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Research Article

A Comparative Study on Dye Degradation by Leaf and Root Extracts of *Parthenium hysterophorus* L

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Abstract

The use of different dyes and pigments is increasing with the increase in industrialization leading to the high production of effluent. The effluent contaminated with dyes and dye-stuff has harmful effects on public health and the environment. Thus, the treatment of effluent is essential. Biological approaches are gaining much interest due to their cost-effective and eco-friendly nature over various physicochemical methods for the treatment of dye-contaminated wastewater. This study highlights on the biodegradation of congo red and malachite green by using leaf and root extracts of *Parthenium hysterophorus*. The extract and the dye were mixed in the ratio of 1:2 and incubated at 40ºC for 90 minutes. Decolorization assay was performed using UV visible spectrophotometer which indicated that decolorization was due to degradation of dyes into non-colored metabolites. The leaves extract exhibited higher decolorizing activity than roots extract. The maximum decolorization for leaves extract was 55.8% (congo red) and 51.6% (malachite green). Furthermore, phytotoxicity test was carried out to determine the effect of dyes and their degradation metabolites on seed germination and seedling growth of chickpea (*Cicer arietinum* L). The germination percentage and seedling growth were more in degradation metabolites than untreated dyes, indicating less toxic nature of degradation metabolites. Hence, it can be inferred that *P. hysterophorus* extracts can be used to treat dye wastewater and treated wastewater can be used for irrigation.

Keywords: *Parthenium hysterophorus*; congo red; malachite green; dye decolorization; phytotoxicity test

Introduction

Dyes have become an inseparable part of human life due to their applications in different industries, such as textile, food, cosmetic, paint, paper, beverage, polymer, pharmaceutical etc. in order to impart colors to various products. It is estimated that more than 10,000 different dyes & pigments are used commercially and over 0.7 million tons of dye-stuff are produced annually worldwide (Robinson *et al*., 2001). The wastewater from textile industry has become one of the major sources of severe pollution problems worldwide due to increased usage of synthetic dyes along with demand for textile products (Santos *et al*., 2007). Approximately 20 to 50 % of reactive dyes can be released into waterways depending upon dyestuff type, the application route and depth of shade required during the process of fixing of reactive dyes to

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fabrics (Soares *et al*., 2004). The presence of a very small amount of dyes in water is highly visible and can affect the aesthetic merit, transparency and gas solubility of water bodies (Banat *et al*., 1996). These dyes can remain in the environment for a long period of time due to their high stability against temperature, light, detergent and microbial attack (Couto, 2009). Furthermore, different dyes from wastewater and their breakdown products are toxic and carcinogenic to life (Weisburger, 2002). Hence, the treatment of such harmful dyes before discharging them into natural water bodies seems to be mandatory.

The different types of physicochemical methods such as flocculation, froth floatation, membrane filtration, ozonation, reverse osmosis, adsorption, and electrocoagulation have been employed for decolorization of wastewater dyes (Gupta *et al*., 2004; Gupta *et al*., 2006). But, these methods are costly, less efficient and produce hazardous wastes which are recalcitrant in nature. Thus, the interest in biological processes is increasing due to their cost-effective and eco-friendly nature (Chen *et al*., 2003). The biodegradation of various dyes using different biological components has been reported, such as degradation of dyes using crude extract and laccase isolated from *Pleurotus nebrodensis* (Yuan *et al*., 2016), removal of malachite green by *Trichoderma sp*. (Saravanakumar and Kathiresan, 2014), decolorization of congo red by *Bacillus sp.* (Sawhney and Kumar, 2011), degradation of malachite green and congo red using *Aloe barabadensis* extracts (Rai *et al*., 2014), removal of malachite green using *Limonia acidissima* shell as adsorbent (Sartape *et al*., 2017), removal of congo red through adsorption by ackee apple seeds (Bello *et al*., 2013) and removal of phenol, 4-nitrophenol and 4-chlorophenol from aqueous solutions by activated neem leaf (Ahmaruzzaman and Gayatri, 2011). Here, we have focused on the biodegradation of two most commonly used dyes: congo red and malachite green by using leaf and root extracts of *Parthenium hysterophorus.*

