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Research Article

SAFRANIN DECOLOURISATION FROM AQUEOUS SOLUTION USING *CLADOSPORIUM* SPECIES – AN *IN VITRO* STUDY

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ABSTRACT

Fungus play a considerable role in the bioremediation of dye contaminated soil and wastewater. In the present investigation decolourisation of safranin using *Cladosporium* species was assessed. Various parameters such as pH, temperature, dye and inoculum concentration, incubation time, carbon and nitrogen sources were optimized for effective decolourisation. Maximum decolourisation was observed when 1% *Cladosporium* species was inoculated in the medium amended with 0.01g of safranin at 30°C on fifth day at pH 5 supplemented with 1% glucose and potassium nitrate. The dye loaded and unloaded fungal biomass was subjected to Fourier-transform infrared (FT-IR) spectral analysis and it was recorded that the hydroxyl, carboxyl and amine groups present on the surface of the cell walls helps in the binding of safranin. Phytotoxicity study reveals that the fenugreek grown using degraded metabolites of safranin solution seems to be non-toxic when compared with control plants. Thus the results suggest that *Cladosporium* species removes safranin from the aqueous solution at optimized conditions.

Keywords: *Cladosporium* species, Decolourisation, Optimization, Safranin, FT- IR, Phyotoxicity.

1. INTRODUCTION

Industries are the wings of modern technology which afford indispensable desires of the populace in terms of clothing, food and shelter. But the extensive industrial activities and mismanagement of resources have resulted in the dumping of solid and liquid wastes, thereby leading to pollution of the ecosystem. Water pollution has turn into one of the critical environmental issue in the globe. The main sources of water pollution come from the domestic sewage and industrial wastewater, and the latter is accounted for the vast majority [1]. Due to rapid industrialization and modernized civilization, there was a boom in textile sector. Textile industry plays a pivotal role in the global economy as well as in day to day life. India ranks as second largest exporter of cotton yarn with nearly 10,000 manufacturers and 2200 bleaching and dyeing industries. Concerning the hazardous effects of dyes on the environment, the consumption and release of effluent from the dyeing and finishing process of textile industry have accelerated pollution in aquatic and terrestrial environment, making water a limited resource. The untreated brightly coloured effluents are dangerous when discharged into open water bodies and diminish the penetration of sunlight which is essential for photosynthesis. Textile dyes are reported to cause skeletal and cerebral abnormalities in foetus. Various methods have been researched in the treatment of textile waste water for decreasing the scarcity of water and its impact on environment [2, 3]. Hence waste water reclamation and reuse has become a striking method to shield the environment and available water resources.

Safranin is a synthetic and heterocyclic dye extensively used in textile industry for dyeing cotton, wool, silk, leather and paper. It is a water soluble dye which is persistent and difficult to degrade due to its structural availability [4, 5]. Ingestion of safranin causes damage to liver, lungs and kidney, produces nausea and vomiting, leads to gastrointestinal discomfort, conjunctival edema, purulent discharge, inflammation of skin, and dermatitis [6]. Degradation of this dye is gaining momentum which is evident from the number of available reports.

Even though various physicochemical methods such as adsorption, membrane filtration, photocatalytic degradation, ion exchange, precipitation, flocculation, floatation and ozonation are quite helpful in decolourisation of dyes, all have some disadvantage such as high cost per unit volume of waste water treatment, unfriendly for nature or unpredictability in operation [7].

Although decolourisation is a challenge for textile industry as well as waste water treatment systems, there is a great potential for developing microbial decolourisation systems with total colour removal within few hours [8]. A successful technique for mitigating the effects of toxic dyes is by employing microorganism to degrade it to non-hazardous compounds [9]. Degradation using microbes proves to be an attractive method, due to their cost effectiveness, diverse metabolic pathways and versatility [10, 11]. Mycoremediation is an effective and affordable technique for degradation and decolorization of dye-bearing effluents. Now-a-days research interest in the decolourisation and degradation of synthetic dyes using fungus has been increased significantly [12-14]. Hence, an attempt has been made to study the ability and potential of *Cladosporium* species towards remediation of safranin dye under optimal conditions and to assess the phytotoxic effect of the degraded metabolites on fenugreek.

2. MATERIAL AND METHODS

2.1. Chemicals and Media

Safranin $C_{20}H_{19}ClN_4$ (Mol. Wt. 350.85 g·mol^{-1}=520 nm) is a cationic dye. Safranin and the microbiological media used in the study were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. The structure of safranin is given in Fig.1.

