

Phytoremediation of Methylene blue dye (triarylmethane) and Congo red (azo) by *Trachyspermum ammi*: Kinetic studies

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Title: Phytoremediation of Methylene blue dye (triarylmethane) and Congo red (azo) by *Trachyspermum ammi*: Kinetic studies

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Abstract

Phytoremediation developed as a green-clean, solar-driven and innovative technique for the eradication of contaminants from the environment from last two decades. In the present research work, phytoremediation potential of an ornamental plant *Trachyspermum ammi* L. (*T. ammi*) was examined for the removal of a triarylmethane-Methylene blue (MB) and di-azo-Congo red (CR) dye. The various parameters such as initial dye concentration, contact time, and pH were focused to explore the dye removal capability of *T. ammi*. in wastewater. The increase in initial dye concentration affects the decolorization potential of the *T. ammi*. The decolorization potential of *T. ammi* found to be reduced with enhancing the initial dye concentration of both the dyes. The plant achieved condition of equilibrium after the 40 h of contact time with both MB and CR dyes. The kinetics of phytoremediation reaction with both MB and CR dyes were studied with the help of pseudo-first order, pseudo-second order, and Elovich models and the results were well appropriated to pseudo-first order with the correlation value $R_2 \geq 0.99$ for MB and $R_2 \geq 0.97$ for CR. The Langmuir and Freundlich isotherms studies of phytoremediation reaction found in favor of Freundlich equilibrium isotherm with correlation $R_2 \geq 0.99$ for both MB and CR dyes. The finding of analytical studies disclosed that the phytoremediation mechanisms of MB and CR by *T. ammi* firstly, involves phytoadsorption through electrostatic interaction and hydrogen bonding and then degradation of dyes occur through desulfurization and denitrification.

Key words: Methylene Blue Dye, Congo Red dye, Phytoremediation, Degradation, Kinetics

54 **Statement and declarations**

55 **Competing interests**

56 The authors declare that there are no relevant financial and non-financial interests.

57 **Authors' contributions**

58 Navjeet Kaur: Experimentation, data collection and writing original draft

59 Jyotsna Kaushal: Conceptualisation, data curation and final editing of manuscript.

60 Pooja Mahajan: The data interpretation and editing of manuscript

61 All authors read and approved the final manuscript.

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64 **Data Availability**

65 The datasets used for present study are presented in manuscript.

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1. Introduction

Synthetic dyes used in textile industry are mostly allergic, carcinogenic, and mutagenic in nature. Furthermore, if these dyes are discharged in wastewater as such in the water bodies, it resulted into a fatal threat to aquatic life such as fishes, microbes, and other creatures of water bodies (Konicki et al. 2013, Aljeboree et al. 2017). The dye contaminated water is also unsuitable for human consumption. Subsequently, their complete expulsion from textile wastewater is a matter of great concern for research community. Various physio-chemical techniques such as adsorption, photocatalysis, advanced oxidation, precipitation, coagulation, solvent extraction, membrane removal etc. were proposed in the literature to remove dyes from textile wastewater (Ali et al. 2019, Kang et al. 2019). But, these types of techniques have less productivity, costly and produce intermediate contaminants. Because to these issues, there are lot of significant methods to develop eco-friendly, cost effective and resourceful method for treatment of textile dyes wastewater (Ullah et al. 2020). Some researchers also proposed the use of enzymes and microbes such as bacteria and fungi as an eco-friendly approach but in actual practice, microbial administration appears to be quite difficult (Sarkar et al. 2017, Arfin et al. 2019). In last two decades, another clean-green innovative plant-based technique known as phytoremediation comes into view as a solar driven and cost-effective method for the removal of the contaminants from wastewater in contrast with physio-chemical and biological methods (enzymatic, microbial) etc (Kabra et al. 2013, Khandare and Govindwar 2015).

