IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 3, February 2026
Impact Factor: 8.2

Degradation of Malachite Green and Congo Red Using Melothria Scabra

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Abstract: *Degradation of trimethyl dye malachite green and azo dye congo red was investigated using water extract of Melothria scabra with different parameters such as buffer system ,temperature, dye and enzyme concentration and pH. Phytotoxicity study was carried out to detect the effect of dye, plant extract treated dye on seed germination and seedling growth . Decolourization assay was performed to calculate percent degradation by taking absorbance using UV-Visible spectrophotometer. Congo red showed a maximum of 27.33%decolorization with plant extract using citrate buffer and skin extract produced 30% decoloration. Maximum pH for highest decoloration varied from 6-8 for both congo red and malachite green and temperature was 40- 50 C. With different dye concentration 0.1% exhibited higher decoloration. The time for decoloration varied from 30 minutes to 2 hours. There was a marked difference in the toxic effect of dyes on seedling compared with plant extract treated dyes products*

Keywords: *trimethyl dye malachite*

I. INTRODUCTION

Decolorization is simply the disappearance of the color in wastewater without the actual breaking apart of the dye molecules, which does not necessarily mean degradation of the complex dye molecules. Degradation is the destruction of the large dye molecule to smaller components, along with the breakdown of the chromophores. While chromophore groups of dyes may be destroyed, the intermediate produced may be more toxic than the original compounds and could present significant problems for receiving water bodies. Mineralization means organic compounds are converted to inorganic compounds, i.e., nitrate, carbon dioxide, and water. In this case, a complete detoxification is achieved and no secondary pollution will be introduced. The Melothria scabra(cucumis sativus) in the family (CUCURBITACEAE) is one of the most researched and used plant among 975 species across 98 genera of Melothria scabrahas been used medicinally for several thousands of years in many cultures from ancient Egypt, Greece, and Rome to China and India. The plant has many common names and is often referred to as, burn plant Melothria scabra are, first-aid plant, or medicine plant. Melothria scabra are thought to have originated in tropical Africa but are now cultivated in warm climate areas of Asia, Europe and America. Melothria scabra(Cucumis sativus) is a widely-cultivated creeping vine plant in the Cucurbitaceae family that bears usually cylindrical fruits, which are used as culinary vegetables. Considered an annual plant there are three main varieties of Melothria scabra —slicing, pickling, and seedless—within which several cultivars have been created. The Melothria scabra originates from South Asia, but now grows on most continents, as many different types of Melothria scabraare traded on the global market. In North America, the term wild Melothria scabra refers to plants in the genera Echinocystis and Marah, thoughthe two are not closely related. The Melothria scabrais a creeping vine that roots in the ground and grows up trellises or other supporting frames, wrapping around supports with thin, spiraling tendrils.

DOI: 10.48175/568

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II. EXPERIMENTAL SECTION

2.1 Materials and Methods

Preparation of crude Extract

Melothria scabra was procured from Alva's Shobhavana, Moodbidri. Three different extracts were prepared namely Melothria scabra pulp extract, Melothria scabra skin extract and Melothria scabra whole plant extract. Melothria scabraplant was cleaned and pulp was separated from skin. About 200gm of the pulp as weighed and crushed in a wet grinder with the addition of 200mlof water. The mixture was filtered using muslin cloth and the filtrate was diluted with distilled water and made up to 500ml and the final concentration of 40% was obtained. In the same way Melothria scabra skin was cleaned and 40gms of the cleaned skin was crushed with 60ml of distilled water in a grinder.

The mixture was filtered using muslin cloth and the filtrate was collected. 200gms of the whole plant was cleaned and crushed in a wet grinder. The extract was filtered using muslin cloth and the filtrate was diluted and made up to 500ml using distilled water giving the final concentration of 40gms/100ml. All the 3 extracts were centrifuged at 8000rpm for 12 minutes. Supernatant was collected and stored at 4 C. It was brought to room temperature before use.