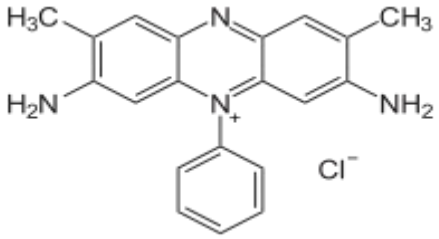


Fig. 1: Chemical Structure of Safranin

2.2. Sampling

The soil samples were collected near a small scale textile dyeing unit at Tiruppur district, Tamil Nadu, India. The collected soil samples were crushed, sieved and stored at 4°C before analysis.

2.3. Isolation, screening and identification of safranin decolourising fungi

Ten grams of the soil sample was serially diluted, spread plated on sterile Rose Bengal Chloramphenicol agar medium and incubated at room temperature (28°C) for 5 days. After incubation period, well grown fungal colonies were isolated and maintained on Rose Bengal Chloramphenicol agar slants at 4°C for further study. A loopful of the fungal isolates was inoculated into Sabauroud dextrose broth amended with 0.1g of safranin separately and screened for its decolourisation efficiency. At the end of the incubation period, the samples were removed and analysed for its colour intensity at 540 nm using UV-Vis spectrophotometer. The decolourisation efficiency of the fungal isolates were calculated using the formula,

Percent decolourisation = $(A_i - A_f)/ A_i$ X 100

Where A_i= Initial absorbance, A_f= Final absorbance

Based on the per cent decolourisation, the fungal isolate which exhibited maximum decolourisation percentage was selected and was subjected to identification by lactophenol cotton blue staining method.

2.4. Optimization of different parameters for decolourisation

To determine the optimal conditions for safranin decolourisation, the selected fungal colony was inoculated into a series of 250ml Erlenmeyer flask containing different concentrations of dye (100mg/l-500 mg/l) at varying inoculum concentrations (1%-5%). The pH varied from 3 to 9 by adjusting with 1N HCl or 1N KOH. At each pH, the fungal isolate was inoculated and incubated at different temperature (15°C-40°C) for different incubation period (1-8 days) to find out the optimum decolourisation activity. The carbon (glucose, sucrose, maltose, mannitol and starch) and nitrogen (ammonium chloride, sodium nitrate, potassium nitrate, sodium chloride, yeast extract, glycine and peptone) sources were added at 1% level for effective decolourisation. At the end of each incubation period, the samples were assessed for its decolourisation efficiency. The isolated fungal species under the optimized conditions was checked for its efficiency to degraded safranin.

2.5. FT-IR spectral analysis

FT-IR analysis was carried out to study the degradation of safranin and resolve the toxicity of the degraded metabolites through the modifications of functional group. The liquid-liquid extraction was executed using ethyl acetate as a mid-polar solvent on the degraded metabolites. The moisture present in the fungal samples were removed by mixing it with potassium bromide and kept in hot air oven for 30 min at 105°C, further powdered and subjected to analysis in the ratio of 5:95 (w/w). The disk was then fixed in a FT-IR spectrometer (FT-IR 8400S SHIMADZU) to analyse the spectrum of

the biomass unexposed and exposed to safranin at 400 to 4000 cm^{-1}, for a total of 16 scans.

2.6. Phytotoxicity study

Phytotoxicity test was carried out on fenugreek (*Trigonellafoenum-graceum*) to assess the toxicity of safranin before and after decolourisation by *Cladosporium* species. For the present investigation, nine pots were set and ten healthy seeds were sown in each pot and kept under laboratory conditions. The seeds were watered regularly twice a day using degraded metabolites of safranin solution (T_3), untreated safranin solution (T_2) and tap water (T_1) which served as control. The treatments were maintained in triplicates. At the end of 7^{th} day the fenugreek seedlings were uprooted and the biometric parameters such as germination percen-tage, shoot length, root length, fresh and dry weight and vigour index were analysed. The data obtained were statistically analysed by one way analysis of variance ($P<0.05$) using statistical software Sigmastat 3.1.

3. RESULTS AND DISCUSSION

3.1. Identification of safranin decolorizing fungi

Five morphologically distinct fungal colonies were isolated and screened for decolourisation from the soil sample. Among them, the isolate 4 removed 78% of dye followed by isolate 2 which decolourised 62% of safranin. Minimum decolourisation of 56%, 45% and 34% was observed in isolate 1, 5 and 3 respectively. The fungal isolate which removed maximum colour from the dye amended medium was identified as *Cladosporium* species. The colonies were greenish black and powdery on Sabauroud dextrose agar medium. The hyphae were large and thick. The conidiophores were erect, branched, floccose and pigmented. The conidia were one celled, smooth, globose and ovate.

