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PHYTOTOXICITY ANALYSIS OF DIFFERENT CARPET DYES AND THEIR FUNGAL EXTRACTED METABOLITES (FUNGAL DEGRADED PRODUCTS) THROUGH RELATIVE SEED GERMINATION AND SEEDLING GROWTH PARAMETERS

Qurratulane Bari* and Nagendra Bhardwaj

Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India - 302004.

ABSTRACT

To assess the phytotoxicity of five different carpet dyes viz. Methyl Red, Methyl Orange, Erichrome Black, Crystal Violet and Malachite Green and their extracted metabolites, (degraded products) an experiment was set up, in which the seeds of *Hordeum vulgarevar. RD 2508* were germinated to assess the comparative toxicity of the dye against its degraded/extracted metabolites by the potent degrader fungal strain *Aspergillus niger*. All the dyes displayed a unique and uniform behavior. The results with the control (DD H_2O) group were the best, followed by the extract group and then the dye group. Best results of germination and seedling growth parameters on *Hordeum vulgare* were obtained with Methyl Orange dye and the least results were obtained with Malachite Green dye. Although all the carpet dyes, showed variable results among the root length, shoot length and dry weight, that indicated their differential toxic behavior towards the seedling germination and seedling growth. The overall pattern indicated that the degraded products or metabolites were undoubtedly less toxic than the dyes themselves .

Keywords: Phytotoxicity, Extracted Metabolites, Degraded products, Toxic, Germination, Root length, Shoot length, Total dry weight.

INTRODUCTION

One of the major problems that humans are facing is the restoration of the contaminated environment. Textile dyes contribute as the most important environment-polluting agents. Several classes of such contaminants have been synthesized, and still new products are being synthesized now and then. The textile industry is a large water consumer and produces large volumes of contaminated water.Synthetic dyes released into the environment in the form of effluents by textile, leather, food, paper and printing industries cause severe ecological damages. Rapid industrialization has necessitated the manufacture and use of different chemicals in day to day life [1]. Wastewater resulting from dyeing and finishing processes has an adverse impact in terms of total organic carbon, biological oxygen demand and chemical oxygen demand. The concentration of dye contained in the effluent varies between 10-200mg/ml depending on the dyeing process [2]. Many dyes and pigments are hazardous and toxic for human as well as aquatic life at the concentration at which they are being discharged to receiving water [3]. The reactive azo dyes-containing effluents cause serious environmental pollution. Therefore, industrial effluents containing azo dyes must be treated before discharging into the environment to remove the dye toxicity from textile effluents [4-7]. The high concentration of dyes is known to cause ulceration of skin, and mucous membrane, dermatitis, perforation of nasal septum, severe irritation of respiratory tract and on ingestion may cause omitting, pain, haemorrhage and sharp diarrhoea [8]. Chronic effects of dyestuffs, especially of azo dyes were seldom directly mutagenic orcarcinogenic [9]. Pinheiro et al [10] reported

Corresponding Author:- Qurratulane Bari Email:- qbari_aries2000@yahoo.com

that not all aromatic amines are toxic and carcinogenic, and found that some aromatic amines are non-toxic and non-carcinogenic. The slow rate of decomposition of dyes present in waste water necessitates treatment methods to accelerate the process [11]. These processes may not guarantee the treatment of toxic dye in the effluent. Moreover, considering the volume of wastes released during the industrial production process these are often laborious and expensive [12]. Biological methods of removal involve use of microorganisms such as bacteria and fungi to convert the pollutants into nontoxic harmless substances. Anoxic degradation of various azo dyes by mixed aerobic and facultative anaerobic microbial consortia was reported [13- 15]. Although, many of these cultures were able to grow aerobically, degradation was achieved only under anaerobic conditions [16,17]. Many microorganisms belonging to the different taxonomic groups of bacteria have been reported for their ability to decolorize azo dyes [18].

Biological processes convert organic compounds to water and carbon dioxide, have low cost sustainable and are easy to use [19]. Biological methods are generally considered environmentally friendly as they can lead to complete mineralization of organic pollutants at low cost [20]. Bioremediation may be the most effective method of treating industrial dyes waste water [21]. The toxicity of the degraded product can be verified by using phytotoxicity study.

In the present study also, the comparative phytotoxicity of five different dyes was analyzed and compared with their degraded products or extracted metabolites by the relative and over all germination behaviour of the seeds of *Hordeum vulgare* along with the seedling growth parameters.

