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Assessment of Phytotoxicity of Bio-Physically Treated Malachite Greendye on *Vigna Radiata*

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Abstract:

Malachite green, a water-soluble dye is considered be an efficient dye that has its usage in various fields be in textile or in medical or in pharmaceutics. The dye has been reported to cause health hazard thus the effluent with such a dye demands proper treatment. But, the fate of such dye treated water is also another concern in the recent times. In this study, the toxicity of such dye was analyzed on *Vigna radiata*(moong) plant on the basis of radical and plumule growth and percent germination. A comparison of the above parameters was done with dye, wheat bran treated, acid and alkali modified wheat bran treated dye and also by organism (*Schizophyllum*-S4) treated dye on the plant. The study has revealed that there was 100% germination of seeds under all the conditions, with 10% and 12.3% increase in radical and plumule growth by *Schizophyllum*-S4, a white rot fungus, treated dye (initial concentration 100mg/L) after an incubation period of 10 days but has shown a better result with alkali treated wheat bran as an adsorbent with around 15% and 11.7% increase in the radicle and plumule growth with 23% and 48% increase in chlorophyll a and chlorophyll b, respectively in comparison to the control (plant without aqueous solution of dye)under the same conditions applied. Thereby representing that treated dye either with agro industrial waste or with basidiomycetes species can be used for farming aiding in the reduction of the harmfulness of the dyes used irrespective of the industries which alone can be deleterious for the water eco-system.

Introduction

Malachite green, a triphenylmethane dyes, is a crystalline green powdered dye, which has innumerable usage, whether as a food colorant or in aquaculture or as a medical disinfectant. But are environmentally persistent in nature and has the capacity to damage nervous system, liver or brain when ingested. Though being cheap in comparison to the natural dyes they are continuously in use in various industries. These dyes contribute to a lot of ill effect to the environment as well as on the users, like that of other dyes by causing dermatitis caused because of the dyes were reported long back in 80's (1, 2). Fig.1 depicts the structure of Malachite Green, with anChloride ion and three phenyl 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

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the plant growth. The plant taken into consideration was *Vigna radiata*(moon dal), though the application of such treated dyes is more appropriately seen in ornamentation plant but still a study was conducted to analyze their effects on such plants.

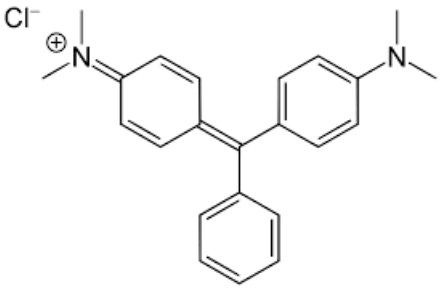

Figure.1 Structure of Malachite Green (Anonymous 1, 2019)

Materials and Methods

Materials

The dye used in the study was of analytical grade obtained from Loba Chemicals, Mumbai, India. The adsorbent used (wheat bran) was procured form 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

Batchadsorption of the disperse orange was done under on adsorbent, wheat bran (WB)[4], on acid modified wheat bran (AWB) [5] and alkali modified wheat bran [6]. Liquid state decolorization of the dye was carried out under an optimized condition. In 100 mL conical flask 20mL working volume with a concentration of 100mg/Lwas 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 decolourization 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 1% sodium hypochlorite solution for 5-10 minutes and rinsed thoroughly with distilled water for many times to remove the excess amount of chemical, before use for the experimental work [8].

In this experiment, the effect of treated disperse orange 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 (1^{st}, 2^{nd} and 3^{rd} 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:

Relative seed germination (%) = Number of seeds germinated in presence of effluent/dye (Eq.1)

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Number of seeds germinated in control

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 effluent/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].

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

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

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

Whereby, D_{645} is theOptical density at 645 nm, D_{663} is theOptical 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 4, incubation time 210 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 orange removal obtained was 95.25, 89.87 and 98.39% respectively (process not disclosed). Thus, the permeate obtained from the study after been used for phytotoxicity analysis has shown 23% more radical growth 48% more plumule growth with dye obtained after adsorption on alkali modified wheat bran in comparison to the control. Whereas showing 3.9% increase and 13.2 % decrease in radical and plumule growth respectively on adsorption on wheat bran. Though on reusability of the dye adsorbed bran at the third cycle has shown a decrease both the radical and plumule growth by 26% and 12.5 % respectively. The alkali modification of the wheat bran on the contrary has given a better result with 15% and 11.7% increase in radical and plumule growth, respectively with 23 and 48% increase in chlorophyll a and chlorophyll b, respectively (Table1; Table 2; Figure 2 and Figure 3) depicting that 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) yieldedcent 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). Another study has shown that sprouting of *Triticum aestivum* was lesser with Malachite green treatment as compared to its treated

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products. Thereby proving that degradation of dyes by a biological agentstems in theirreclamation (14).