3.2. Optimization studies for safranin decolourisation

The optimization parameters for decolourisation of safranin by *Cladosporium* species was presented in Fig. 2a-2g. Dye concentration greatly influences the decolourisation rate by the fungal isolate. Maximum decolorization (77%) was observed in the medium amended with 0.01g/100 ml safranin. When the dye concentration was increased from 0.02-0.06g/100ml the decolorization rate by the *Cladosporium* species was gradually reduced. As for the effect of varying concentrations of dye, the trend obtained followed a typical and normal course of sorption process whereby the least concentrated showed the highest uptake and the more concentrated showed the lowest uptake of dye. The decrease in decolourisation rate may be due to the toxic effect of dyes on the fungal isolate or the blockage of active sites of the enzymes by the dye molecule [15-17] or the presence of one or more sulphonic acid in the dye may act as a detergent and affects the synthesis of DNA and thereby inhibits the growth of the fungus [18].

Cladosporium species exhibited highest decolourisation activity at 30°C (71%) on 5^{th} day of incubation at pH 5. Above 30°C, there is a decrease in percentage decolourisation which might be due to thermolabile nature of *Cladosporium* species, or the slowdown of enzyme catalysis which in turn slowed the growth of the fungus [19]. When the temperature is increased, gradually the stability of the fungal cell wall, its configuration and the dye binding sites are affected and causes ionisation of chemical moieties and thereby it decreases the rate of colour removal from the dye amended medium which is an energy-independent physiochemical mechanism. The results of the present study fall in line with the findings of Kumar *et al.,* [20] who reported the optimum temperature for Congo red decolourization by *Curvularia sp*. was 30°C within 8 days. The results indicated that the removal of safranin by *Cladosporium* species was high on the fifth day (73%) which might encountered the boundary layer effect and then diffused into the porous structure of the biosorbent.

Among the different carbon and nitrogen sources (1%) tested, glucose and potassium nitrate was found to show maximum percent decolourisation (72% and 73%) after 5 days of incubation. With other supplements of carbon and nitrogen source, decolorization efficiency was decreased. For the reduction of azo dyes, it requires the presence and availability of a co-substrate which acts as an electron donor. Degradation of azo dyes by microbes is difficult, since they are deficient in carbon and nitrogen sources [21]. Carbon sources afford energy for the growth and survival of the microbes and as electron donors, which are indispensable for the breakage of the azo bond. These sources produce reducing equivalents which are transferred to the dye during the process of decolorization [22].

Cladosporium species under optimized conditions degraded safranin to 82%. For effective decolourisation dye concentration (0.01g/100ml), inoculum concentration (1%), incubation period (5 days), pH (5), temperature (30°C) and glucose and potassium nitrate concentration (1%), should be in optimal level (fig. 3).