Study area

Phytotoxicity analysis of five different carpet dyes and their extracted metabolites (degraded products) through seed germination was conducted at Tonk district, which is located in north-eastern part of the Rajasthan state between 75°07' to 76°19' east longitude and 25°41' to 26°34' north latitude.

MATERIALS AND METHODS

In the experiment, five different Carpet dyes, i.e. Methyl Red, Methyl Orange, Erichrome Black, Crystal Violet and Malachite Green were inoculated with *Aspergillus niger* (best adapted fungal strain for degradation of these dyes) and then these were subjected to bioremediation/decolorization experiment in which the fungal culture was grown in liquid DMM or DMM broth in static condition for 8 days at 30^0C.

To prepare DMM or Dye Modified Media, in a liter of distilled water, potato dextrose agar or potato dextrose broth was dissolved accordingly and the pH was adjusted to 5.6 using 0.1 M HCl and 0.1 NaOH. Besides, the carpet dyes were added in required amounts. Peptone (10 g) and dextrose (40 g) were also added to the media.
The strain decolorized the dyes according to its degrader potential in optimum conditions, leaving a light colored solution in the media. The extract was taken out and filtered after the completion of the experiment i.e. 7 days; so that no more media traces could have been left, and then the filtrate was autoclaved to destroy the fungal spores and biomass and to keep only metabolites of dye active.

After this, three preparations were made from each dye filtrate and germination study was carried out. The seeds of *Hordeum vulgare var. 2508* were surface sterilized (0.1% $HgCl_2$), washed repeatedly. These seeds were dried using blotting paper. Seeds were lined with filter paper over pre-sterilized cotton pads. 20 healthy seeds were kept equidistantly on the top of the filter paper. For each dye 3 sub groups were made viz. control, dye and extract group and triplicates were prepared for each sub group. So a total of 9 x 5 =45 Petri plates were needed**.** As pretreatment, 5 ml of DDH_2O was inoculated/added in control group petri plates, 5 ml of dye solution (DMM) was added in dye group petri plates and 5 ml of extract solution was added in extract group petri plates on the first day, and 3 ml of the same solution was added on 2^{nd}, 4^{th} and 6^{th}day of sowing. These Petri plates were kept in BOD incubator at 20^0C under proper humid conditions. Relative seedling emergence was recorded after 48 hours and overall growth after 7 days. Root and Shoot length and dry weight were also recorded.

Observation and Results

It was observed that all the dyes displayed a unique trend in a uniform way. The results with the control (DD H_2O) group were the best, followed by the extract group and then the dye group (Table 1 and Figure 1). Best results of germination and seedling growth parameters on *Hordeum vulgare* were obtained with Methyl Orange dye and the least results were obtained with Malachite Green dye.

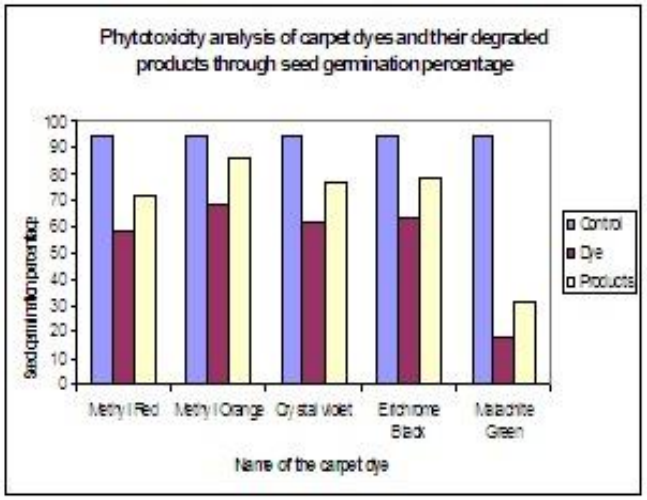
Although the carpet dyes, showed variable results among the root length, shoot length and dry weight, that indicated their differential toxic behavior towards the seedling germination and seedling growth, the overall pattern indicated that the degraded products or metabolites were undoubtedly less toxic than the dye itself.

