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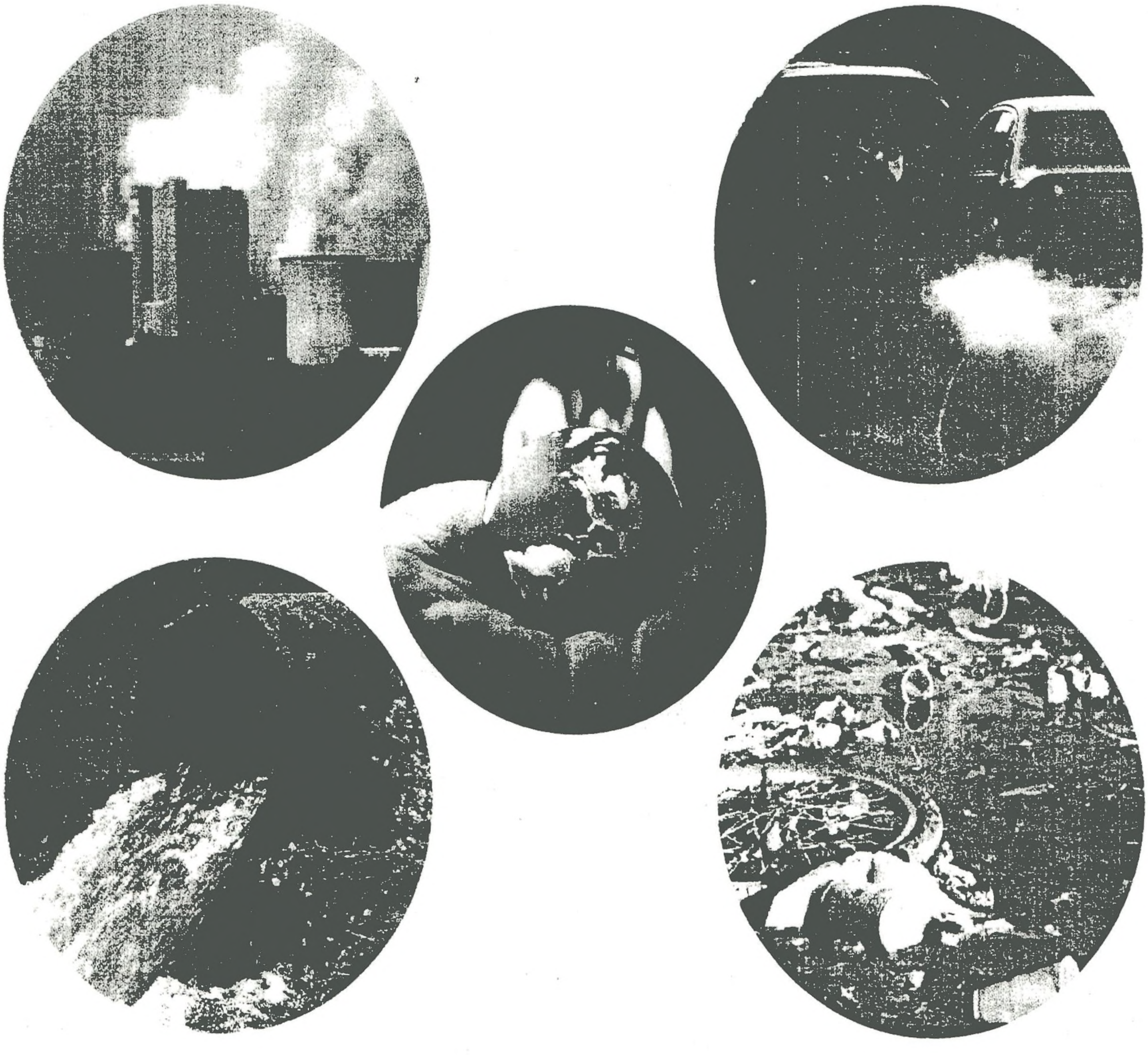

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Decolourisation of Reactive Dye by *Aspergillus niger* and its Impact on the Growth of *Zinnia elegans*