Phytoremediation technology is the best suitable, accepted as natural and sustainable development strategies to change over contaminated areas into the fruitful one with use of different varieties of plants. In literature, aquatic plant species such as *Azolla pinnata* (Al-Baldawi et al. 2018), *Chara vulgaris* (Mahajan et al. 2019, Mahajan and Kaushal 2020), *Lemna minor* (Imron et al. 2019), *Eichhornia crassipes* (Tan et al. 2016, Sharma et al. 2021), *Pistia stratiotes* (Mahajan and Kaushal 2019, Ekanayake et al. 2021), *Salvinia molesta* (Kaushal and Mahajan, 2015, Al-Baldawi et al. 2020), *Typha angustifolia* (Chandanshive et al. 2017) are more focussed for the phytoremediation of dye wastewater due to their easy administration in aqueous form. Many terrestrial plants also proved their potential for dye removal due to the effective enzymatic system in their roots. As illustration, the ornamental flowering plant *Blumea malcolmii* was reported to degrade the Direct Red 5B with involvement of number of enzymes present in its roots (Kagalkar et al. 2009). *Aster amellus*, *Portulaca grandiflora*, *Glandularia pulchella*, *Petunia grandiflora* and *Nopaleaco chenillifera* were some other terrestrial garden ornamental plant species reported for the degradation of Remazol Red, Navy Blue HE2R, Green HE4B, Brilliant Blue Green and Red HE7B respectively (Kabra et al. 2011, Khandare et al. 2011a, b, Kabra et al. 2011, Adhi et al. 2012). Recently, some other commonly grown ornamental plants such as *Asparagus officinalis*, *Alcea rosea*, and *Bacopa monnieri* plant species reported for remediation of hazardous azo dyes (Watharkar et al. 2018, Mahmoudabadi et al. 2019, Shanmugam et al. 2020).

In the present study, *Trachyspermum ammi* ornamental plant species of Apiaceae family explored for the removal of dyes. Previously, *T. ammi* was reported for the removal of heavy metals from the soil (Javed et al. 2020). Revathi et

al. 2018 utilised the biomass of leaves of *T. ammi* as an adsorbent for removal of Grey BL dye from aqueous solution. As per our knowledge, no work is reported in literature for removal of dyes from aqueous solution by *T. ammi* plant in the living state. Hence, the present study was planned to examine the phytoremediation ability of *T. ammi* plant for removal of two structurally different dyes, a diazo dye Congo Red (CR) and a triaryl methane dye Methylene Blue (MB) in aqueous form. To optimise the phytoremediation process, the effect of contact time, initial dye concentration and pH were studied. The mechanism of dye removal by plant in living conditions was analysed through study of kinetic and equilibrium isotherms. FTIR, SEM, HPLC and GC-MS studies were also executed to support the dye removal mechanism of *T. ammi* plant.

2. Material and methods

2.1 Chemicals

The two structurally different dyes, a triarylmethane dye, Methylene Blue (MB) (chemical formula $C_{16}H_{18}N_3SCl$, MW. 319.85 g/mol.) and a diazo dye Congo Red (CR) (chemical formula $C_{32}H_{22}N_6Na_2O_6S_2$, MW. 696 g/mol.) were used for present research studies (Fig. 1). The solid form of MB and CR dyes used in experimental work was in a high analytical grade and acquired from Merck Limited, Mumbai. Analytical grade Hi-media Hoagland No.2 Basal Salt Mixture was used to prepare Hoagland solution for propagation of plant. All other chemicals such Ethyl acetate, Ethanol, Methanol, Sodium sulphate, KBr etc. used for analytical procedure were of high purity HPLC grade and procured from Hi-media Lab.