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 MG (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	4.9 ± 0.5	12.8 ± 2.21	100
Untreated	3.2 ± 0.3	9.3 ± 1.1	100
Unmodified WB (first cycle) treated	5.1 ± 0.8	11.1 ± 1.0	100

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Unmodified WB (second cycle) treated	4.2 ± 0.7	14.2 ± 0.6	100
Unmodified WB (third cycle) treated	3.6 ± 0.7	11.2 ± 0.9	100
Acid modified WB treated	3.7 ± 0.2	10.9 ± 1.2	100
Alkali modified WB treated	5.8 ± 0.3	14.5 ± 1.2	100
Organism treated	5.5 ± 0.9	14.6 ± 1.7	100

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

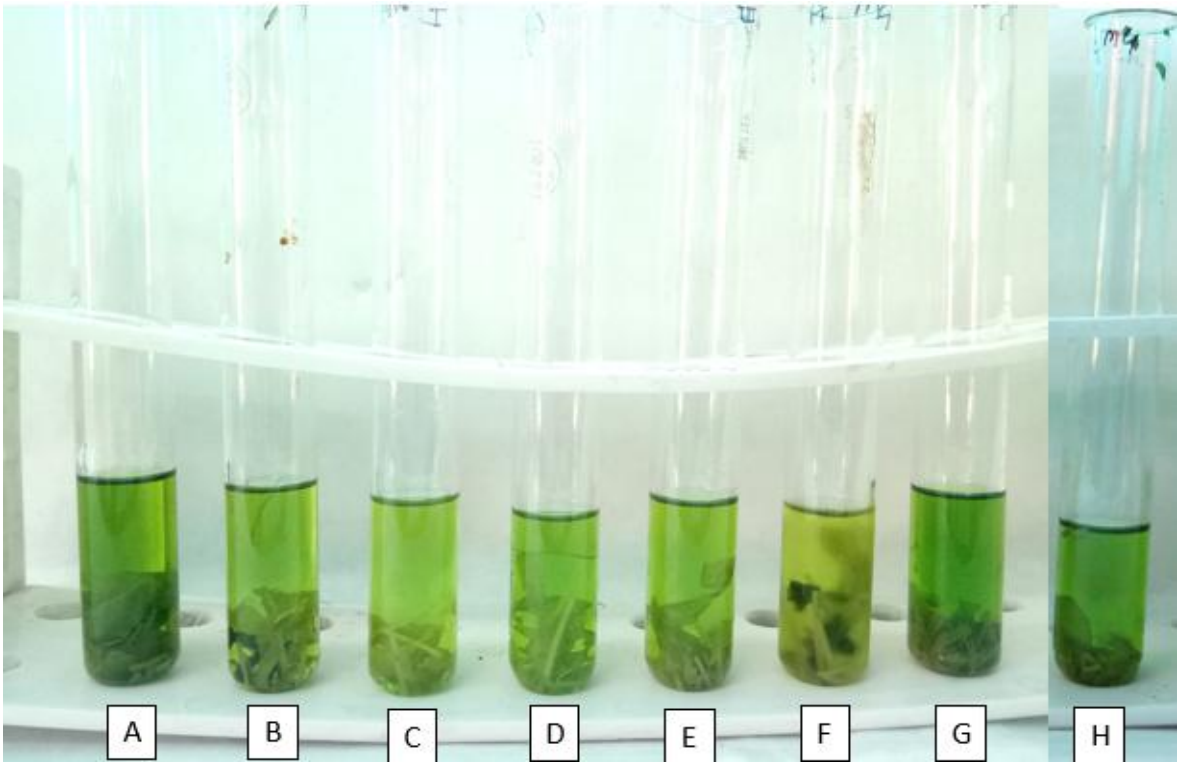

Figure 3: Effect of MG (100 mg/L) on chlorophyll content; A-Control, B-Untreated, C-Unmodified WB treated (1st cycle), D-Unmodified WB treated (2nd cycle), E- Unmodified WB treated(3rd cycle), F- Acid modified WB treated, G-Alkali modified WB treated, H-Organism treated

Plant treatment	**Chlorophyll a**	**Chlorophyll b**
Control	0.945 ± 0.084	0.264 ± 0.016
Untreated	0.547 ± 0.047	0.179 ± 0.015
Unmodified WB (first cycle) treated	0.525 ± 0.041	0.172 ± 0.017

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Unmodified WB (second cycle) treated	0.704 ± 0.063	0.226 ± 0.026
Unmodified WB (third cycle) treated	0.612 ± 0.039	0.207 ± 0.021
Acid modified WB treated	0.277 ± 0.037	0.083 ± 0.008
Alkali modified WB treated	1.229 ± 0.023	0.391 ± 0.074
Organism treated	0.879 ± 0.050	0.214 ± 0.011

Table 15.30 Chlorophyll content of plant treated with MG (100mg/L)

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