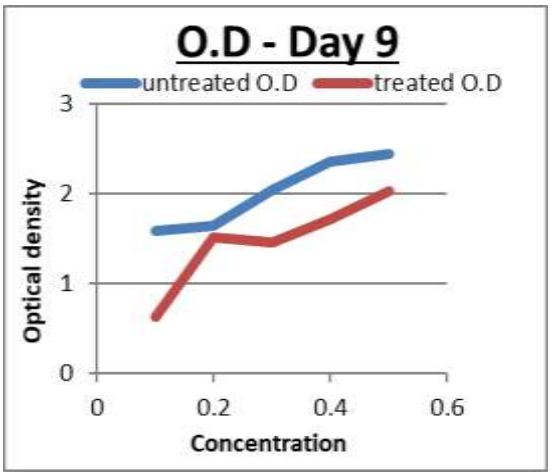


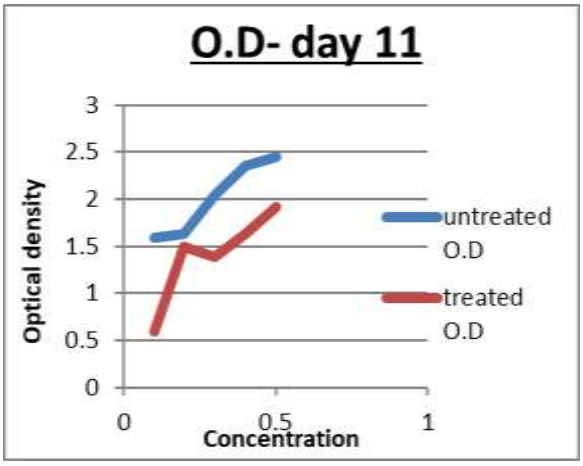
2. ***Aspergillusflavous***:

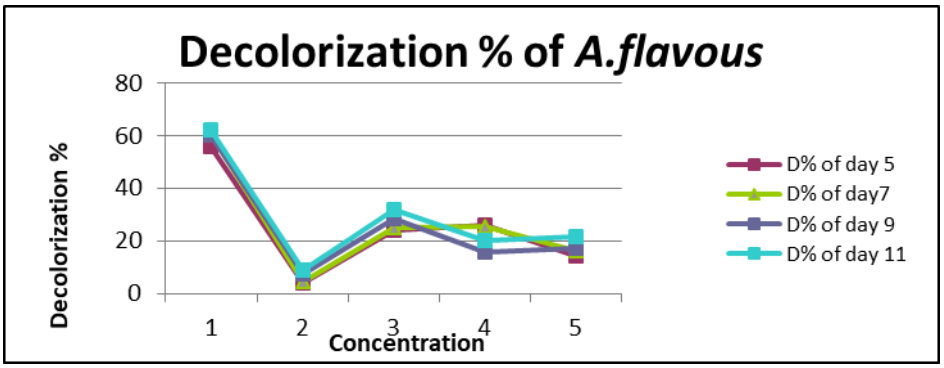
DAY	CONCENTRATION	PRE INOCULATION ABSORBANCE	POST INOCULATION ABSORBANCE	DECOLORIZATION %
5	0.1	1.586	0.703	55.6
	0.2	1.638	1.576	3.7
	0.3	2.041	1.551	24.0
	0.4	2.351	1.736	26.1
	0.5	2.448	2.099	14.2
7	0.1	1.586	0.619	**60.9**
	0.2	1.638	1.566	4.3
	0.3	2.041	1.527	25.1
	0.4	2.351	1.827	25.6
	0.5	2.448	2.054	16.0
9	0.1	1.586	0.634	**60.0**
	0.2	1.638	1.520	7.2
	0.3	2.041	1.457	28.6
	0.4	2.351	1.721	15.6
	0.5	2.448	2.023	17.3
11	0.1	1.586	0.596	**62.4**
	0.2	1.638	1.489	9.0
	0.3	2.041	1.389	31.9
	0.4	2.351	1.627	20.2
	0.5	2.448	1.918	21.6