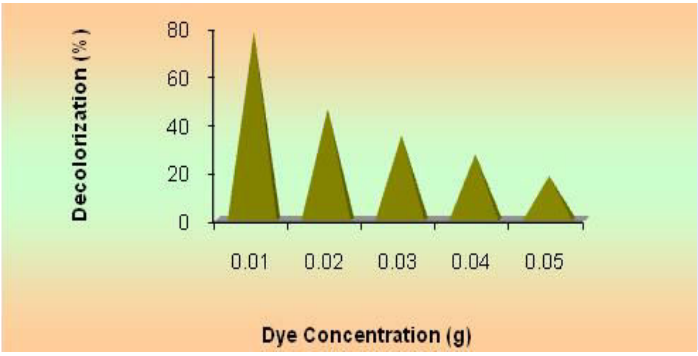


Fig. 2a: Effect of dye concentration

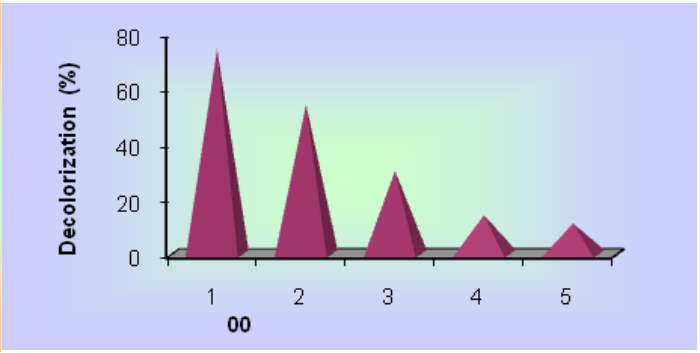


Fig. 2b: Effect of inoculum concentration

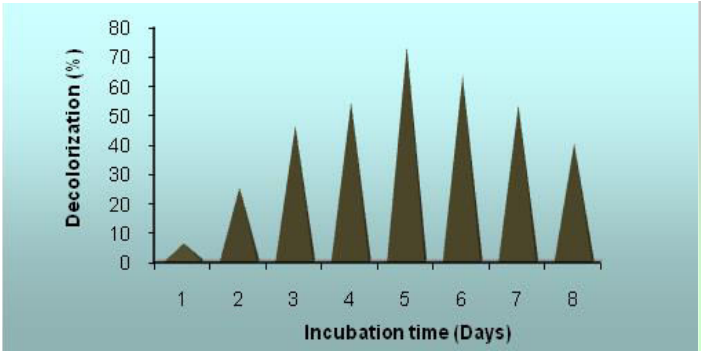


Fig. 2c: Effect of incubation time

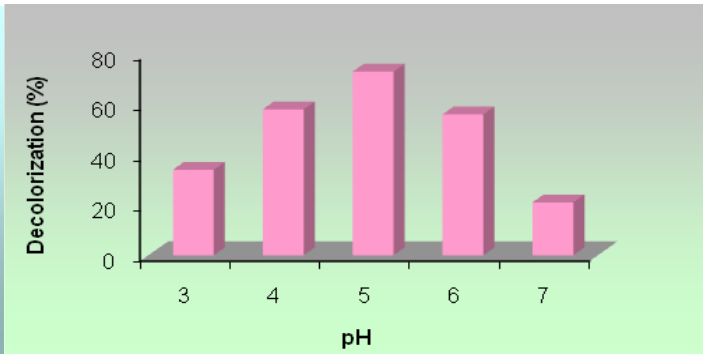


Fig. 2d: Effect of pH

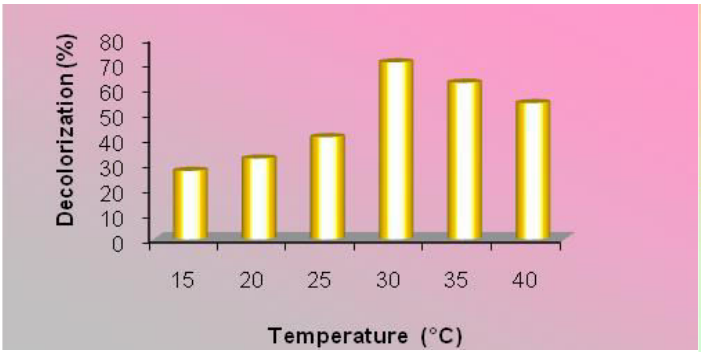


Fig. 2e: Effect of temperature

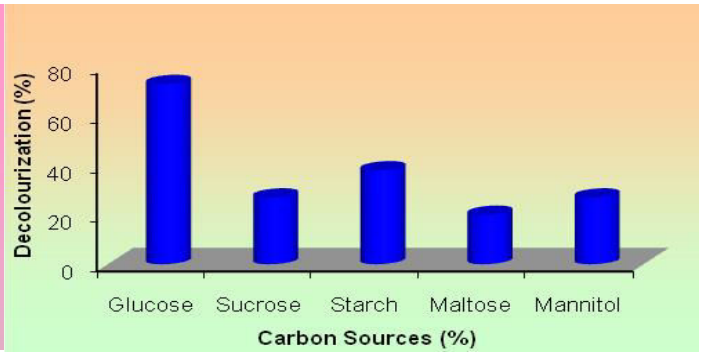


Fig. 2f: Effect of carbon sources

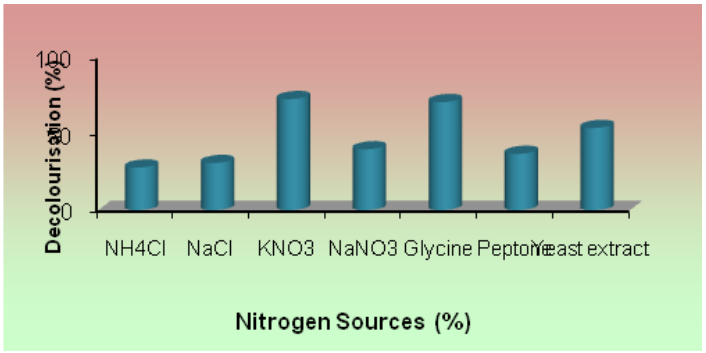


Fig. 2g: Effect of nitrogen sources

Fig. 2a-2g: Safranin removal using *Cladosporium* species at different operational parameters

A-Untreated Dye B-Dye treated with Cladosporium sp.