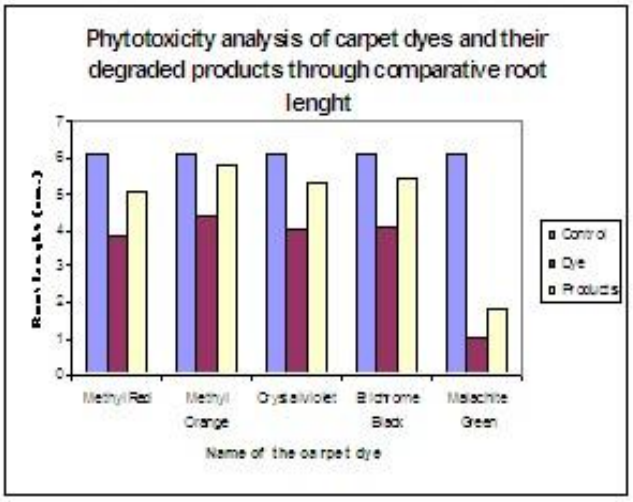
DISCUSSION

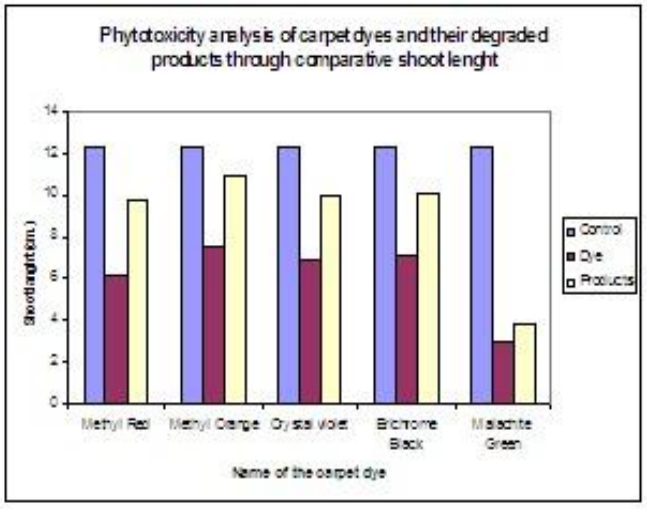
In the present study, phytotoxicity experiments were set up that were based upon the relative germination behaviour of *Hordeum vulgare* seeds that was set in the group of three viz. control, dye group and the product of the dye itself. It was found in all five dyes that the control group displayed the best results in terms of germination percentage and seedling growth parameters followed by the product group (degraded group) and the least results were observed with the dye group.

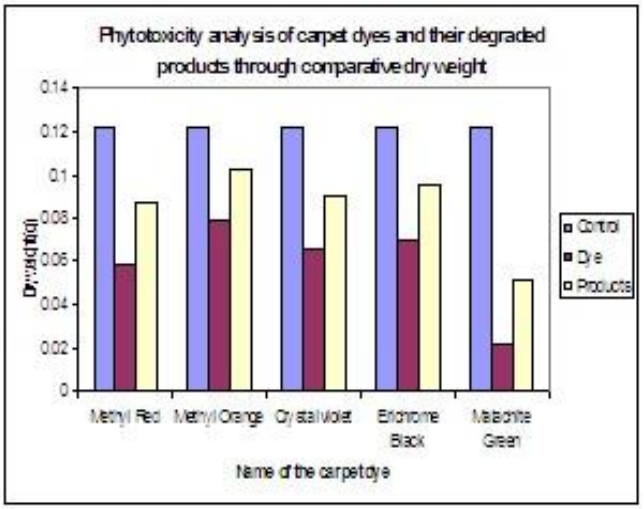
Table 1. Phytotoxicity analysis of carpet dyes and their extracted metabolites (degraded products) through seed germination experiments on the plant *Hordeum vulgarevar RD 2508*

Growth Parameter	Carpet Dye Tested	Methyl Red		Methyl Orange		Crystal Violet		Erichrome Black		Malachite Green	
	Control	Dye	Products	Dye	Products	Dye	Products	Dye	Products	Dye	Products
%Germination (48 h)	95.00 (57/60)	58.33 (35/60)	71.6 (43/60)	68.33 (41/60)	86.66 (52/60)	61.66 (37/60)	76.66 (46/60)	63.33 38/60	78.33 47/60	18.33 11/60	31.66 19/60
% Decrease in seed germination	-	38.5	24.6	28.07	8.77	35.09	19.3	33.33	17.54	80.7	66.67
Root length	6.1	3.86	5.13	4.38	5.8	4.02	5.31	4.1	5.46	1.08	1.86
% Decrease in Root length	-	36.72	15.9	28.19	4.91	34.09	12.95	32.78	10.49	82.29	69.5
Shoot length	12.3	6.21	9.8	7.58	10.86	6.89	10.03	7.1	10.14	3.03	3.85
% Decrease in shoot length	-	49.5	20.3	38.3	11.7	43.98	18.45	42.27	17.56	75.36	68.69
Total dry weight(g)	0.122	.058	0.087	.079	.103	.066	.090	.07	.095	.022	.051
% Decrease in total dry weight	-	52.4	28.6	35.24	15.5	45.90	26.22	42.62	22.13	81.96	58.19









The results are similar to the study where the degradation of Reactive Blue 171 *by Marinobacter sp. NB-6* lead to the formation of non-toxic products as revealed by toxicity testing on *Azotobacter sp., Pseudomonas sp.* and *Rhizobium sp* [22].