Congo red is an acidic diazo dye whereas malachite green is a basic triphenylmethane dye. These dyes have been used as coloring agents in different industries and have harmful effects on public health and the environment. Congo red has been reported as toxic and carcinogenic due to the presence of aromatic amine group (Buan *et al*., 2010). Malachite green has been used as a very effective antiseptic, anti-parasitic and anti-protozoan agent. But, the toxicological and carcinogenic effects of malachite green and its reduced form, leucomalachite green on organisms have been demonstrated (Sudova *et al*., 2007). *P. hysterophorus* is an annual herbaceous weed which grows vigorously in a diverse range of habitats. It is a prolific weed belonging to Asteraceae family, yielding thousands of small white capitula each producing five seeds after maturity (Patel, 2011). *P. hysterophorus* has been reported as a novel adsorbent for the removal of heavy metals and dyes (Bapat and Jaspal, 2016). In this study, the biodegradation of congo red and malachite green by extracts of *P. hysterophorus* has been explored. Furthermore, the comparative study on dye decolorization by leaf and root extracts has been carried out along with phytotoxicity test to analyze the effect of dyes and their degradation metabolites on seed germination and seedling growth of chickpea (*Cicer arietinum* L).

Materials and Methods

Chemicals

Congo red was purchased from Ranbaxy Fine Chemicals Limited, India. Malachite green and sodium hypochlorite were purchased from Thermo Fisher Scientific Pvt. Ltd. India. Sterile deionized water was used throughout the experiments.

Plant Material Collection

P. hysterophorus was collected from Pepsicola, Kathmandu. The collected plant material was authenticated by National Herbarium and Plant Laboratories, Lalitpur, Nepal.

Extracts Preparation

Fresh leaves and roots of *P. hysterophorus* were surfaces washed with running tap water to remove debris followed by deionized water and then air dried for sometimes. 5 gm of each leaf and root (finely crushed) were kept in two separate conical flasks each containing 100 ml deionized water and then kept at room temperature for 24 hours. The stirring of the mixture was done at different time intervals. Finally, the extracts were filtered using muslin cloth followed by Whatman no. 1 filter papers and then stored at 4ºC until further use.

Decolorization Assay

The stock solution of 1 mg/ml of each dye (congo red and malachite green) was prepared. From the stock solution, the different concentrations (50, 100, 200, 400 & 800 µg/ml) of each dye were prepared by diluting with deionized water. The extracts and the dyes were mixed in the ratio of 1:2 (1 ml of extract was added to 2 ml of dye). The mixtures were stirred thoroughly and incubated at 40ºC for 90 minutes. After incubation, the absorbance of the reaction mixtures was measured spectrophotometrically at a maximum wavelength of each dye (λ_{max} for congo red; 497 nm and for malachite green; 615 nm). The percentage decolorization was calculated by using the following equation:

$$\text{Decolorization (\%)} = 100(A_1 - A_o)/A_1$$

Where A_1 is the initial absorbance of the dye solution and A_o is the absorbance after incubation at a specific time. All determinations were carried out in triplicate.

Phytotoxicity Test

The phototoxicity test was performed by petri dish method. This test was carried out with 50 mg/L concentration of each dye and its degradation metabolites. The sterile petri dishes

containing a double layer of Whatman no. 3 filter papers were prepared. Three dishes were prepared: dish soaked with water (control), dish soaked with dye solution and dish soaked with degradation metabolites. The healthy seeds of chickpea (*Cicer arietinum* L.) were selected and surface sterilized with 1.2 % sodium hypochlorite solution and then washed thoroughly with deionized water. The sterilized seeds were soaked in respective solutions for 1 hour and then transferred to soaked petri dishes (5 seeds on each petri dish). The petri dishes were placed in the dark for germination. After 3 days, the germination percentage was recorded by observing the length of radicles. Seeds with radicle greater than 1mm were considered as germinated (Wu *et al.*, 2007). The germinated seeds were then transferred to petri dishes containing loam soil at normal room condition and irrigated daily with 10 ml of each dye and its degradation metabolites. The shoot and root length measurements were recorded after 10 days. All determinations were performed in triplicate.