Decolorization assay

All decolorization experiments were performed in two sets. The change in absorbance was monitored spectrophotometrically at their maximum wavelength of each dye. The set without the addition of enzyme in the respective dye served as control. The decolorization efficiency was determined using the following equation: The percentage decolorisation/ degradation was studied at different parameters like maximum part of plant showing degradation, effect of buffers, effect of pH, effect of temperature, effect of dye concentration, effect of enzyme quantity and effect of time period.

$$\% \text{ Decolourization} = \frac{\text{Initial absorbance} - \text{observed absorbance}}{\text{Initial absorbance}} \times 100$$

USE OF APPROPRIATE BUFFERS FOR EACH DYE

A buffer is needed for the activity of enzyme. Three buffers (Acetate buffer, phosphate buffer and citrate buffer) of pH 7 were used. Four different dyes (Congo red, Brilliant blue, crystal violet and Malachite green) were initially used. Each dye was examined with all the three buffers. Each set contains one tube with 5ml of 0.1% dye solution, next tube contains 3ml of buffer: 0.5ml of 0.1% dye solution, the third tube contains 3ml of buffer: 0.5ml of 0.1% dye solution: 1ml of enzyme extract was added. Temperature and time was kept constant at 30 C and 1hr respectively. All the set was performed in duplicates. Decolorations were examined using different buffers.

EFFECT OF DIFFERENT MELOTHRIA SCABRA EXTRACT

Different part of the plant has different composition. The effect of the different Melothria scabra extracts was examined with the two dyes. For the study each set contains one tube with 5ml of 0.1% dye solution, next tube contains 3ml of buffer: 0.5ml of 0.1% dye solution, the third tube contains 3ml of buffer: 0.5ml of 0.1% dye solution: 1ml of each enzyme extract was added. Temperature and time was kept constant at 30 C and 1hr respectively. All the set was performed in duplicates. Decolorations using different buffers were examined.

EFFECT OF pH

The pH factor was optimized to obtain maximum decoloration. pH range from 4- 9 was studied. Buffers of different pH range were prepared. 6 tubes with 3ml of buffer of different pH were taken in 2 tubes. To each tube 0.5ml of 0.1% dye solution was added. In one of the tube 1ml of enzyme extract was added according to previously observed result. The same was repeated for both the dye solution. Temperature and time was kept constant at 30 c and 1hr respectively. All the set was performed in duplicates. Decolorations at different pH were examined.

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EFFECT OF TEMPERATURE

Temperature plays an important role in the enzyme activity. Temperature range for 30 ºC- 60ºC was taken. 5 tubes with different temperature were taken with uninoculated enzyme taken as control and the tube with extract as test. Other parameters were kept constant according to previously observed results. All the set was performed in duplicates. Decolorations at different temperature were examined.

EFFECT OF DYE CONCENTRATION

Dye concentration from 0.1ml- 1ml/ 100ml was used for the studies. Optimal temperature, pH was kept constant. All the set was performed in duplicates. Decolorations at different dye concentration were examined.

EFFECT OF ENZYME QUANTITY

40% enzyme concentration was previously prepared. Hence decolorisation rate at different enzyme quantity from 0.5ml to 3ml was conducted. Each tube contains buffer and dye in control and buffer, dye and enzyme was taken. Optimal temperature, pH and dye concentration was kept constant. All the set was performed in duplicates. Decolorations at different enzyme quantity were examined.

EFFECT OF TIME

Decoloration rate was studied at the time interval of 30 min, 1hr. 1.5hr and 2hrs. Each tube contains buffer and dye in control and buffer, dye and enzyme was taken. Optimal temperature, pH dye concentration and enzyme quantity was kept constant. All the set was performed in duplicates. Decolorations at different time interval were examined.