Similiar results were obtained in a study conducted on C.I. Direct Black 38 azo dye through decolorization in an anaerobic/aerobic sequential reactor system where it was observed that the degradation products of the dye were less toxic than the dye itself [23].

In an Innovative Approach to Biodegradation of Textile Azo Dyes by native bacterial strains, it was found that the degradation products of the dyes were less toxic than the dye itself when tested on growing Sorghum bicolour [24]. These results coincide with the results of the present study where all the tested dyes were found to be more toxic than their formed degraded products.

It has been observed however that the toxicity of all the tested dyes varied greatly towards *Hordeum vulgare* as the percent decrease in seed germination ranged from 28.07% (in case of Methyl Orange) to 80.7% (in case of Malachite Green).

In a study, degradation of Remazol Red dye by *Galactomyces geotrichum* MTCC 1360 resulted in increased iron uptake in *Sorghum vulgare* and *Phaseolus mungo* from soil. This is due to the conversion of the complex dye molecule into less toxic products [25].

The results are similar to the present study where the maximum vegetative parameters are obtained on the degraded products of dyes, as percent decrease in root length was observed 36.72% with Methyl Orange dye while it was observed only 15..9% with its degraded products, the values for the decrease in shoot length with Methyl Orange dye was 49.5% and for the degraded products it was 20.9%,as far as the decrease in total dry weight is concerned again the maximum values are with the dye Methyl Orange that displayed a reduction percentage of 52.4% in the total dry weight (g) and with the degraded products the reduction percentage was 28.6% only. The results showed a similar pattern with all the dyes that displayed the intermediate values and the least results were observed with Malachite Green dye that displayed a 82.29 % decrease in root length and 69.5% with its degraded products. The values for percent reduction in shoot length with the dye and the products were 75.36% and 68.69 % respectively. The decrease in percent total dry weight (g) with the dye and the product was 81.96% and 58.19 % respectively.

Studies on degradation of azo dyes with methyl orange as model dye using *Saccharomycers cerevisiae*. The outcome of this research showed that baker's yeast S*accharomyces cerevisiae* has satisfactory catalytic effort in degradation of organic compound and the degraded end product is also less toxic to the environment [26]. These results are in the line with the present study.

Biodegradation and Decolorization of Reactive Orange 16 by *Nocardiopsis alba* Soil Isolate was performed in a study and the LC-MS analysis indicated the presence of 1-amino-1-napthalene sulphonic acid in degraded product of the dye. The degraded product is less toxic to the growth of *Vigna mungo* seeds when compared to the non degraded dye [27]. In another study, Decolourization and degradation of azo Dye, Synozol Red HF6BN, by *Pleurotusos treatus* was performed and the observation of no zones of inhibition on agar plates and growth of *Vigna radiata* in the presence of dye extracted sample indicated that the fungal degraded dye metabolites are nontoxic to beneficial micro-flora and plant growth [28]. The results were similar to the study conducted by Shah et al, where *Bacillus spp. ETL-1979* was employed for Degradation and Decolorization of Methyl Orange, Malachite Green and Congo Red and the degraded products were found non-toxic in nature.

All the results of the abovementioned study correlate with the results of the present study where the finally degraded fungal products of all the tested dyes not only displayed a significant increase in the germination percentage (a maximum value of 86.66%, in case of Methyl Orange dye) but also all the vegetative parameters of the seedling showed a trend of increase when the products were compared with the dyes. As it is also evident with the % decrease of the root length, shoot length and total dry weight of the seedling.

It was also found that Methyl Orange dye was least toxic and Malachite Green dye was the most toxic dye in terms of germination percentage and overall growth parameters of seedling growth on *Hordeum vulgare*.

CONCLUSION

It is therefore concluded from the study that the degraded products of all the tested different dyes were less toxic, hence the dye drainage reservoir should be treated with the native degrader fungal strains under optimum conditions for a definite period of time, so that the toxicity of the dye- effluent can be decreased up to the level that it can be released in the local water bodies without affecting the local flora and fauna of the area with its toxic effects.

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