Results and Discussion

Decolorization Assay

The decolorization of dyes was carried out at different concentrations of each dye (50, 100, 200, 400 & 800 µg/ml) using UV visible spectrophotometer by determining the change in absorbance. Results obtained showed the decrease in percentage decolorization with an increase in dye concentration. The maximum decolorization for leaves extract was obtained at 50 µg/ml with 55.8% (congo red) and 51.6% (malachite green) whereas minimum at 800 µg/ml with 25.3% (congo red) and 16.8% (malachite green). Similarly, the roots extract exhibited higher decolorization at 50 µg/ml with 48.5% (congo red) and 45.2% (malachite green) whereas minimum at 800 µg/ml with 17.5% (congo red) and 13.9% (malachite green). The percentage decolorization was observed more in congo red than malachite green for both leaf and root extracts. Furthermore, the leaves extract demonstrated the higher decolorizing activity for both congo red and malachite green than roots extract. The difference in decolorizing activity of leaf and root extracts towards congo red and malachite green was observed. This is possible due to various factors determining the rate of decolorization since the rate of decolorization depends on contact time, incubation temperature and p^H of the reaction medium as well as on the molecular weight, the presence of functional groups and chemical structure of the dye. The decrease in absorbance after incubation along with decolorization of the reaction mixture was observed indicating the biodegradation of dyes into non-colored metabolites (Ogugbue and Sawidis, 2011). Moreover, the biodegradation of dyes can be attributed to different organic compounds present in the extract having the potential to reduce dyes.

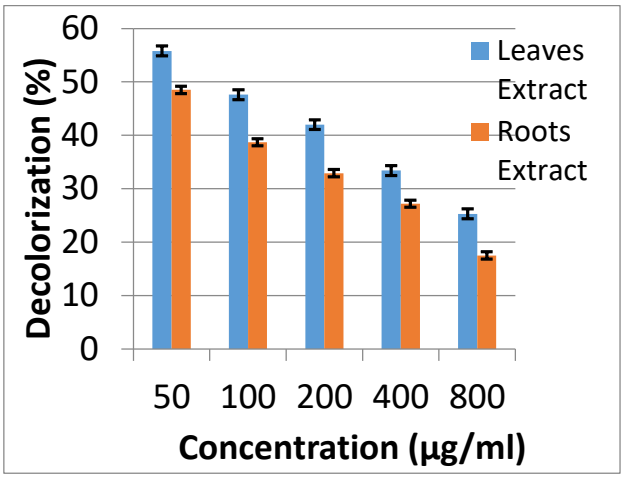

Fig. 1: Decolorization of congo red by leaf and root extracts of *P. hysterophorus*. Data represent mean ± S.D. of triplicate determinations**.**

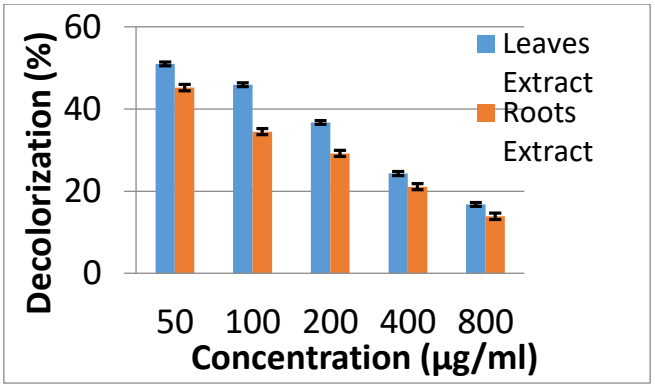

Fig. 2: Decolorization of malachite green by leaf and root extracts of *P. hysterophorus*. Data represent mean ± S.D. of triplicate determinations.

