Trends in Biotechnology Research
An International Peer-Reviewed Journal
Online and Print Version
www.sciencejournal.in

PHYTOTOXIC EFFECT OF SYNTHETIC TEXTILE DYE EFFLUENT ON GROWTH OF FIVE PLANT SPECIES

Jadhav Umesh U[1]., Dhawale Rhushikesh N[2]., Dawkar Vishal V[3]., Chougale Ashok D[4]. and Padul Manohar V.[5] *

[1]Department of Power Mechanical Engineering, National Tsing Hua University, No. 101, Sec.2, Kuang Fu Rd., 30013, Hsinchu, Taiwan ROC.
[2]P.G. Department Of Biochemistry, N.A.C. and S. College, Ahmednagar-414 001, Affiliated to University of Pune, India.
[3]Plant Molecular Biology Unit, Division of Biochemical Sciences, National Chemical Laboratory, Pune, India.
[4]Mass Spectrometry And Proteomics Group Organic Chemistry Division, National Chemical Laboratory, Pune, India
[5]Department of Biochemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004, India.
*(Corresponding Author E-Mail: manoharpadul@yahoo.co.in)

ABSTRACT
The textile effluent constitutes synthetic dyes which are liberated in the environment without proper treatment. Anything added to the environment will certainly affect the flora and fauna. In the present study, the toxicity of synthetic textile dye effluent was investigated using a bioassay method with five plant species namely Triticum aestivum, Vigna radiata, Vigna aconitifalia, Vigna sinensis, and Cicer arietinum. The effect of various concentrations of synthetic effluent on germination index (GI) varied with plant species. The increasing concentration of effluent exerted toxicity effects on development of root and shoot. Also α-amylase activity was determined during seed germination and at inhibition of seed germination. We found that there was inhibition of α-amylase activity along with germination inhibition.

KEY WORDS: α-amylase inhibition, Phytotoxicity, seeds, Synthetic textile dye effluent,

Introduction

Among the industrial chemical wastes, the textile industry effluents raise great concern because of their diverse environmental hazards. Additionally, their aromatic amines, dye by-products are mutagenic and carcinogenic(Levine 1991). As industrial effluents are a mixture of toxic components, the contribution of each component to toxicity varies with its dilution and dispersion in water and is also affected by the diversity of the discharge environments(Reemtsma 2001).

The ecotoxic effect of the textile dyes on environment and its possible hazard to human health has been frequently reported(Rao and Farnandes 1996; Zuskin et al 1997; Seidenari 1997). Living organisms are almost always exposed to possibly genotoxic environmental agents both at cellular and molecular levels. Genotoxic potential studies are important to predict the impact of certain agents on animals, vegetables, and consequently on human beings(Kim et al 2006).

Seed germination (% seed germination) and root shoot ratio tests have been used as simple, rapid, reliable and reproducible techniques to evaluate the damage caused by toxic compounds present in various textile wastes(Wang and Keturi 1990). Inhibition of seed germination and effects on root elongations are the main areas of interest in the studies of phytotoxicity(Vermeulen et al 1993). Chang et. al. used the seed germination / root elongation and plant genotoxicity bioassays to evaluate the remediation of some of toxic elements from contaminated soil(Chang et al 1997). The same test was employed by many other researchers(Kong et al 2007; Roony et al 2007) to assess the phytotoxicity of different chemicals. The aim of present study was to evaluate the toxicity of synthetic textile effluent on germination and root/shoot elongation of five plant seeds.

MATERIALS AND METHODS

Preparation of synthetic effluent

Synthetic textile dye effluent was prepared by using five commercial dyes. The dyes used were Blue 2RNL, Blue GL (Solo), Golden yellow, Direct Red 5B and Scarlet RR. Various concentrations of synthetic effluent were prepared by varying the concentration of respective dyes.

Plant Material

The seeds of *T. aestivum, V.radiata, V. aconitifolia, V. sinensis* and *C. arietinum* were used in this study.