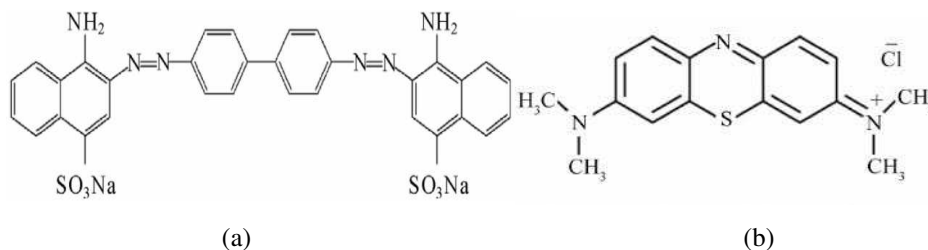


Fig. 1 Chemical Structure of (a) Congo red (CR) (b) Methylene blue (MB)

2.2 Plant collection and cultivation

T. ammi plant saplings grown in soil culture medium (soil and coconut husk in ratio of 1:2) of same size and grown plants were collected from the local nursery of Amritsar, Punjab (India). Initially, plants were gently washed under the tap water thoroughly to remove soil culture medium for 10-15 minutes and after that kept in distilled water for 24 h so that plants become free from any soil particles. Before experimentation, plants were propagated in glass jars each containing 100 mL Hoagland nutrient solution for 15 days under normal room temperature conditions ($27 \pm 30^\circ C$ with 30% humidity) (Leggett and Frere 1971). After every 3-days, Hoagland solution was replaced with fresh solution for extensive growth of plants. After 15 days, plants were transferred into distilled water (100 mL) in the beakers with support system before 1-day for experimentation.

2.3 Phytoremediation experiments

Phytoremediation experiments were performed to study the effect of initial concentration, contact time and pH on decolorization potential of *T. ammi* plant with both the dyes MB and CR. All the experiments were carried out in triplicates to minimize percentage error and abiotic controls were maintained without addition of plants. For examine the effect of initial dye concentration (10, 20, 30, 40 and 50 mg L⁻¹) and contact time (8, 16, 24, 32 and 40 h), plants with same growth (a length of approx. 9-11 cm, width of 3–5 mm and 6-7 g wet weight) were used for experimentation purpose and each plant was transferred in 250 mL beaker containing 100 mL volume of dye solution. The samples of dye solution up to 2 mL were taken every 8 h up to 40 h for measuring absorbance with double beam UV-Visible spectrophotometer. These samples were centrifuged at rpm 5000 to remove unwanted particles. The calibration curve was determined using the maximum absorbance at λ max 665 nm for MB dye and 495 nm for CR dye absorbance and then decolorization percentage was calculated as given in equation (1) (Kabra et al. 2011).

$$\text{Decolorization (\%)} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100 \quad (1)$$

The quantity of dye removed by the *T. ammi* plant was determined with help of equations (2) and (3) respectively (Mahajan and Kaushal 2020):

$$q_t = \frac{(C_0 - C_t)}{w} \times v \quad (2)$$

$$q_e = \frac{(C_0 - C_e)}{w} \times v \quad (3)$$

Where ‘ q_t ’ (mg g⁻¹) and ‘ q_e ’ (mg g⁻¹) are the amount of dye removed at time t (minute) and equilibrium respectively. ‘ C_0 ’ is the initial dye concentration, ‘ C_t ’ is the dye concentration any time ‘ t ’ and ‘ C_e ’ is the equilibrium dye concentration in mg L⁻¹, ‘ v ’ is the volume of the solution in mL and ‘ w ’ is the weight of the plant in g. T

The fresh wet weight of the plant is taken after drying several times in folds of filter paper. To study the effect of pH on decolorization potential of *T. ammi*, on MB and CR dye remediation was evaluated by performing experiment in pH range 3 to 10 with 100 mL of 20 mg L⁻¹ both dye solutions. The pH values were determined with the help of digital pH-meter (pH 211 Hanna instrument).

2.4 Plant’s relative growth rate (PRGR) studies

To study toxicity impact of dyes on *T. ammi* plants, plant’s relative growth rate (PRGR) was calculated with equation (4) (Imron et al. 2021). PRGR value describes the impact of dye toxicity on plant growth after exposure to dye solution.

$$\text{PRGR (per hour)} = \left(\frac{\ln(W_t) - \ln(W_0)}{t} \right) \quad (4)$$

Where ‘ W_0 ’ describes the wet weight of plant taken before treatment and ‘ W_t ’ was weight of plant taken after exposure to dye solution for ‘ t ’ days. For phytotoxicity of present study, *T. ammi* exposed to different concentrations (10, 20, 30, 40 and 50 mg L⁻¹) of both the dyes MB and CR. Biotic control was also maintained with distilled water for time of 40 h. To check photo transpiration, volume of water was maintained with distilled water. After period of 40 h,

plants were shifted to soil culture medium and treated dye water were used to provide watering to plants for next 120 h. Then, PRGR was observed and compared with control plants.