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The present investigation was carried out to assess the decolourisation of reactive dye using *Aspergillus niger* and its impact on the growth of *Zinnia elegans*. The textile effluent spilled soil sample was assessed for the presence of fungus and screened for the decolourisation of reactive yellow dye. The fungal species, *Aspergillus niger* exhibited maximum decolourisation in the medium amended with 0.01 g of dye at pH 6.0 at 30°C. Inoculum concentration of 1% amended in the medium for 5 days exhibited maximum percentage of decolourisation. Among the different carbon and nitrogen sources the maximum decolourisation activity was observed in the medium supplemented with 1% glucose and 0.3% ammonium chloride. *Zinnia elegans* grown with microbially treated reactive yellow dye (T_1) exhibited maximum biometric parameters, such as germination percentage, shoot length, root length, dry weight, fresh weight and vigour index when compared with control plants (T_3). The plants grown with untreated reactive yellow dye (T_2) recorded minimum growth when compared with T_1 and control on 7th day after sowing.

KEYWORD

Decolourisation, Reactive dye, Optimization, Biometric parameter, *Zinnia elegans*.

INTRODUCTION

The Indian textile industry has an overwhelming presence in the economic life of the country. Apart from providing one of the basic necessities of life, the textile industry also plays a vital role through its contribution to industrial output, employment generation and export earnings to the country. Synthetic dyes have been used intensively in the textile, power loom and dyeing industries because of their ease and cost effectiveness in synthesis. The textile dyeing industries utilizes large volumes of water in its wet processing operations and thereby, generates substantial quantities of wastewater which is the principle route by which dyes stuffs enters the environment (Kousar and Charya, 2002). The discharge of highly coloured synthetic dye effluent from these industries may have toxic, carcinogenic and genotoxic effects (Ozfer *et al.,* 2003). The dyes may enter the food chain and gets biomagnified through bioaccumulation, thereby increasing the magnitude of problem. In view of their high toxicity, environment mobility, non-biodegradability and stability, their removal becomes an absolute necessity (Vasudevan *et al.,* 2001). Even though some physical and chemical methods, such as adsorption, membrane filtration, photo catalytic degradations, ion exchange, precipitation, flocculation, floatation and ozonation are quite effective in decolourisation of dyes, all have some disadvantages, such as high cost per unit volume of wastewater treatment, unfriendly for nature or unreliability in operation (Aksu, 2005). Also the existing physico-chemical techniques for colour removal are very expensive and commercially unattractive (Emrah *et al.,* 2007). Biological treatment methods are attractive due to their cost effectiveness, diverse metabolic pathways and versatility of micro-organisms (Singh *et al.,* 2004; Mandezpaz *et al.,* 2005; Pandey *et* *al.,* 2007).

MATERIAL AND METHOD

Collection of the soil sample

The soil sample was collected from the textile dye effluent discharged area, Tirupur at a depth of about 50 cm from the surface and dried at ambient temperature. The lumps in the soil sample was crushed by using a porcelain mortar and pestle and stored in cloth bags for subsequent analysis.

Isolation of reactive yellow dye decolourising fungi

Ten gram of effluent contaminated soil sample was weighed and dispersed in 100 mL of sterile distilled water and stirred well. The sample was serially diluted upto 10^{-8} dilutions and 1 mL of the sample was drawn from each dilution and was pour plated on sterile rose bengal chloramphenicol agar medium and incubated at room temperature (28°C) for 5 days. After 5 day of incubation, the well grown fungal colonies were isolated and screened for their dye decolourizing effect and maintained on rose bengal chloramphenicol agar slants at 4°C for further study.

Preparation of inoculum

A loopful of the isolated fungal colonies was inoculated into 100 mL of Sabauroud dextrose broth separately. The broth was incubated for 5 days at room temperature and the fungal mats grown on the medium were collected and it served as inoculum.

Screening and identification of the isolated fungal colonies for their decolourisation effect

To screen the fungus which exhibits maximum decolourisation, 100 mL of Sabauroud dextrose broth amended with 0.1 g of reactive yellow dye was taken in 250 mL Erlenmeyer flasks and inoculated with 1 g of each inoculum separately. The flasks were incubated for 5 days and after incubation period the percent decolourisation was determined by measuring the absorbance at 520 nm in UV vis spectrophotometer.

$$\text{Percent decolourisation} = \frac{\text{Initial absorbance} - \text{Obsereved absorbance}}{\text{Initial absorbance}} \times 100$$

Based on the percent decolourisation, the fungal strain which showed maximum decolourisation percentage was selected for the present study. The fungal colonies appeared on the plates were subjected to identification by lactophenol cotton blue staining method (Cappuccino and Sherman, 1999).