Phytotoxicity study

In this experiment, the effect of different concentrations of synthetic textile dye effluent on germination of *C. arietinum, V. sinensis, V. aconitifolia, V. radiata* and *T. aestivum* was evaluated. The seeds were germinated in sterile 10 cm petri dishes, layered with germination paper. Seeds were sterilizedSomasegaran and Hben 1985) before transferring to the surface of the paper in the petri dishes (10 seeds per plate). The phytotoxicity bioassay was evaluated using the seed germination technique(Zucconi et al 1981a; Zucconi et al 1981b). This method involves incubating the synthetic dye effluent at various concentrations with seeds at 25 ^{0}C for 5 days in the dark, and then measuring the number of seeds germinated and root growth thereafter (Equations 1 and 2, respectively).

$$\text{Relative seed germination (\%)} = \frac{\text{Number of seeds germinated in synthetic dye effluent}}{\text{Number of seeds germinated in control}} \times 100 \quad \text{.....(1)}$$

$$\text{Relative root growth (\%)} = \frac{\text{Mean root length in synthetic dye effluent}}{\text{Mean root length in control}} \times 100 \quad \text{.....(2)}$$

After 5 days of incubation in the dark, the seed germination and root length of the five plants in the effluent were determined. The seed germination percentage and root elongation of the plants in distilled water were also measured and used as the control. Seeds were considered germinated when the radical and hypocotyl together appeared. The percent seed germination and root shoot ratio was recorded at regular interval of 24 h for five days continuously. The relative seed germination, relative root elongation and germination index (GI, the product of relative seed germination and relative root elongation, Equation 3) were calculated as follows(Tiquia 2010):

$$\text{Germination index (GI)} = \frac{\text{(\% Relative seed germination) X (\% Relative root growth)}}{100} \quad \text{.....(3)}$$

Determination of total sugar and total starch content

The seeds were soaked for 4 hours in effluent. Embryo was separated from seed and a part of seed without embryo was crushed to make fine paste and 0.5 gm paste was extracted in 10% ethanol. It was centrifuged at 2000 rpm for 10 min. The supernatant was collected and used for estimation of total sugar. Total sugar content was determined by Phenol-H_2SO_4 method(Dubios et al 1951).

The residue collected from above method was subjected for the determination of total starch content. The residue was dissolved in 5 mL distilled water. Then 6.5 mL of 52% perchloric acid was added. This solution is centrifuged at 2000 rpm for 10 min. The process is repeated thrice and then total volume is made up to 100 mL with distilled water. From this solution 1 mL aliquot was taken. To this 1 mL, 5% phenol and 5 mL of concentrated H_2SO_4 was added. The tubes were incubated in boiling water bath for 30 min. Then optical density was measured at 470 nm(McCready et al 1950), the values were plotted on the standard graph prepared by using known standard concentrations.

Assay for α-amylase enzyme activity

Amylase activity was determined by detecting the amount of reducing sugars liberated. The reaction mixture (1 mL) contained 0.25 mL of 1% soluble starch, 0.25 mL of 0.4 M Tris-HCl buffer (pH 8.0) and 0.5 mL of enzyme. The reaction was terminated by addition of 2 mL of 3, 5-dinitrosalicylic acid reagent after incubation at 35 ^{0}C for 30 min(Sengupta et al 2000) The protein concentration was measured with bovine serum albumin (BSA) as a standard(Lowry et al 1951).

Determination of inhibition of α-amylase activity

The amylase activity was determined in control seeds as well as in seeds treated with synthetic dye effluent during seed germination. Activity of amylase in control seed was considered 100% and residual activity in treated samples was

measured and subtracted from control activity to get percent inhibition. The percent inhibition of amylase activity was calculated by using following formula (Equation 4)

$$\text{Percent inhibition (\%)} = \frac{\text{Enzyme activity in control seeds} - \text{Enzyme activity in treated seeds}}{\text{Enzyme activity in control seeds}} \text{ X 100} \quad ..(4)$$

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparisons. Readings were considered significant when **P was <0.01, and ***P<0.001. Graphpad software was used for this analysis.