Phytotoxicity Test

The phytotoxicity study was carried out to determine the toxicity of dyes and their degradation metabolites on seed germination and seedling growth of chickpea. The germination percentage, shoot length and root length of the seedlings irrigated with dyes solution and their degradation metabolites were recorded and compared with that of control (Table 1). The germination percentage of seeds was less for raw dye as compared to extract treated dye. The lengths of shoot, root and seedling growth were more in degradation metabolites when compared to raw dyes, indicating less toxic nature of degradation metabolites than dyes. Among the dyes, untreated malachite green exhibited more toxic effect than untreated congo red on seed germination and growth of seedling, whereas treated congo red showed the least toxic effect. Furthermore, the germination rate and seedling growth for treated dyes were comparable with control, whereas significantly different for untreated dyes. Results obtained suggest that disposal of dye wastewater on land may have a negative impact on soil fertility and growth of plants. Thus, it seems to be necessary to treat dyes before their disposal.

Table 1: Toxic effect of treated and untreated dyes on seed germination and seedling growth of *Cicer arietinum.*

Test Parameter	Control	Congo red		Malachite green	
		Untreated	Treated	Untreated	Treated
Germination (%)	100	60	87	53	80
Shoot length (cm)	17.7 ± 1.2	7.9 ± 1.1	15.5 ± 0.8	6.1 ± 1.2	13.6 ± 0.7
Root length (cm)	9.5 ± 0.9	3.2 ± 1.3	6.3 ± 1.0	3.8 ± 0.9	4.5 ± 1.1

Data represent mean ± S.D. of triplicate determinations.

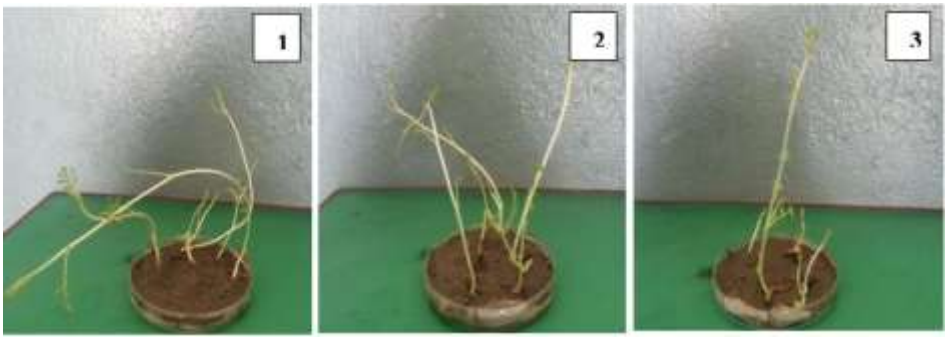

Fig. 3: The seedling growth of *C. arietinum*: Control (1), treated dye (2) and untreated dye (3).

Conclusion

Various physicochemical methods have been employed for the treatment of industrial wastes containing different dyes and dye-stuff, however, these methods are very costly and end up producing recalcitrant wastes. Thus, the cost-effective and eco-friendly methods seem to be an urgent need for today's world. In this study, the degradation of the two most commonly used dyes: congo red and malachite green by using leaf and root extracts of *P. hysterophorus* was demonstrated. The UV visible spectrophotometer indicated that decolorization was due to biodegradation of dyes into non-colored metabolites. The maximum decolorization was obtained in leaves extract for both congo red and malachite green. Besides, the phytotoxicity study was carried out to determine the toxicity of dyes and their degradation metabolites on seed germination and seedling growth of *C. arietinum*. The germination percentage and seedling growth were more in degradation metabolites than untreated dyes, indicating less toxic nature of degradation metabolites. Results obtained suggest that *P. hysterophorus* extracts can be used to treat dye wastewater and treated wastewater can be exploited for agricultural purposes. However, detailed research is required on various issues like the mechanism of degradation, the toxicity of dye at the cellular and molecular level etc.

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