TOXICITY STUDY

Phytotoxicity studies were carried out with 1gms/L of each dye and its extracted metabolites using seeds of Triticum aestivum, and Lens esculenta. The seeds were surface sterilized with 1.2% sodium hypochlorite solution to discourage fungal growth. Five seeds of each plant species were placed in each pot and watered separately with 5ml samples of each dye and its degradation product per day.. The glass were kept in the dark and observed for germination. Seeds with radical (>1mm) were considered germinated (Chimezie and Thomson, 2011). The germinated seeds were then exposed to day and night cycle length of 10/14 h, respectively. The length of plumule (shoot) and radical (root), and the germination rate (%) were recorded after 7 days.

III. RESULTS AND DISCUSSION

Buffers play a very important role in the activity of the enzyme. In the present study, among the three buffers solutions used for the four dyes, congo red showed a maximum decoloration with citrate buffer with 27.73%. In case of Brilliant blue maximum degradation was seen with acetate buffer. On the other hand, Crystal violet did not showed decoloration with any of the used buffers but Malachite green gave maximum degradation with phosphate buffer (Fig.

Table.1 Effect of dyes on the germination, shoot length and root length of *Triticum aestivum*

Test parameter	control	malachite green		congo red	
		dye	treated	dye	treated
Germination (%)	100	58	90	65	87
Shoot length (cm)	12.4	7.5	10.5	7	11
root length (cm)	10.8	6.1	9.2	5.8	9.6

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FIGURE.1 EFFECT OF BUFFERS ON DECOLORATION

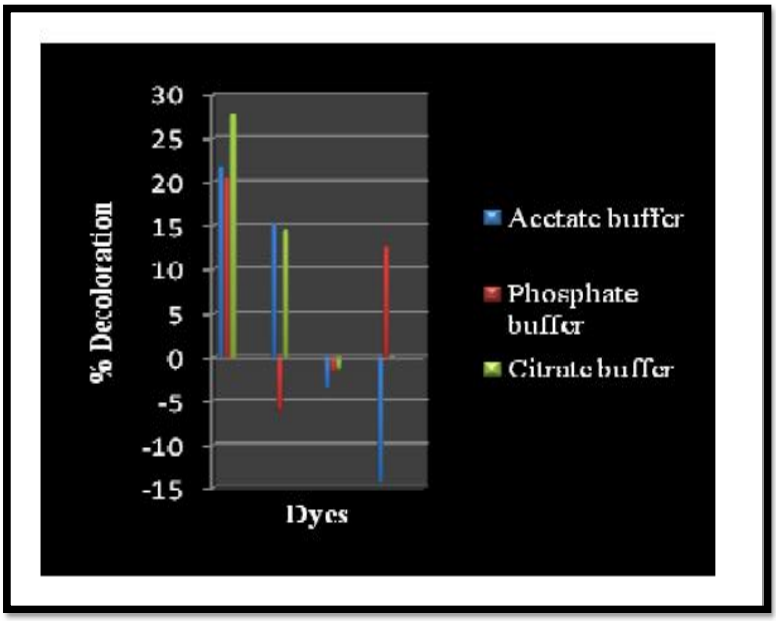
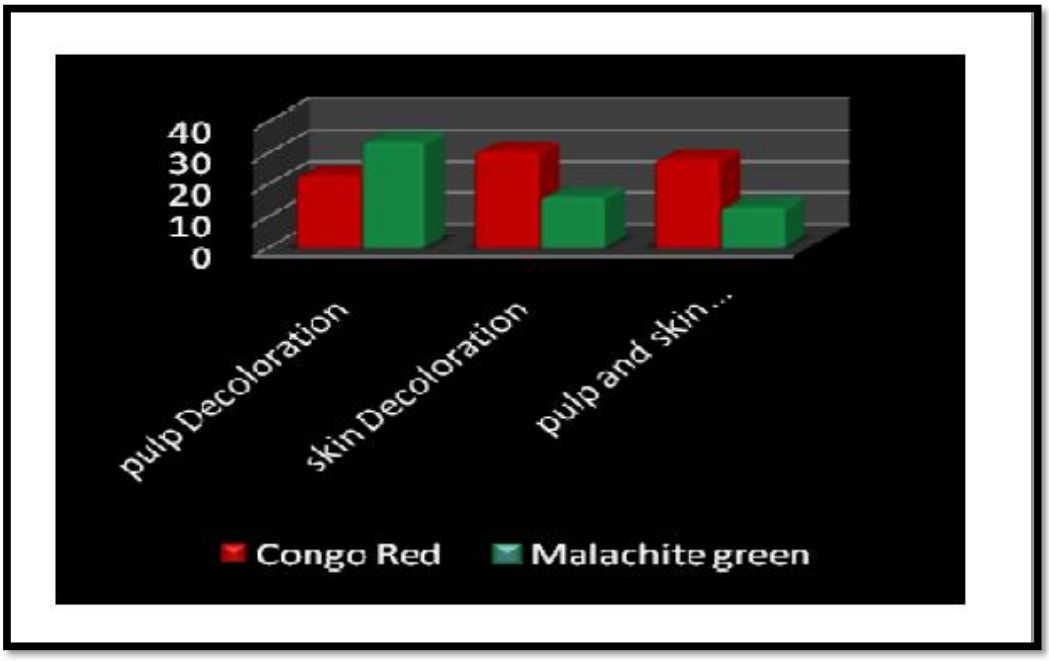