RESULTS AND DISCUSSION

Toxicity bioassay using seed germination test

Table 1 Germination index of various plant seeds treated with increasing concentration of synthetic effluent

Concentration of synthetic effluent (ppm)	Germination index				
	T. aestivum	*V. radiata*	*V. aconitifolia*	*V. sinensis*	*C. arietinum*
Nil	100	100	100	100	100
100	88.72 ± 1.41**	88.04 ± 0.84***	85.24 ± 0.43***	97.09 ± 0.58**	63.11 ± 0.91**
1000	76.65 ± 0.41***	80.72 ± 0.82***	75.50 ± 1.28**	67.75 ± 0.54**	40.91 ± 0.24***
3000	45.20 ± 0.29	68.16 ± 0.22**	68.05 ± 0.29***	58.06 ± 0.38**	29.16 ± 0.17***
5000	23.08 ± 0.22***	65.74 ± 0.36**	57.04 ± 0.71**	52.01 ± 0.41**	14.17 ± 0.20***
7000	6.63 ± 0.54***	44.58 ± 1.38**	41.81 ± 0.42**	36.86 ± 0.32**	2.64 ± 0.37***
9000	5.26 ± 0.44***	34.21 ± 0.86***	32.14 ± 0.31**	15.09 ± 0.65***	0
15000	2.80 ± 0.39**	19.77 ± 0.59**	6.54 ± 0.67***	0	0
20000	1.26 ± 0.27***	9.30 ± 0.28**	0	0	0
30000	0	0	0	0	0

The effect of xenobiotics on environment is usually performed by chemical analysis. This is not enough either to evaluate the environmental risk or to evaluate the efficiency of remediation processes. Information on the bioavailability of complex mixtures of xenobiotics and degradation products cannot be totally provided by chemical analytical data, but results from bioassays can integrate the effects of pollutants in complex mixtures(Vega-Loyo et al 2005). In the present study, the effect of different concentrations of synthetic textile effluent on germination of five plants namely *T. aestivum*, *V. radiata*, *V. aconitifolia*, *V. sinensis* and *C. arietinum* was evaluated. In all the plants tested, the GI tended to decrease with increasing concentration of synthetic dye effluent. The germination of *C. arietinum* seeds was completely inhibited at 9000 ppm concentration (Table 1). The germination of *V. sinensis* was completely inhibited at 15000 ppm concentration. The germination of *V. aconitifolia* seeds was completely inhibited at 20000 ppm concentration, while the germination of seeds of *T. aestivum* and *V. radiata* was inhibited at 30000 ppm (Table 1). Similar study was conducted by Ren et al. who demonstrated the toxicity of polycyclic aromatic hydrocarbons (PAHs), anthracene (ANT), benzo[a]pyrene (BAP), and fluoranthene to the duckweed *Lemna gibba* L. and *Brassica napus* L. seeds (Ren et al 1996). In some cases, especially with the anaerobic sludges, a depressive effect on seed germination has been seen, which has been attributed to the release of ammonia(Fuentes 2006).

Toxicity effect on root & shoot length

A competitive chemical effect is observed not only on seed germination, but also on all other aspects of growth parameter. After germination of the seeds the root and shoot development in the seeds was studied. The effect of synthetic effluent application on plant shooting varied with the plants used in the experiment. The root and shoot lengths for control as well as treated *V. radiata, V. aconitifalia, T. aestivum, V. sinensis and C. arietinum*, seeds are shown in fig. 1. The increased concentration of effluent exerted toxicity effects on shooting.

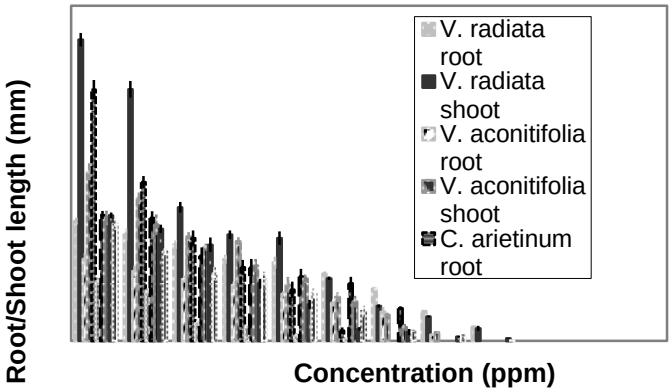

Figure 1. Root shoot length of various plants treated with increasing concentration of synthetic effluent.

Also the significant toxicity effect on radical length values was recorded with the higher concentrations of the effluent. Similar results were reported using several textile dyes(Mowad et al 2003).

