

Assessment of Phytotoxicity of Biophysically Treated Disperse Dark Red dye on *Vigna Radiata*

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Abstract:

Disperse dyes are characterized by their lesser water solubility but still are considered efficient dye that can be used for the coloration of the different types of fibers like polyester or cellulosic fibers. It is a known fact that the polyester fibers are extremely difficult to tint because of lack of free ions which supports towards their repulsion towards water. They are under the azo dyes, which for them being more toxicogenic in nature. In this study, the toxicity of such dye was analyzed on *Vigna radiata* (moong) plant based on the radical and plumule growth and percent germination along with content of chlorophyll a and chlorophyll b. A comparison of the above parameters was done with the pure dye, adsorbed on wheat bran, acid and alkali modified wheat bran and by organism (*Schizopyllum*- S4) treated dye on the growth of the plant. The study has revealed that under all the different categories there were 100% germination of seeds, with around 20.8% and 8.5% increase in radical and plumule growth by *Schizopyllum*-S4 treated dye (initial concentration 100mg/L) after 10 days of growth, with only 8.04% and 15.38% increase in chlorophyll a and chlorophyll b, respectively under the same condition in comparison to the control (plant without aqueous solution of dye). Though with around 19.47% and 33.7% increase in pigment chlorophyll a and chlorophyll b, respectively with dye treated on acid modified wheat bran, depicting that alkali modified wheat bran can be a potential alternative for the disperse dark red dye treatment, the permeate obtained from which can aid in the reduction of the toxicity of the dyes used in the industries especially on textiles, which alone are deleterious if released untreated into the open water system.

Introduction

Disperse dark red is a type of synthetic, less soluble type of azo dye. The presence of unrestricted ionizing group on it contributes towards its feeble attraction towards the polymers like paper, cellulose, cellophane, mostly of cellulosic backbone. However, it exhibits good affinity towards cellulose acetate¹³ and polyethylene terephthalate. The conditions required for its usage is low pH and higher temperature. It is quite cheap in comparison to the naturally occurring dyes. It has quite a good demand in the textile industries. Though it has shown innumerable mal effects to the environment as well as on the consumers, like allergic responses, like “nylon allergy”, which was specifically dermatitis caused because of such type of dye, which was reported long back in 80’s (1, 2). Fig.1 depicts the structure of Disperse Dark Red, with an azo group, that contributes towards its resistance to degradation. Various types of remediation methods have been employed for the degradation of such dyes, this study mainly targeted the phytotoxicity study of the dye after various types of treatments such as adsorption on wheat bran (WB), on acid treated and

alkali treated wheat bran, organism like a white rot fungus treated dye and also the effect of reusability of the same dye adsorbed bran as an adsorbent in order to compare at which phase a check can be given for the usage of the permeate obtained after the adsorption, so that they can have certain application in the plant growth. The plant used in this study was *Vigna radiata* (moon dal), though the application of such treated dyes is more appropriately seen in plants meant for ornamentation but still this study was conducted to analyze their effects on this edible plant.

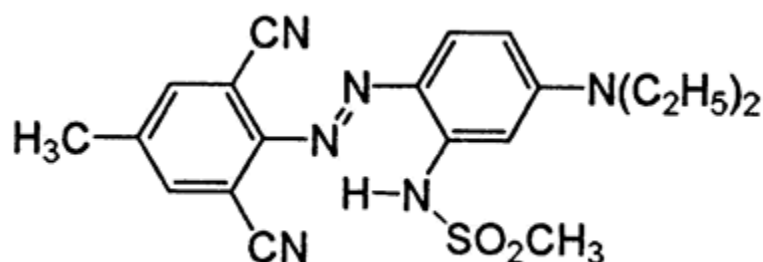


Figure.1 Structure of Disperse Dark Red (Anonymous 1, 2019)

Materials and Methods

Materials

98% pure dye, used in the study was of analytical grade obtained from Loba Chemicals, Mumbai, India. The adsorbent used (wheat bran) was procured from the local market from Jalandhar Cantt., Punjab, India. The organism, *Schizophyllum-S4*, was an already isolated and identified strain obtained from the laboratory of Lovely Professional University, Phagwara, Punjab, India.

Methodology

Batch adsorption of the disperse orange was done under on adsorbent, wheat bran (WB) [4], on wheat bran modified with citric acid (AWB) [5] and sodium hydroxide modified wheat bran [6]. Liquid state decolorization of the dye was executed under an optimized condition. In 100 mL conical flask 20 mL working volume with a concentration of 100 mg/L was taken in presence of wheat bran. After autoclaving in the laminar air flow the culture was inoculated with 2-3 pieces of button shaped bodies with help of cork borer. The flasks were incubated at 28°C for 7 days and reduction in colour intensity was monitored by taking optical density after every day for a period of 16 days with a UV- Spectrophotometer [7]. All the experiments were performed in triplicates to reduce the error.

Toxicity study

Healthy seeds of moong (*Vigna radiata* or *Phaseolus aureus*) were collected from local store. The seeds were surface sterilized with sodium hypochlorite (1%) solution for 5-10 minutes and rinsed thoroughly with distilled water for several times for the removal of excess amount of chemical, before use for the experimental work [8].