FIGURE.2 EFFECT OF PLANT EXTRACT ON DECOLORATION



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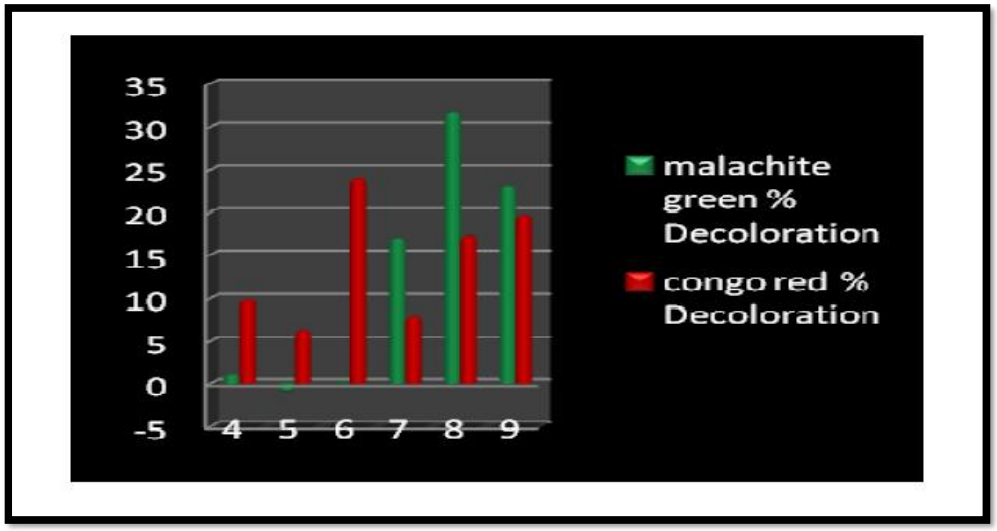