For every plant seed used, the root and shoot development was inhibited at various concentrations of synthetic effluent. The inhibitory concentrations were as follows, *T. aestivum* and *V. radiata* (30000 ppm), *V. aconitifolia* (20000 ppm), *V. sinensis* (15000 ppm), and *C. arietinum* (9000 ppm). Phytotoxic effect of dyes is reported (Kosinkiewicz et al 1984). The reduction in root, shoot length with increasing concentration of phenolic compounds was also reported(Colpas et al 2003). The results of these studies suggest that, while the exposure of seeds to low concentration of dye during germination was less toxic to seed germination as compared with the higher concentrations, the low concentration of the dye could adversely affect the shooting percent significantly.

Table 2. Total sugar and total starch content of the various plant seeds

No	Seeds	Total sugar µg/g	Total starch µg/g
1	*V. sinensis*	2393 ± 9.89**	1840.5 ± 6.36**
2	*C. arietinum*	2377.5 ± 3.53***	1610 ± 1.41***
3	*V. aconitifolia*	1765 ± 7.07***	1351.5 ± 2.13***
4	*V. radiate*	1695 ± 7.08***	1528.5 ± 2.12
5	*T. aestivum*	1435.5 ± 6.36**	1280.5 ± 0.70***

Values are of three experiment ± SEM. The values are significantly different from control at **P<0.01, and ***P<0.001. These values were calculated by one-way ANOVA test using graphpad software.

Determination of total sugar, total starch and α-amylase activity

Study was carried out to determine total sugar and starch content of the seeds tested for phytotoxicity. The total sugar and total starch present in the seeds (germinated in distilled water) were shown in Table 2. This experiment was carried out to find out the amount of starch present in the seeds as compared to the total sugar.

It is accepted that α-amylase plays an important role in the degradation of storage starch in the endosperm of germinating cereal seeds. α-amylase is an endoamylolytic enzyme that hydrolyzes the α-1, 4-glucosidic linkages of

starch (Fincher 1989). In germinating cereal seeds, α-amylase secreted from the aleurone cells initiates the degradation of starch granules in endosperm. It liberates soluble glucans that can be further degraded by other hydrolytic enzymes such as debranching enzymes(Yu et al 2005). By keeping this in mind the effect of increasing concentration of synthetic effluent on α-amylase activity was studied in all five plant seeds. The α-amylase activity was inhibited in all five plant seeds. The inhibition of activity for *T. aestivum, V. radiata, V. aconitifolia, V. sinensis* and *C. arietinum* was 80.45 (± 0.63**), 74.49 (± 0.40**), 82.75 (± 0.35***), 67.74 (± 0.33***), and 69.61 ± (0.55**) percent respectively. Since synthetic textile effluent inhibited α-amylase activity, the starch present in seeds would not be utilized which in turn might inhibited seed germination and further growth of plant.

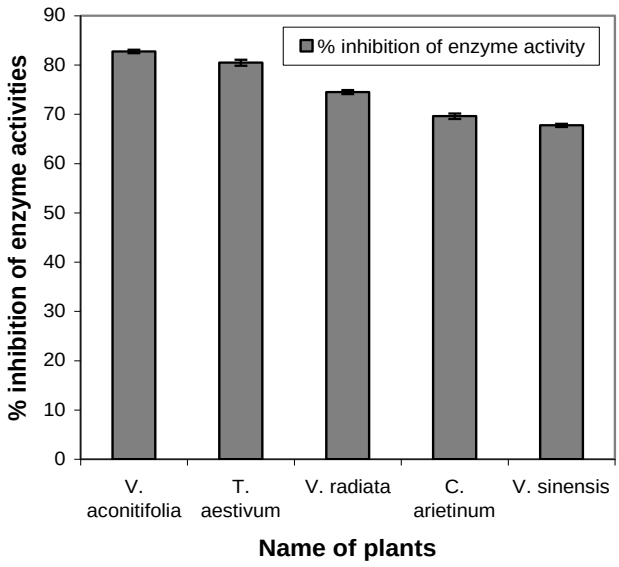

Figure 2. Inhibition of amylase activity (%) in various plant seeds.

The present results confirm that synthetic textile dye effluent exert toxic effect on all the plants tested. The results suggest that the phytotoxicity bioassay can be used as an efficient toxicity test for synthetic textile dye effluent, and also as a monitor of environmental contamination. The toxic effect of dyes reported in this study also suggests the need for remediation of textile dyes in the industry effluents before discharging it into the environment. The screening of tolerant seeds having the ability to grow in contaminated soil can help for getting knowledge about suitable crop species to such contaminated soils.

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