Maintenance of the fungal isolate

The identified fungal isolate was subcultured on rose bengal chloramphenicol agar slants and incubated at room temperature. After sufficient growth was obtained, the slants were stored in refrigerator which served as stock culture and subculture was routinely made for every month.

Optimization of different parameters for reactive yellow dye decolourisation using *Aspergillus niger*

There are environmental and biochemical factors that influence the growth of microorganisms. For the uptake of reactive yellow dye, different operational parameters, such as dye and inoculum concentration, incubation period, pH, temperature, various carbon and nitrogen sources were optimized. Into a series of 250 mL conical flasks containing sterile Sabauroud dextrose broth, different concentrations of reactive yellow dye (0.01 g, 0.02 g, 0.03 g, 0.04 g and 0.05 g) were added. The flasks were inoculated with varying inoculum concentrations (1%, 2%, 3%, 4% and 5%) for different incubation periods (1, 3, 5, 7, 9, 11, 13 and 15 day). The pH was set at different ranges from 3 to 8 (3, 4, 5, 6,7 and 8) by adjusting with 1N HCl or 1N NaOH and at each pH, the isolated fungal species was incubated at different temperatures (15°C, 20°C, 25°C, 30°C, 35°C and 40°C). Various carbon sources (glucose, fructose, sucrose, maltose, mannitol and starch) and nitrogen sources (ammonium chloride, ammonium nitrate, diammonium phosphate, sodium nitrate, potassium nitrate, yeast extract, glycine and peptone) were added at 1 % level separately to enhance decolourisation. At the end of the each incubation period the samples were removed, centrifuged at 10,000 rpm for 10 min and the supernatants were analysed for the percentage decolourisation of reactive yellow dye. The isolated fungal species was subjected to dye decolourisation under optimised conditions.

Impact of reactive yellow dye on the growth of *Zinnia elegans*

Red soil and sand (3:1) for the pot culture experiment was collected from Avinashilingam Deemed University, Coimbatore district. The soil sample was sieved and mixed with sand in equal proportion. *Zinnia elegans* commonly known as daisy was selected for the present study. Seeds were collected from Tamil Nadu Agricultural University (TNAU), Coimbatore. Nine pots were set for the present investigation. Seven healthy seeds were sown in each pot and were kept under laboratory conditions. Thinning was done on the 7th day after germination leaving only 5 plants per pot. They were watered regularly twice a day. The plants were grown with microbially treated reactive yellow dye (T_1), untreated reactive yellow dye (T_2) and tap water (T_3) which served as control. Each treatment was replicated thrice. The plants were uprooted on 7th day and the biometric parameters, such as germination percentage, vigour index, shoot length, root length, fresh weight and dry weight were analysed. After 7 day of sowing, germination percentage of the seedlings was calculated using the formula :

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seed sown}} \times 100$$

(The protrusion of radical through seed coat was taken as the criterion of germination).

The vigour index of each seedling was calculated using the formula :

Vigour index = Germination percentage x (root length + shoot length) (Abdul - Baki and Anderson, 1973).

The data obtained were statistically analysed by one way analysis of variance ($P<0.05$) using statistical software Sigmastat 3.1.

RESULT AND DISCUSSION

Screening and isolation of reactive yellow dye decolourizing fungi

About 7 morphologically distinct fungi were isolated from the textile effluent contaminated soil by serial dilution technique. A loopful of fungal cultures was inoculated into 100 mL of the reactive yellow dye with the concentration of 0.01 g each. The contents were incubated at room temperature for 5 days and the percentage decolourisation was observed. The fungal isolate 1, 2, 3, 4, 5, 6 and 7 removed 25%, 30%, 35%, 67%, 39%, 23% and 15% of colour, respectively. Among them, the fungal isolate 4 showed highest percentage of decolourisation and subjected for identification.