In this experiment, the effect of treated disperse dark red on germination of *V. radiata* was evaluated. The surface sterilized seeds were evenly placed in sterile 10 cm petri dishes containing untreated dye, and laboratory scale treated (wheat bran treated dye (1st, 2nd and 3rd

cycle) and organism treated dye) layered with double layer of Whatman filter paper 1 while tap water was taken as control and incubated at 28°C in the dark for germination. The relative seed germination %, relative root growth %, using the plant growth data after 48 hrs and 168 hrs.

The Relative seed germination percentage calculated using the formula:

$$\text{Relative seed germination (\%)} = \frac{\text{Number of seeds germinated in presence of dye}}{\text{Number of seeds germinated in control}} \quad (\text{Eq.1})$$

Pot Study

The phyto-toxicity assay was carried out in triplets using medium sized plastic container. Soil in each container was incubated with 10 seeds of moong (*Vigna radiata* or *Phaseolus aureus*). The assay was checked using untreated, unmodified wheat bran treated (1st cycle, 2nd cycle and 3rd cycle), modified (acid and alkali) wheat bran treated and organism treated dye samples. The assay was performed at room temperature (30 ± 2 °C) and 5 ml of sample was watered separately per day. At the same time plain water was used to carry out the control set. After 10 days of incubation, length of plumule (shoot), radicle (root), Chlorophyll content (mg/g) and germination (%) was recorded [9,10].

$$\text{Germination \%} = \frac{\text{Number of seeds germinated in presence of dye}}{\text{Number of seeds germinated in control}} \quad (\text{Eq. 2})$$

$$\text{Chlorophyll a} = \frac{(12.3 \times D_{663} - 0.86 \times D_{645}) \times V}{D \times 1000 \times W} \quad (\text{Eq.3})$$

$$\text{Chlorophyll b} = \frac{(19.3 \times D_{645} - 3.60 \times D_{663}) \times V}{D \times 1000 \times W} \quad (\text{Eq. 4})$$

Whereby, D_{645} is the Optical density at 645 nm, D_{663} is the Optical density at 663 nm, V is the final volume in ml, W is the fresh weight of leaf and D is the path length (cm)

Result and Discussion

After adsorption under pH 5, incubation time 180 mins, adsorbate dosage 100mg/L, adsorbent dosage, 12 gm/L at a temperature of 30°C on WB, AWB and AMWB, the maximum percentage of disperse dark red removal obtained was 93.55±0.1%, 95.47±0.4% and 72.36±0.1% respectively (process not disclosed). When direct dye was used it has shown a decrease in radical and plumule growth by 75% and 36.7% respectively. Whereas, when the permeate obtained after the first cycle of wheat bran adsorption, it has shown 14.5% and 3.12% increase in the radical and plumule growth. On the other hand if it is compared with the 3rd cycle usage of the dye adsorbed wheat bran, the permeate obtained from here on analysis of phytotoxicity has shown only 2% and 10.9 % increase in the radical and plumule growth. On alkali modified wheat bran, on the other hand there was a decrease by 41.6% and 21% in radical and plumule growth, depicting that the alkali treatment might go for some adverse reaction with the dye showing such a lesser percentage of growth of the plant. Whereas, treatment with *Schizophyllum*-S4 has shown an increase of around 20.8% and 8.5% increase in the radical and plumule growth. Again, with respect to the pigment content like chlorophyll a and chlorophyll b, acid modification has shown 19.47 and 33.7 % whereas, the organism treated dye has shown around 8.04% and 15.38% increase respectively. Table1; Table 2; Figure 2 and Figure 3, depicts that both acid treated and organism treated dye can be used for plantation as may be a consequence of degradation of the dye by the enzyme produced by the organism which does not affect the plant adversely. In another similar study it has been shown that Direct Yellow 4 (DY4) treated by citrus POD

reduced the toxicity of DY4, indicating that the biodegradation of dyes are quite efficient in reduction of toxicity of the dyes (11). In another study with *Sorghum vulgare* Pers seeds, the effect of acid orange 10 and treated dye has shown that there was also 100% germination in control while the treated dye yielded 27% and untreated dye yielded only 06% germination (12). Another study reported that *Triticum spp.* (Wheat) yielded cent percent germination of seeds in control and but the seeds treated with dye showed no germination. In the same study with *Vigna radiata* (Moong seeds), 100% germination in control was observed whereas, the seeds treated with dye at 500-1000ppm only 40% and with 5000ppm dye only 20% germination was recorded (13).

Conclusion

Thus, treated dyes shows better result with respect to phytotoxicity. Thus such treated aqueous dyes can be used for ornamental trees, which can be avoided to be disposed off the environment like that.



