Int.J.Curr.Microbiol.App.Sci (2024) *13*(05): 208-212

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 13 Number 5 (2024)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2024.1305.026

Phytotoxicity and Cytotoxicity Assay to Elucidate Detoxification of Metanil Orange by Textile Effluent Acclimatized Bacterial Isolate JHP-1

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Keywords

Cytotoxicity, Detoxification, MCF-7 cell line, Metanil Orange and Phytotoxicity

Article Info

Received:
20 March 2024
Accepted:
22 April 2024
Available Online:
10 May 2024

A B S T R A C T

Toxic effluents containing azo dyes are discharged from various industries and they adversely affect water resources, soil fertility, aquatic organisms and ecosystem integrity. They pose toxicity (lethal effect, genotoxicity, mutagenicity and carcinogenicity) to plants, aquatic organisms as well as animals. Considering the potential applications of bioremediation processes in wastewater treatment, the present investigation targets on the complete detoxification of Metanil Orange by phytotoxicity and cytotoxicity studies. Phytotoxicity studies indicated that the extracted metabolites (degraded dye) contains non-toxic metabolites, resulting in good germination rate as well as significant root and shoot length of *S. vulgare* and *P. mungo* when compared to dye sample (untreated), where inhibition in all these parameters was observed. Cytotoxicity assay using MCF-7 cell line confirmed the detoxification of Metanil Orange.

Introduction

Ever since the beginning of mankind, people have been using colorants for painting and dyeing their surroundings, their skins and their clothes. The first evidence of the use of colorant materials by man goes as far as 15000-9000 BC, in the walls of the Altamira cave in Spain (Shyamala *et al.,* 2014). The pioneering synthesis of mauveine by W. H. Perkins started the era of synthetic dyes, with chemical and physical properties better suited to contemporary demands, better level of quality and more reproducible techniques of application (Vijayanand and Hemapriya, 2013). Now there are more than 1, 00,000 commercially available dyes whilst over 7 x 10^5 metric tons of dyestuffs are produced annually (Hemapriya and Vijayanand, 2013; Aswinkumar *et al.,* 2017).

Toxicants generated from textile industry effluent get into aquatic organisms, pass through the food chain and ultimately reach humans, leading to various physiological disorders like hypertension, sporadic fever, renal damage, cramps, etc., Bioaccumulation of toxicants depends on the availability and persistence of the contaminants in water, food and physico-chemical properties of the toxicants (Puvaneswari *et al.,* 2006). One of the major problems with textile effluents is that they have a toxic effect on germination rates and biomass

of several plant species; whereas plant play many important ecological functions such as providing the habitat for wildlife, protecting soil from erosion and providing bulk of organic matter that is so significant to soil fertility (Pourbabaee *et al.,* 2006; Kalyani *et al.,* 2009). Chlorophyll content of plants decreased in response to industrial effluent could be associated with higher concentrations of dissolved solids or increase in chlorophyll degradation or decrease in the endogenous cytokinins involved in stimulating chlorophyll synthesis (Puvaneswari *et al.,* 2006).

A wide range of methods has been developed for the removal of synthetic dyes from waters and wastewaters to decrease their impact on the environment (Bavani *et al.,* 2021; Aulprakash *et al.,* 2022). According to Kalyani *et al.,* (2009), Bioremediation of textile effluents has been of considerable significance since it is inexpensive, eco-friendly and produces a less amount of sludge.

The effectiveness of microbial decolorization depends on the adaptability and the activity of selected microorganisms including bacteria, actinomycetes, fungi, yeasts and algae capable of degrading azo dyes (Daneshwar *et al.,* 2007; Hemapriya *et al.,* 2010; Vijayanand and Hemapriya, 2014; Vijayanand *et al.,* 2017; Barathi *et al.,* 2020a). Considering the advantages and potential applications of bioremediation processes in wastewater treatment, the present investigation targets on the complete detoxification of Metanil Orange, by phytotoxicity and cytotoxicity studies.

Materials and Methods

Bioremediation of Metanil Orange

The effluent samples were serially diluted and incubated over basal nutrient agar medium containing 50 ppm of Metanil Orange at 37°C for 5 days. Colonies surrounded by halo (decolorized) zones were picked and streaked on nutrient agar plates containing azo dyes. Different colonies of dye decolorizing bacteria were picked and re-streaked several times to obtain pure cultures.