Identification of the selected fungal isolate

Based on the morphology the organism was found to be *Aspergillus niger.* The surface of the colonies on rose bengal chloramphenicol agar was found to be black in colour. Immature colonies were found to be covered with white fluffy aerial mycelium and the mature colonies were black which had salt peppery effect. The reverse side of the plate was buff coloured. The vesicles were small and globose shaped. Sterigmata were found to be arranged in 2 series. The conidiophores were short and had thick smooth walls.

Optimization of different parameters for reactive yellow dye decolourisation using *Aspergillus niger*

The results of the decolourisation assay at different operational parameters were presented in table 1.

Effect of dye concentration

The reactive yellow dye at the concentra-

Table 1. *Percentage removal reactive yellow dye using* Aspergillus niger *under optimal condition*

pH	% removal of dye	Temperature, ^{0}C	% removal of dye	Incubation period, day	% removal of dye	Dye concentration, g
3	30.29	15	29.41	1	22.34	0.01
4	35.84	20	35.29	3	46.66	0.02
5	38.43	25	46.73	5	52.27	0.03
6	44.12	30	78.57	7	42.33	0.04
7	36.92	35	68.75	9	36.18	0.05
8	33.41	40	52.33	11	21.36	
				13	19.91	
				15	10.32	

Table 1. *Continued.*

% removal of dye	Inoculum concentration, g	% removal of dye	Carbon source	% removal of dye	N_2 source	% removal of dye
55	1	42.85	Glucose	47.82	NH_4Cl	82.35
48.27	2	36.92	Sucrose	33.33	NaCl	33.33
43.90	3	32.14	Starch	40	KNO_3	25.88
27.39	4	26.28	Maltose	43.47	$NaNO_3$	77.77
14.54	5	21.08	Mannitol	27.27	Glycine	31.57
					Peptone	35
					Yeast extract	30.05

tion of 0.01 g showed maximum decolourisation (55%) by *A. niger* within 5 days of incubation and a minimum decolourisation of 14.54% was observed in the medium amended with 0.05 g of dye. As the concentration of the dye increases the colour removal efficiency by the fungal isolate decreased. The decrease in the removal of colour at higher concentration of dye might be toxic to the metabolic activities of the microbes and may affect the adsorption mechanism (Ramya *et al.,* 2007). Aksu *et al.* (1999) reported that the high decolourisation efficiency may be due to the adsorption attributed by the electrostatic attraction between the negative charges of the dye with positive charge of cell wall constituents (chitin, acidic polysaccharides, lipids or amino acids) of the microbe. The results of the present study falls in line with Pandey and Upadhyay (2009) who reported that the textile dyes showed complete decolourisation by *Phanerochaete chrysosporium* after 5 day of incubation period.

Effect of inoculum concentration

In order to find out the optimum inoculum needed for the faster and higher percentage decolourisation, *Aspergillus niger* was inoculated at different inoculum concentrations varying from 1% to 5%. The percentage decolourisation was maximum in 1% inoculum concentration (42.85%), followed by 2% (36.92%) and 3% (32.14%). The uptake of dye was minimum at 4% (26.28%) followed by 5% (21.08%) of inoculum concentration. High percent decolourisation was due to dye sorption by mycelium of fungi as well as reduction of dye intensity in the solution (Balan *et al.,* 2001). Verma and Madamwar (2002) stated that the lower decolourisation efficiency by the fungal isolates is due to higher dyestuff concentration in dye bearing industrial effluent. Sani and Baneyee (1999) reported that there was no proportionate increase in the percentage of decolourisation with increase in inoculum size when Kurthia species was inoculated into triphenyl methane dyes.

Effect of incubation period

Maximum decolourisation of 52.27% was observed on fifth day of incubation period followed by third day (46.66%). The percentage decolourisation decreased in other incubation periods was shown in table 1. Fungi play an important role in decolourization reaction by adsorbing the dye by entrapment in the cellular structure and subsequent sorption onto the binding sites present it. Mohandass *et al.* (2007) have reported that the decolourisation of reactive blue by *Aspergillus* sp. was maximum at third day of incubation. Experiments conducted by Devi and Kaushik (2005) showed that treatment of textile dye effluent with *A. niger* resulted in 98 % decolourisation within 8 day of incubation. Sukumar *et al.* (2008) also reported that decolourisation of the dye effluent by fungal culture was significantly increased with incubation period from 2nd to 7th day which was in accordance with the findings of the present study. Similar result was observed in the synthetic dye inoculated with *Irpex lactus* (Novonty *et al.*, 2010), textile dyes with *A. niger* (Prabakaran *et al.*, 2009), green dye with *Cryptococus neoformans* and Swiss pink with *Curvularia lunata* (Leon and Saravannan, 2009) in which the maximum decolourisation was observed at 5 day of incubation period.