Figure 2:Effect of DDR (100 mg/L) on plant; A-Control, B-Untreated, C-Unmodified WB treated (1st cycle), D-Unmodified WB treated (2nd cycle), E- Unmodified WB treated(3rd cycle), F- Acid modified WB treated, G-Alkali modified WB treated, H-Organism

Plant treatment	Radical (cm)	Plumule (cm)	Germination %
Control	4.8 ± 0.5	12.8 ± 2.21	100
Untreated	1.2 ± 0.2	8.1 ± 1.3	100
Unmodified WB (first cycle) treated	5.5 ± 0.8	13.2 ± 1.8	100
Unmodified WB (second cycle) treated	2.1 ± 0.8	13.5 ± 1.3	100
Unmodified WB (third cycle) treated	4.9 ± 0.7	14.2 ± 1.7	100
Acid modified WB treated	5.4 ± 1.2	14.7 ± 0.9	100
Alkali modified WB treated	2.8 ± 0.7	10.1 ± 1.1	100
Organism treated	5.8 ± 1.4	13.9 ± 1.5	100

Table 1:Radical length, plumule length and germination % of plant treated with DDR (100 mg/L)

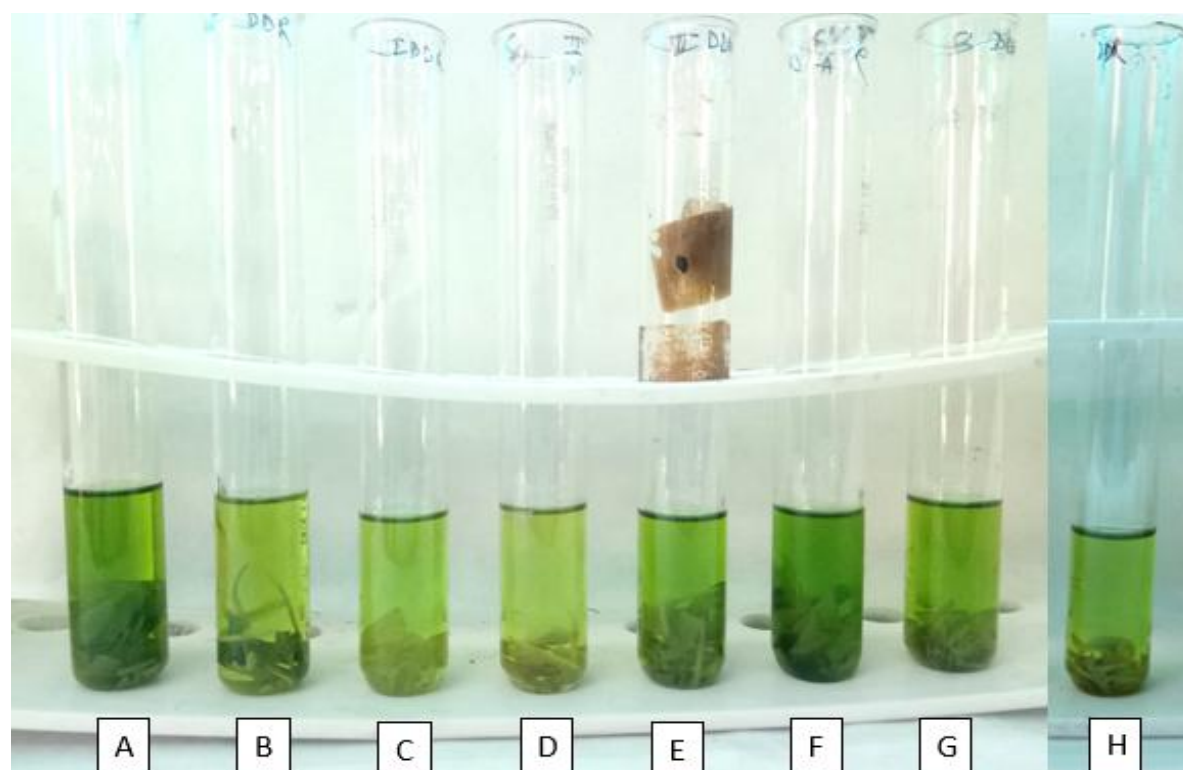


Figure 3:Effect of DDR (100 mg/L) on chlorophyll content; A-Control, B-Untreated, C-Unmodified WB treated (1st cycle), D-Unmodified WB treated (2nd cycle), E- Unmodified WB

treated(3rd cycle), F- Acid modified WB treated, G-Alkali modified WB treated, H-Organism treated

Plant treatment	Chlorophyll a	Chlorophyll b
Control	0.945 ± 0.084	0.264 ± 0.016
Untreated	0.233 ± 0.028	0.075 ± 0.0037
Unmodified WB (first cycle) treated	0.410 ± 0.039	0.125 ± 0.004
Unmodified WB (second cycle) treated	0.474 ± 0.041	0.227 ± 0.022
Unmodified WB (third cycle) treated	0.706 ± 0.057	0.223 ± 0.027
Acid modified WB treated	1.129 ± 0.087	0.353 ± 0.031
Alkali modified WB treated	0.801 ± 0.089	0.236 ± 0.019
Organism treated	1.021 ± 0.044	0.312 ± 0.013

Table 15.26 Chlorophyll content of plant treated with DDR (100mg/L)

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