2.5 Kinetics and Equilibrium studies

Several scientists have proposed various kinetic models to describe the mechanism of solute adsorption. The rate and mechanism of adsorption depends upon various factors like physical and chemical properties of adsorbent as well as mass transfer process. The results of these kinetic models are significant for the design and optimization of the wastewater treatment process caused by harmful dyes in bulk at industrial level.

The kinetics of *T. ammi* for the removal of the MB and CR dyes was interpreted by using the pseudo-first order, pseudo-second order and Elovich models as demonstrated by equations 5, 6 and 7 respectively (Kaushal and Mahajan, 2022):

$$\log (q_e - q_t) = \log q_e - K_1 t \quad (5)$$

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{1}{q_e} t \quad (6)$$

$$q_t = \frac{1}{\beta} \ln (\alpha \beta) + \frac{1}{\beta} \ln t \quad (7)$$

where ‘K₁’ and ‘K₂’ are the rate constants for pseudo-first order and pseudo-second order, respectively. In equation (7) ‘α’ determine the chemisorption, and ‘β’ find out that how much adsorption takes place at root surface of plant. The application and importance of suitability of the kinetic models to experiment data are predicted with the regression value (R²) and standard deviation of normalized value (Δ q_t%). The normalized standard deviation value is computed with following equation (Mahajan and Kaushal 2020):

$$\Delta q_t \% = 100 \sqrt{\frac{\sum \left| \frac{q_t(\text{exp}) - q_t(\text{theor})}{q_t(\text{exp})} \right|^2}{n-1}} \quad (8)$$

where q_t (exp) and q_t (theory) are the practical and theoretical values of the amount of dye remediated from aqueous solution where “n” corresponds to the numeral count of experiment. The rationality of kinetics models is based upon the value of Δq_t%. The lower is its value for the kinetic model, higher is the acceptability of the data for that model.

The equilibrium studies of phytoremediation reactions were determined with the help of Langmuir and Freundlich models represented by equations (9) and (10) respectively (Wang et al. 2018 a).

$$\frac{C_e}{q_e} = \frac{1}{b Q_0} + \frac{1}{Q_0} C_e \quad (9)$$

$$\log q_e = \log k_f + \frac{1}{n} \log C_e \quad (10)$$

The Langmuir adsorption constants 'b' and 'Q₀' were related to the high ability of adsorption of the dye at equilibrium and rate of adsorption correspondingly. The value 'n' finds the spontaneity in behaviour of dye molecules toward phytoremediation process in case of Freundlich adsorption isotherm, and 'K_f' is the adsorption capacity for the plant. The valuable Langmuir isotherm parameter 'R_L' is suggested for equilibrium parameter and determined by following equation (Mahajan and Kaushal 2020):

$$R_L = \frac{1}{b + C_0} \quad (11)$$

When there is irreversible process, the 'R_L' value corresponds to the zero, and it is for favourable conditions. The value becomes more than one for the unfavourable process (Kaur et al. 2015).

2.6 Analytical Procedures

Decolorization of both the MB and CR dye solutions were observed using the centrifuged supernatant of the dye solutions with the help of UV–Vis spectroscopic analysis (Shimadzu-2550 UV-visible double beam spectrophotometer). The mechanism of dye adsorption through roots cells were recognized with help of Scanning electron microscopy (SEM) and degradation of the dyes was confirmed by comparing results of Fourier transform infrared spectroscopy (FTIR) and the results of High-performance liquid chromatography (HPLC). Gas chromatography–mass spectroscopy (GC-MS) was carried out for identification of dye degradation products.