Fig. 3: Decolourisation of safranin dye using Cladosporium species under optimal conditions

3.3. FT- IR spectral analysis

A change in the absorption bands was observed in the FT-IR spectra of dye loaded and unloaded biomass which resulted in the shift of many functional groups based on the wavelength and percentage of transmittance (fig.4a and fig.4b). There was a sharp decrease in the band intensity at 3394 cm^{-1} (3350 cm^{-1}), 2854 cm^{-1} (2338 cm^{-1}) and 1745 cm^{-1} (1744 cm^{-1}) corresponding to -NH and -COOH stretching after dye binding. On the basis of the shift in the band, it was suggested that the co-ordination of the dye with hydroxyl or carboxyl or amine groups may increase the hydrogen bonding or form ligand between the dye and the cell wall.

The presence of the enormous cell surface in fungal biomass provides various sorbent groups for adsorption making it a potent biological adsorbent [23]. Approximately 90% of the dry matter of the cell wall contains chitin-chitosan, which has been concerned in sequestering various substances. This predominantly occurs either through ion exchange, chelation, coordination and adsorption process or by the entrapment in inter and intra-fibrillar capillaries of cell wall [24]. Besides these, glucosamines, phosphate and carboxyl groups present in the cell wall of the biosorbent also enhance the dye adsorption capacity [25, 26]. Thus the peaks obtained in the samples represent the presence of functional groups such as phenols, alcohols and carboxylic acids which eventually confirmed the biosorption of safranin onto the biosorbent.