Effect of pH

The pH is the most important parameter in the biosorptive process which greatly affects the uptake of dyes by microbes (Kapoor and Viraraghavan, 1995). *A. niger* recorded a maximum uptake of 44.12 % of dye at the optimum pH 6 and minimum uptake of dye was observed at pH 3 (30.29%). Deepak *et al.* (2005) reported that *Pleurotus florida* was found to grow at maximum pH 6.0. Chang and Miles (1989) reported that the mycelial growth of *Pleurotus* species was found to be maximum at pH 5.5 to 6.0. Alexander (1999) reported that the biodegradation rate was highest at pH near neutrality for fungus. However, Fu and Viraraghavan (2001) reported that the dye anions present in the dye solution are being adsorbed by positively charged cell surfaces due to the electrostatic attraction at lower pH.

Effect of temperature

Decolourisation at different temperatures (15°C, 20°C, 25°C, 30°C, 35°C and 40°C) showed 30°C as optimum temperature for *A. niger.* Above 30°C, there is a decrease in percentage decolourisation. This reduction may be due to thermolabile nature of *Aspergillus niger.* Further increase in temperature from 30°C may alter the surface activity of biomass which results in reduction of colour removal, indicating that the process is exothermic in nature. Khalaf (2008) has reported similar result for biosorption of reactive dye from textile dye effluent using non viable biomass of *A. niger* and *Spirogyra* species. Maximum decolourisation of congo red was observed by native and modified mycelial pellets of *Trametes versicolor* in various reactive modes at 30°C (Binupriya *et al.,* 2008).

Effect of different carbon source

The influence of different carbon sources on the decolourisation activity was presented in table 1. All the carbon sources used in the study encouraged the growth of fungus. *Aspergillus niger* exhibited maximum decolourisation activity (47.80%) when glucose was supplemented in the medium as co-substrate. Followed by glucose, sucrose (33.33%), starch (40%), maltose (43.47%) and mannitol (27.27%) recorded better decolourisation in the medium inoculated with *Aspergillus niger*.

Basically, carbon source is necessary for providing electrons for azo compound re-

Table 2. *Biometric parameter in 7 day old seedlings of* Zinnia elegans *grown with different treatment*

Treatment	Germination percentage	Shoot length, cm	Root length, cm	Fresh weight, g	Dry weight, g	Vigour index
T_1	95.23	5.3	3.6	0.54	0.27	848
T_2	80.95	2.5	1.2	0.28	0.07	300
T_3	90.47	3.3	2.3	0.43	0.14	507
SED		0.0073	0.0128	0.0037	0.0010	
CD (5%)		0.0204	0.0356	0.0103	0.0027	

T_1 - Microbially treated reactive yellow dye, T_2 - Untreated reactive yellow dye, T_3 - Control (tap water)

duction and glucose served as a carbon source and act as electron donor. This is in conformity with the findings of Carliell *et al.* (1995) who reported that the metabolism of glucose results in the production of reduced nucleotides (NADH, FADH) which leads to enhanced decolourisation efficiency. These reduced nucleotides are reported to be redox mediators involved in reduction of azo bonds. Nigam *et al.* (1996) reported that glucose is required as a co-substrate for decolourisation of dyes which supports the findings of the present study. Thus the results obtained indicated that the dye decolourisation required a minimal amount of glucose. If glucose level gets depleted cessation of decolourisation in the medium may occur.

Effect of various nitrogen source

The effect of different sources on the decolourisation activity and biomass production of the isolated fungus was represented in table 1. Among the different nitrogen sources, ammonium chloride (NH_4Cl) recorded the highest decolourisation (82.35%) activity in the medium inoculated with *Aspergillus niger.* Decolourisation efficiency was not as effective as that obtained with NH_4Cl when NaCl, KNO_3 and $NaNO_3$ was used as nitrogen source for decolourisation (Table 1).