Decolorization extent of the isolates were determined by measuring the absorbance of the culture supernatant at 470 nm using UV-visible spectrophotometer (Hitachi U 2800), according to Hemapriya *et al.,* (2010). Following visible decolorization, the sample is subjected for toxicity analysis to ensure complete detoxification of Metanil Orange.

Phytotoxicity Studies

Phytotoxicity tests were performed in order to assess the toxicity of the untreated and treated dye samples. The ethyl acetate extracted products of degraded azo dyes were dried and dissolved in 5 ml sterile distilled water to make a final concentration of 100 ppm.

The Phytotoxicity tests were carried out on *Sorghum vulgare* Pers. (monocot) (Parshetti *et al.,* 2006). 10 healthy plant seeds were treated separately with 5 ml of control dye and degraded products respectively/per day. Control sets were carried out using distilled water at the same time. Germination percentage as well as the length of plumule and radical was recorded after 7 days (Saratale *et al.,* 2009).

Cytotoxicity in MCF-7 Cell line

Cell line and culture

MCF-7 cell line selected for the study was acquired from the National centre for cell sciences, Pune (NCCS). The cells were maintained for its viability in DMEM media supplemented with 10 % Foetal Bovine Serum, penicillin (100 µ/ml), and streptomycin (100 µg / ml) in a humidified atmosphere of 50 µg / ml CO_2 and incubated at 37 °C.

In vitro assay for Cytotoxic Activity (MTT Assay)

MCF-7 Cells (1 x 10^5/well) were plated in 24 well plates and incubated at 37 °C with 5 % CO_2 condition. At once the cell reaches the confluence; various concentrations of the samples (were added and incubated for 24 h. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. 100 µl / well (5 mg/ml) of 0.5 % 3- (4, 5- dimethyl-2-thiazolyl) -2, 5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 h.

After incubation, 1 ml of DMSO was added to all the wells. Then, 50 µl Metanil Orange dye was added to the test wells and 50 µl media was added to the positive control wells and incubated overnight. After incubation, the absorbance at 570 nm was measured with UV-Spectrometer using DMSO as the blank. Measurements were performed and the concentration required for a 50 % inhibition (IC_{50}) was determined graphically. The % cell viability was calculated using the following formula:

% Cell viability = A_{570} of treated cells / A_{570} of treated cells /A_{570} of control cells x 100

Results and Discussion

Environmental pollution has been recognized as one of the major problems of the modern world. The increasing demand for water and the dwindling supply has made the treatment and reuse of industrial effluents as an attractive option. Textile effluents are of global concern because they color the drains and ultimately the receiving water bodies (Barathi *et al.,* 2020a and Barathi *et al.,* 2020b).

Phytotoxicity Assay

Phytotoxicity tests with *Sorghum vulgare and Phaseolus mungo* seeds were performed in order to assess the toxicity of the untreated and treated Metanil Orange dye samples.

Phytotoxicity test with *Sorghum vulgare*

Sorghum vulgare seeds treated with tap water showed 100% germination, the mean plumule length of 20.5 ± 0.05 cm and the mean radical length of 6.30 ± 0.4 cm.

In contrast, the seeds treated with untreated dye sample showed only 70% germination, the mean plumule length of 8.5 ± 0.1 cm and the radical length of 4.2 ±0.2 cm. Whereas, the seeds treated with treated dye sample (degraded) showed 100% germination, the mean plumule length of 19.3 ± 0.5 cm and the radical length of 5.8±0.2 cm (Table 1).

Phytotoxicity test with *Phaseolus mungo*

Phaseolus mungo seeds treated with tap water showed 100% germination, the mean plumule length of 18.2 ± 0.2 cm and the mean radical length of 6.0 ±0.1 cm.

In contrast, seeds treated with untreated dye sample showed only 80% germination, the mean plumule length of 10.2 ± 0.4 cm, the mean radical length 4.5 ±0.2 cm, whereas, seeds treated with treated dye sample (degraded) showed 100% germination, the mean plumule length of 17.2 ±0.2 cm, the radical length of 5.0 ±0.3 cm (Table 2).

The result indicated that the extracted metabolites (degraded dye) contains non-toxic metabolites, resulting in good germination rate as well as significant root and shoot length of *S. vulgare* and *P. mungo* when compared to dye sample (untreated), where inhibition in all these parameters was observed.

This result was in complete concurrence with the earlier findings (Parshetti *et al.,* 2006; Saratale *et al.,* 2009; Shyamala *et al.,* 2014).

Table.1 Phytotoxicity Study of Metanil Orange and its Degradation Products on Sorghum vulgare Pers.