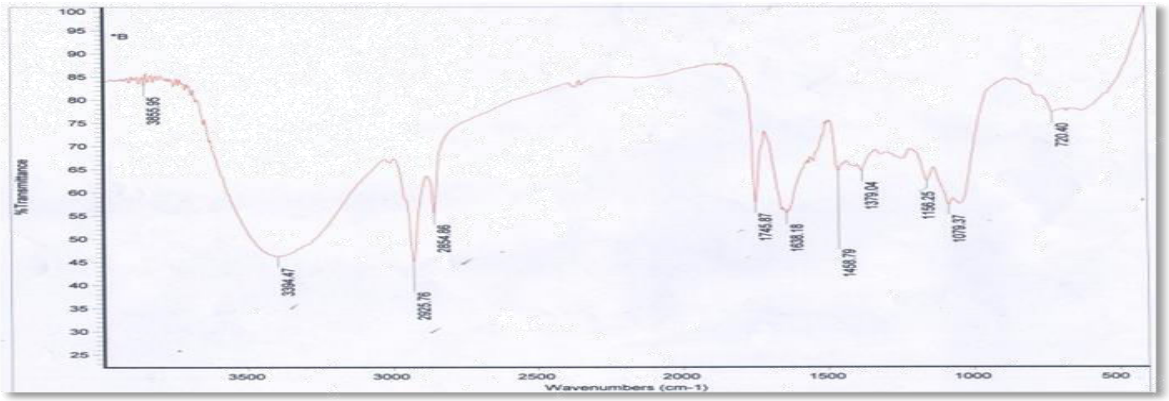


Fig. 4a: FT-IR spectrum of *Cladosporium* species before biosorption

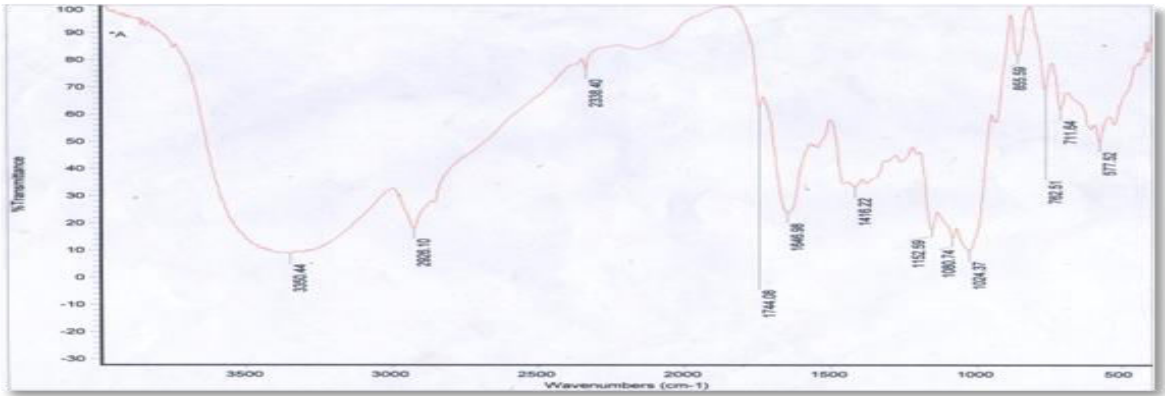


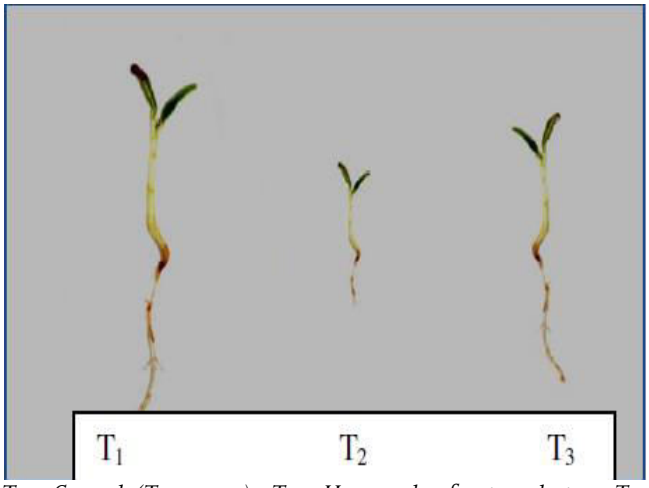
Fig. 4b: FT-IR spectrum of *Cladosporium* species after biosorption

3.4. Phytotoxicity studies

The untreated dye solution may cause serious health hazards and environmental threats when used for the agricultural purposes. Thus, it was concern to assess the phytotoxic effect of the untreated and decolourised safranin solution due to its toxic nature. The germination percentage was 78% in the seedlings grown using untreated safranin solution (T_2) whereas it was 90% in the degraded metabolites of safranin solution (T_3). The shoot and root length of fenugreek seedlings was high in control plants (T_1), followed by T_3 plants and it significantly reduced in T_2 plants. Similar trend was noticed in the fresh and dry weight of fenugreek plants (table 1 and fig.5). The vigour index was maximum in T_1 plants (741) followed by T_3 plants (646) and minimum in T_2 plants (343).

In T_2 plants, seed vigour, shoot length, root length, fresh weight, dry weight and germination percentage decreased due to the interaction of the pollutants in the plant growth [27, 28]. Bankole *et al.* [29] also observed an effective germination in *Triticum aestivum* and *Phaseolus mungo* exposed to scarlet RR treated with *P. prosopidis*.

Thus the results prove that the degraded metabolites of safranin solution indicate its non-toxic nature and hence can be used for agricultural practices.



T_1 - Control (Tap water), T_2 - Untreated safranin solution, T_3 - Degraded metabolites of safranin solution

Fig. 5: Root length and shoot length of 7 days old fenugreek seedlings

Table 1: Phytotoxicity study of safranin and the metabolites obtained after its degradation

Treatments	Germination Percentage	Shoot length (cm)	Root Length (cm)	Fresh weight (g)	Dry weight (g)	Vigour Index
T_1	92	4.50	3.55	0.45	0.21	741
T_2	78	2.47	1.93	0.21	0.11	343
T_3	90	3.73	3.45	0.36	0.19	646
CD (5%)		4.50	3.55	0.45	0.21	

The values are mean of triplicates.

4. CONCLUSION

Thus the approach of using microorganisms proves to be a cost effective and environment friendly technique which scores greater attraction over physicochemical process that exerts lots of sludge. Hence, the results of the present study will form the basis for the development of cost effective and robust indigenous technology for biosorption of dye effluent.

5. ACKNOWLEDGEMENT

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Conflict of interest

The authors declare that there is no conflict of interest of any sort on this research.

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