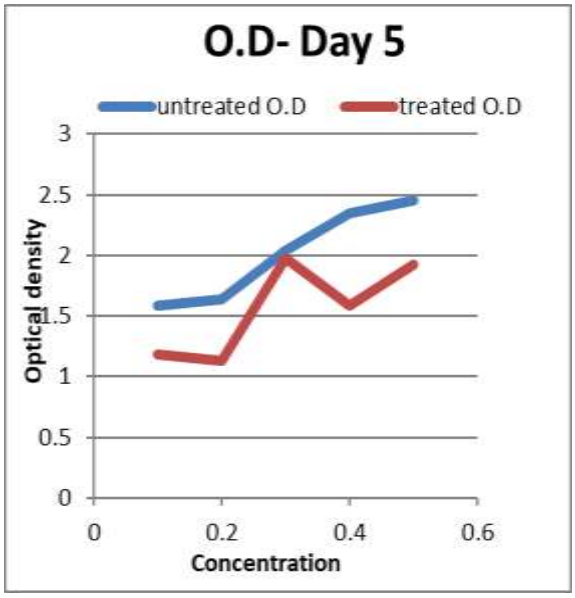


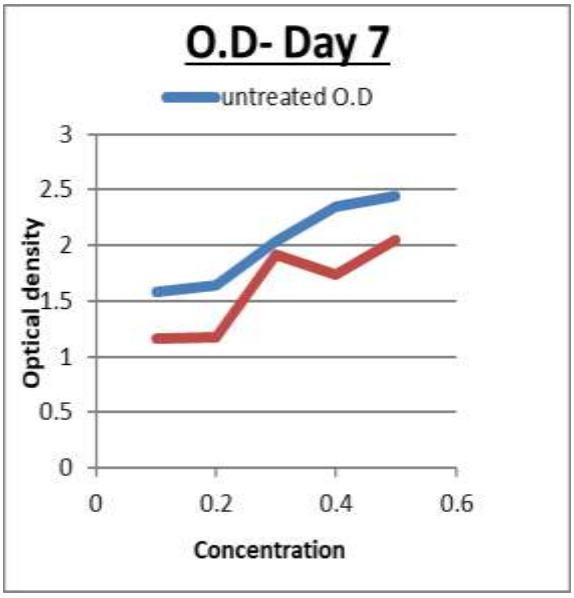


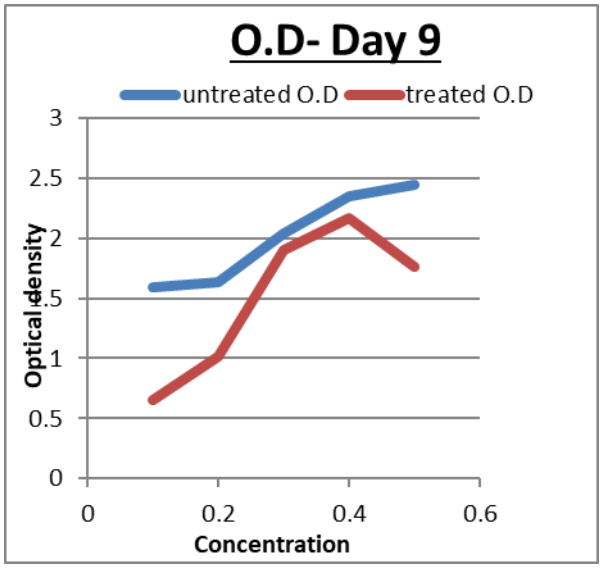
3. ***Fusarium sps:***

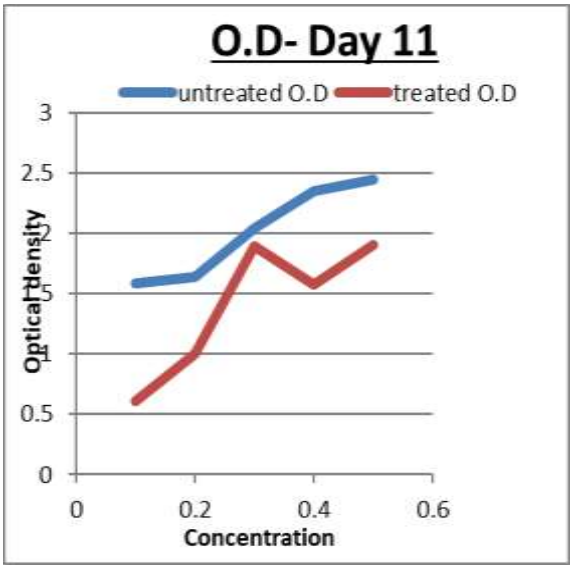
DAY	CONCENTRATION	PRE INOCULATION ABSORBANCE	POST INOCULATION ABSORBANCE	DECOLORIZATION%
5	0.1	1.586	1.184	25.3
	0.2	1.638	1.128	31.1
	0.3	2.041	1.972	3.3
	0.4	2.351	1.586	32.5
	0.5	2.448	1.927	21.2
7	0.1	1.586	1.158	26.9
	0.2	1.638	1.173	28.3
	0.3	2.041	1.919	5.9
	0.4	2.351	1.733	26.2
	0.5	2.448	2.049	16.2
9	0.1	1.586	0.655	58.7