Decolourisation performance with peptone, glycine and yeast extract was not very effective and the colour removal efficiency was around 30%. The biomass production of *A. niger* in the medium amended with nitrogen sources was depicted in table 1. The results of the present study coincide with the findings of Assadi *et al.* (2003) who reported that ammonium chloride showed better results when compared with other nitrogen sources in the process of textile effluent decolourisation using microbial cultures.

Decolourisation of reactive yellow dye using *Aspergillus niger* under optimal condition

Aspergillus niger under optimized conditions degraded reactive yellow dye to 87%. For effective decolourisation glucose and ammonium chloride concentration (1 g and 0.03 g/100 mL), dye concentration (0.01 g/100 mL), inoculum concentration (1%), incubation period (5 day), pH (6) and temperature (30°C) should be in optimal level. Before developing a bioremediation technology for treating the dyes, it is necessary to isolate and identify the suitable microorganism having good degrading ability. Therefore, the present study was conducted to assess the degradation ability of synthetic wastewater using indigenous *Aspergillus niger,* which could be employed for decolourisation of the effluent containing dyes. Under the optimal environmental conditions maximum degradation was obtained which might be due to the presence of enzymes, like laccase, cellulose and peroxidase secreted by *As-*

pergillus niger (Zope *et al.,* 2007). The degradation studies in synthetic wastewater medium would be useful to textile industry effluents and for agricultural purposes. This process does not require sophisticated machinery, instrumentation, addition of chemicals and aeration and separation of mycelial mats is also easy through simple filtration. Thus the approach of using microorganisms is cost effective, eco-friendly and scores greater attraction over physico-chemical process which exerts lots of sludge.

Impact of reactive yellow dye on the growth of *Zinnia elegans*

The result of germination percentage, root length, shoot length, fresh weight, dry weight and vigour index of *Zinnia elegans* seedlings showed maximum growth in plants grown with microbially treated reactive yellow dye (T_1) followed by tap water (T_3). Plants grown with untreated reactive yellow dye (T_2) showed a minimum growth on 7th of the experiment (Table 2). T_1 showed a significant increase in the biometric parameters ($P<0.05$) when compared to T_2 and T_3 plants.

Seed germination is a single step process in the life cycle of a plant, which is very complex and influenced by many environmental factors (Ramagopal, 1988). Among the growth processes, seed germination and early seedling growth have been considered critical for raising successful agricultural crop. The processes of germination and growth of young seedlings are susceptible to toxic materials present in the effluent (Balashouri and Prameeladevi, 1994). The germination percentage of the present study decreased in the seeds grown using untreated reactive yellow dye (T_2) which could be attributed principally to physical constraints as well as biological harm caused to seeds by the physical and chemical constituents of effluents as opined by Debojit and Rao (1994).

The increase of germination percentage in T_1 plants of the present study was in consonance with the findings of Jothimani and Elayarajan (2003) who reported the same in dye effluent treated with biological systems. From the above results it was observed that the reduction in shoot and root length of seedlings grown using T_2 might be due to the presence of higher dye concentration. The reduction in shoot and root length of T_2 plants might be due to the increased concentration of dye which could have restricted the rooting and shooting (Evers *et al.,* 1997). Microbially treated effluent has been reported to favour root and shoot length of black gram and green gram (Madhappan, 1993).

The reduction in the fresh and dry weight of the *Zinnia elegans* plants selected for the present study may be due to the toxic effect of dye which might have inhibited water uptake resulting in the retardation of plant growth (Ganesh *et al.*, 2006). Increase in fresh and dry weight of plants grown in microbially treated effluent is in consonance with the results of Ramakrishnan *et al.* (2001) in paddy grown in sugar mill effluent treated with yeast.

CONCLUSION

It is evident from the study that decolourisation of reactive yellow dye could be done using *Aspergillus niger* and the treated dye could be utilized for agriculture. It possessed high biosorption efficiency, reusability and stability. In addition, *Aspergillus niger* is generally regarded as safe and its biomass production is simple and cheap. The results of this study will form the basis for the development of cost effective and robust indigenous technology for biosorption of reactive dye effluent.

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