Sl. No	Parameters Studied	Tap Water	Metanil Orange (100ppm)	Treated sample (100ppm)
1	Germination (%)	100	90	100
2	Plumule (cm)	20.5 ± 0.2	8.5 ± 0.1	19.3± 0.5
3	Radical (cm)	6.3 ± 0.3	4.2 ± 0.2	5.8 ± 0.2

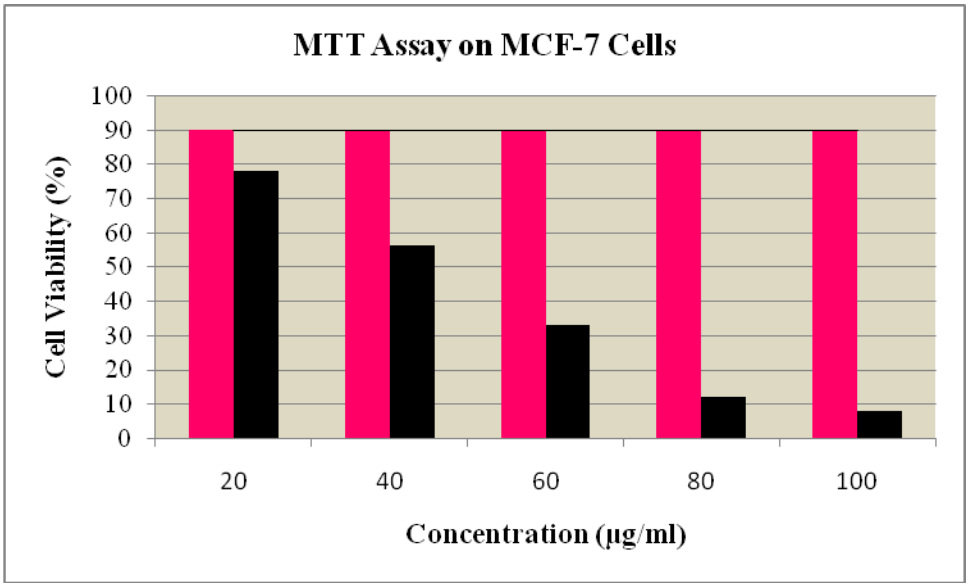
Table.2 Phytotoxicity Study of Metanil Orange and its Degradation Products on *Phaseolus mungo* L.

Sl. No	Parameters Studied	Tap Water	Metanil Orange (100ppm)	Treated sample (100ppm)
1	Germination (%)	100	60	100
2	Plumule (cm)	18.2 ± 0.2	10. ± 0.4	17.2 ± 0.2
3	Radical (cm)	6.0 ± 0.1	4.5 ± 0.2	5.0 ± 0.3

Table.3 MTT Assay on MCF-7 Cells using Test and control sample

S. No.	Dye Concentration (µg/µl)	Cell Viability (%)	
		Treated Dye	Untreated Dye
1.	20	90	78
2.	40	90	56
3.	60	90	33
4.	80	90	12
5.	100	90	8

Figure.1 Cytotoxicity Assay in MCF-7 cell line



Cytotoxicity in a cell line (MCF-7 cell line)

MCF-7 cells treated with control dye sample displayed misrepresentation of cells and decrease in cell viability equated to those treated with treated Metanil Orange solution (Experimental). MCF-7 cells exposed to treated dye solution exhibited no substantial damage to the cells.

MTT assay on MCF-7 cell lines revealed that in the control group, the cell viability diminished with an upsurge in concentration whereas, in the test sample, the cell viability was steady even in increasing concentrations of treated dye solution (Table 3).

Author Contributions

Hena. S. Das: Investigation, formal analysis, writing—original draft. Reshma Girirajan: Validation, methodology, writing—reviewing. Rajalakshmi Balaji:—Formal analysis, writing—review and editing. S. Vijayanand: Investigation, writing—reviewing. J. Hemapriya: Resources, investigation writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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How to cite this article:

Hena. S. Das, Reshma Girirajan, Rajalakshmi Balaji, S. Vijayanand and Hemapriya, J. 2024. Phytotoxicity and Cytotoxicity Assay to Elucidate Detoxification of Metanil Orange by Textile Effluent Acclimatized Bacterial Isolate JHP-1. *Int.J.Curr.Microbiol.App.Sci.* 13(5): 208-212. **doi:** https://doi.org/10.20546/ijcmas.2024.1305.026