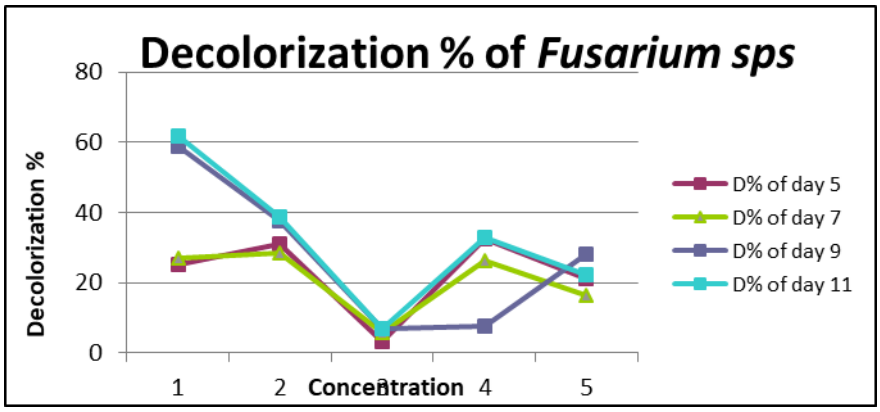
	0.2	1.638	1.019	37.7
	0.3	2.041	1.898	7.0
	0.4	2.351	2.171	7.6
	0.5	2.448	1.759	28.1
11	0.1	1.586	0.606	61.7
	0.2	1.638	1.002	38.8
	0.3	2.041	1.9	6.9
	0.4	2.351	1.577	32.9
	0.5	2.448	1.907	22.09