FIGURE.3 EFFECT OF pH ON DECOLORATION

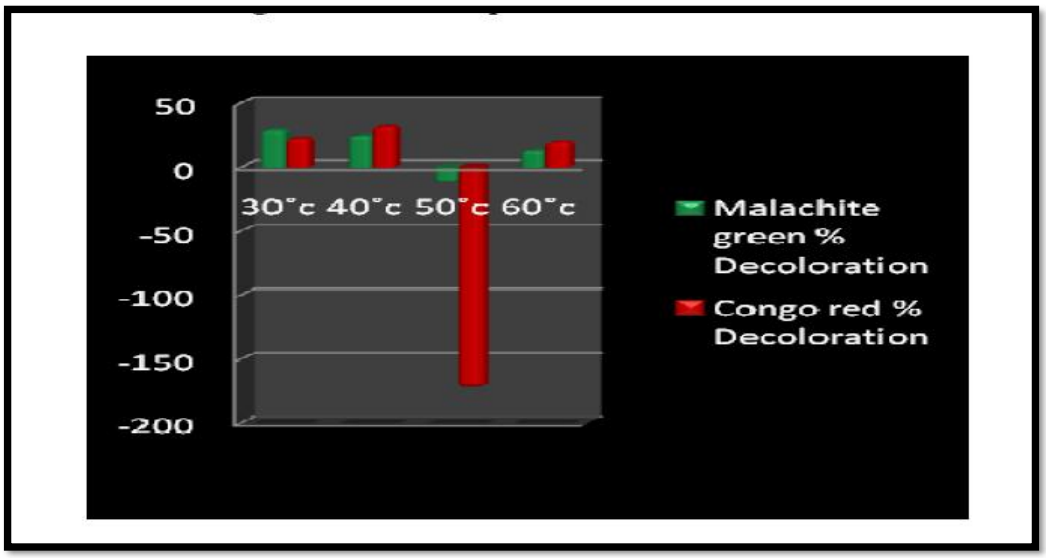

FIGURE.4 EFFECT OF TEMPERATURE ON DECOLORATION

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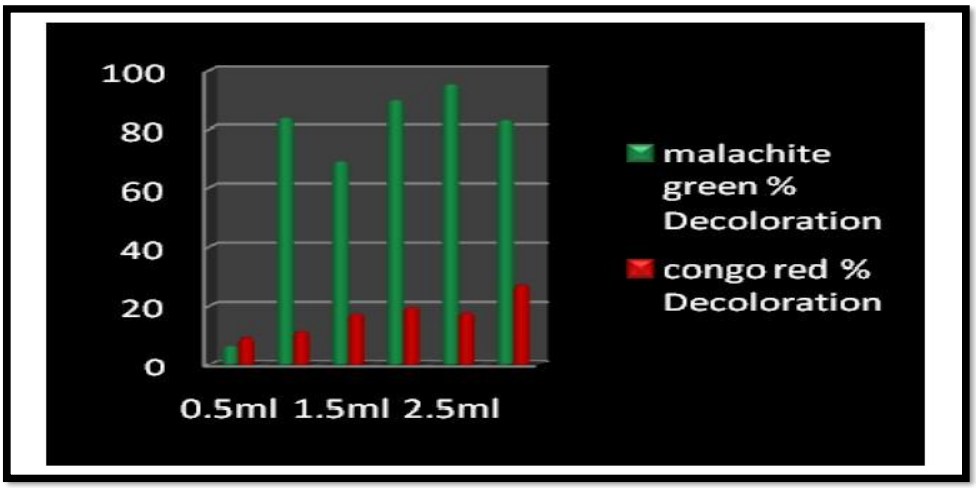