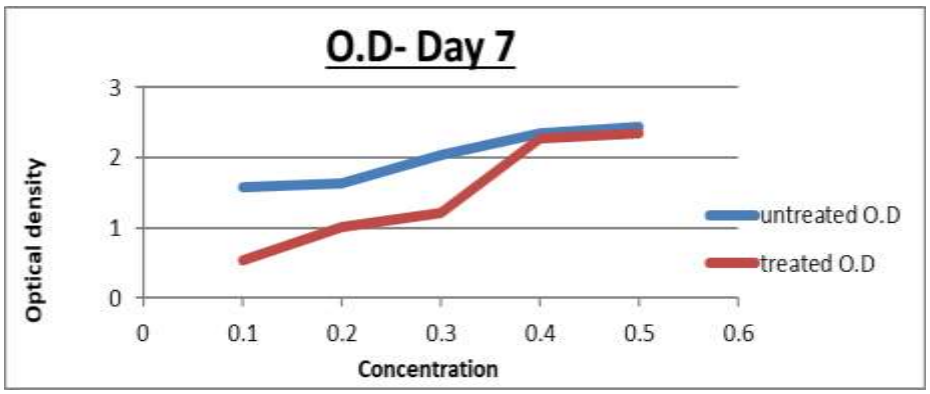


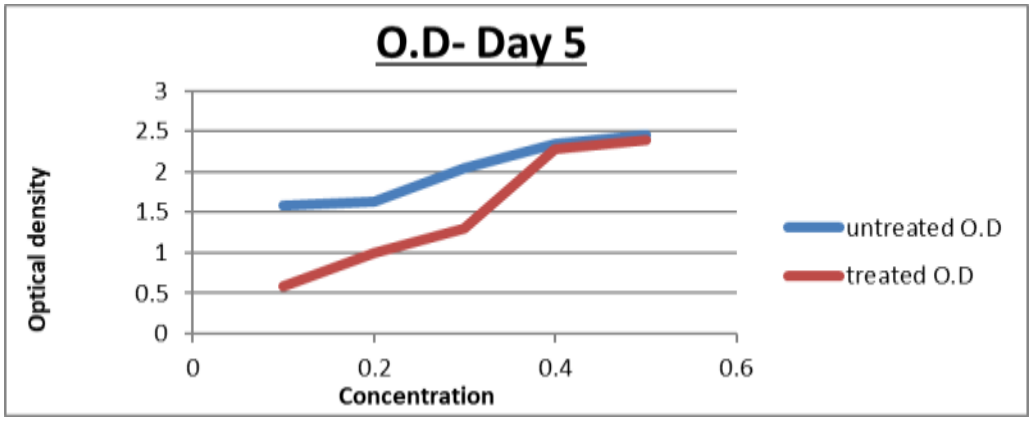


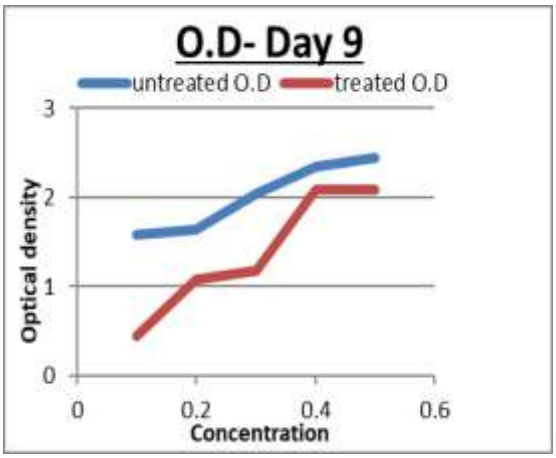


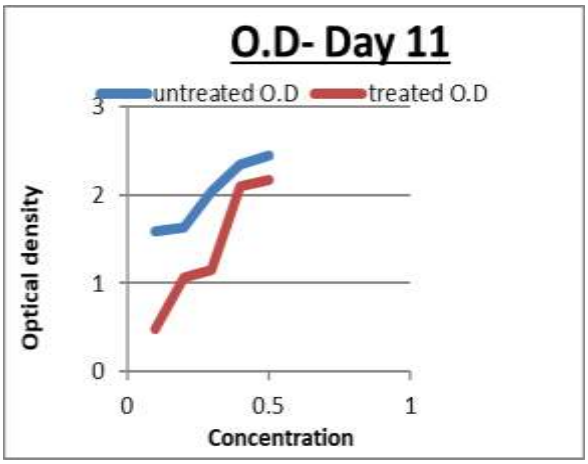
4. Cladosporium sps:

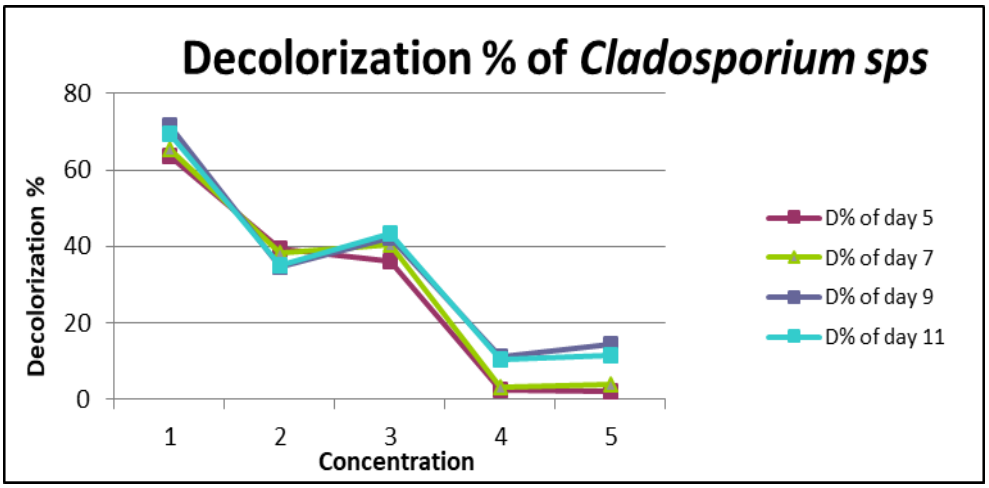
DAY	CONCENTRATION	PREINOCULATION ABSORBANCE	POST INOCULATION ABSORBANCE	DECOLORIZATION %
5	0.1	1.586	0.578	63.5
	0.2	1.638	0.993	39.3
	0.3	2.041	1.305	36.0
	0.4	2.351	2.288	2.6
	0.5	2.448	2.398	2.0
7	0.1	1.586	0.546	**65.5**
	0.2	1.638	1.009	38.4
	0.3	2.041	1.213	40.5
	0.4	2.351	2.279	3.0
	0.5	2.448	2.354	3.8
9	0.1	1.586	0.449	**71.6**
	0.2	1.638	1.071	34.6
	0.3	2.041	1.177	42.3
	0.4	2.351	2.085	11.3
	0.5	2.448	2.091	14.5
11	0.1	1.586	0.486	**69.3**
	0.2	1.638	1.066	34.9
	0.3	2.041	1.155	43.4
	0.4	2.351	2.103	10.5
	0.5	2.448	2.166	11.5











Conclusion:

From the obtained result, it can be concluded that; locally isolated fungal strains from sewage water posses high Decolourization and degradation discharge of textile dyes.

This study investigated for the Decolourization of direct scarlet dye 4BS by fungal strains under all optimized conditions of pH, inoculation of fungal strain and consecutive days observation and calculation of absorbance.

Optical density was detected for direct red dye solutions after treatment by the fungal strains of *A.niger, A.flavous, Fusarium and Cladosporium*,and successful decrease in Optical Density was observed which give the result in the decolourization of dye .

It is confirmed the mycoremediation of textile dye by fungal strains provided a cost effective, easily applicable and eco friendly method.

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