FIGURE.5 EFFECT OF ENZYME QUANTITY ON DECOLORATION

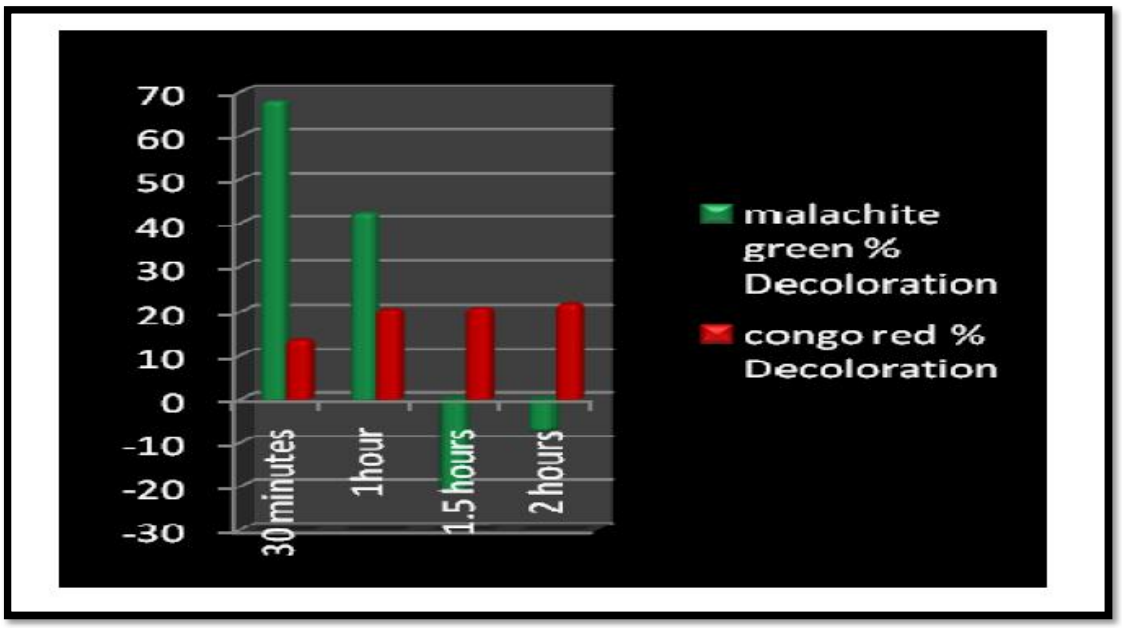

FIGURE.6 EFFECT OF TIME

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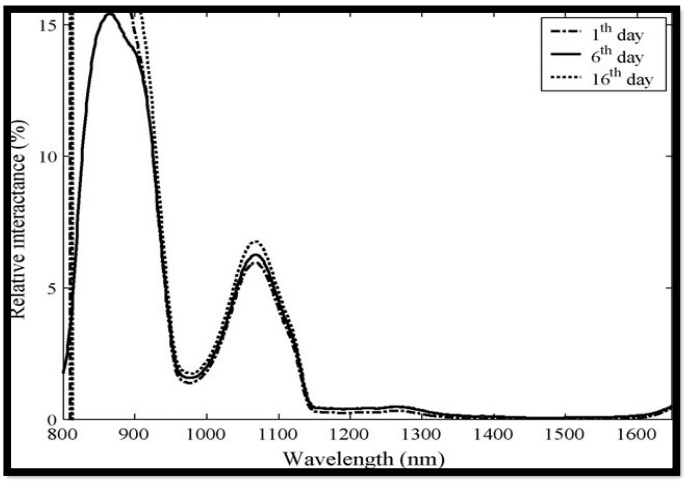

FIGURE 7. Average relative interactance spectra of at the Melothria scabra 1st, 6th and 16th day of the storage period as obtained by the NIR spectrometer

IV. CONCLUSION

The results revealed the potential of Melothria scabra an agricultural solid waste, to be a low- cost adsorbent for removing malachite green and congo red from aqueous solutions. The adsorption was dependent on the initial pH, contact time and dye concentration. Equilibrium data agreed well with Langmuir isotherm model and the kinetic modeling study has shown that the experimental data were found to follow the pseudo-second-order model suggesting a chemisorption process. The result of the intra particle diffusion model suggested that intra particle diffusion was not the only rate controlling step. This study proved that the eco friendly adsorbents RCS and CCS showed a better performance and the raw material Coccinia grandisis easily available in large quantity and its treatment method is very economical.

Application of traditional waste water treatment requires enormous cost and continuous input of chemicals which becomes uneconomical and causes further environmental damage. Hence, economical and eco-friendly techniques using plant extracts can be applied for fine tuning of waste water treatment. Biotreatment offers easy, cheaper and effective alternative for colour removal of textile dyes. Thus, by this present study I concluded that the Coccinia grandisextracts proved very well in the decoloration ability to decolorize/degrade Malachite green and Congo red and can be used in waste water treatment.

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