The FTIR of dried sample of *T. ammi* roots was examined before and after phytoremediation of both MB and CR dyes. *T. ammi* roots with and without dyes adsorption were dried in oven for 24 h at 45 °C. After that, the dried *T. ammi* was ground with KBr in the ratio of 5:95 to a fine powder. The infrared spectra were taken with Agilent (CARRY–630) spectrophotometer with range of 400– 4000 cm⁻¹. The surface analysis of the controlled and treated dried powder form of *T. ammi* roots was examined with the help of scanning electron microscope. The standard screens were used to sieve the crushed powder and only small fractions which was lower than 62 µm were used for adsorption purposes and the *T. ammi* particles were placed on stubs and coated with gold foil by the gold- coating machine- JEOL-JSM-6100 Ltd, Japan (Titchou et al. 2020). Biodegradation of both dyes MB and CR was predicted by means of HPLC. Extraction of dyes/metabolites was carried out using equal volume of ethyl acetate and the extract was evaporated and dried. The solid residue obtained was dissolved in 1 mL HPLC grade methanol and the sample was used for analytical studies. HPLC analysis was carried out on a C18 column (4.5 × 250 mm²) by using methanol at 1 mL min⁻¹ (Khandare et al. 2014). GC–MS technique analysis was carried out to predict the MB and CR dyes from synthetic wastewater. This analysis is used to evaluate the pathways for the phytoremediation mechanism of MB and CR dyes by *T. ammi*. The 50 mL of samples of dyes were extracted twice by using separating funnels (Pyrex, Germany) with 30 mL of ethyl acetate (Merck, Germany). After the extraction process, the solution form two layers, both dyes (MB and CR) were extracted from upper layer and the bottom layer containing the water was discarded. After that, the extracted dyes were added with Na₂SO₄ anhydrous (Merck, Germany) to bind water residue. Later, the extracted dyes were put into the evaporator with 45°C temperature to evaporate the ethyl acetate residue. At the end, the extracted MB and CR dyes were analyzed

using GC–MS instrument (Agilent 5975C, USA). A non-polar DB-5MS UI column (30 m × 250 μm × 0.25 μm) was used to separate organic compounds and the carrier gas is Helium. The volume of injected sample into GC-MS was 1 μL . The temperature for the injector and detector was kept at 250 $^{\circ}\text{C}$. The initial temperature of the oven was 70 $^{\circ}\text{C}$ for 2 min and ramped up to 200 $^{\circ}\text{C}$ with 10 $^{\circ}\text{C min}^{-1}$ and after that 20 $^{\circ}\text{C min}^{-1}$ to 280 $^{\circ}\text{C}$ for 10 min (Imron et al. 2021).

3. Result and discussion

3.1 Effect of contact time, initial dye concentration and pH

The results of effect of contact time and initial dye concentration of MB and CR dye in terms of decolorization potential was depicted in Fig 2 a and b respectively and amount of MB and CR dye adsorbed by plant as a function time is shown in Fig. 3 a and b respectively. The amount of dye removed by the plant was determined up to 40 h because maximum decolorization takes place up to 40 h and further no significant results has been observed.

It has been clearly depicted from Fig. 2 and 3 that with an increase in the initial concentration of both the dyes, the decolorization potential of *T. ammi* got declined (Fig. 2) whereas the amount of dye absorbed increased (Fig. 3). Maximum MB decolorization was observed in 10 mg L $^{-1}$ after that, it reduces in 20, 30, 40 and 50 mg L $^{-1}$ (Fig. 2 a). Fig 2 (b) predicts the decolorization of CR with time. After 24 h the concentration 10 mg L $^{-1}$ and 20 mg L $^{-1}$ follow same pattern of decolorization. Afterwards the percentage decolorization of 10 mg L $^{-1}$ becomes high than 20, 30, 40 and 50 mg L $^{-1}$. The maximum 99% dye decolorization percentage was observed is with 10 mg L $^{-1}$ MB dye solution.

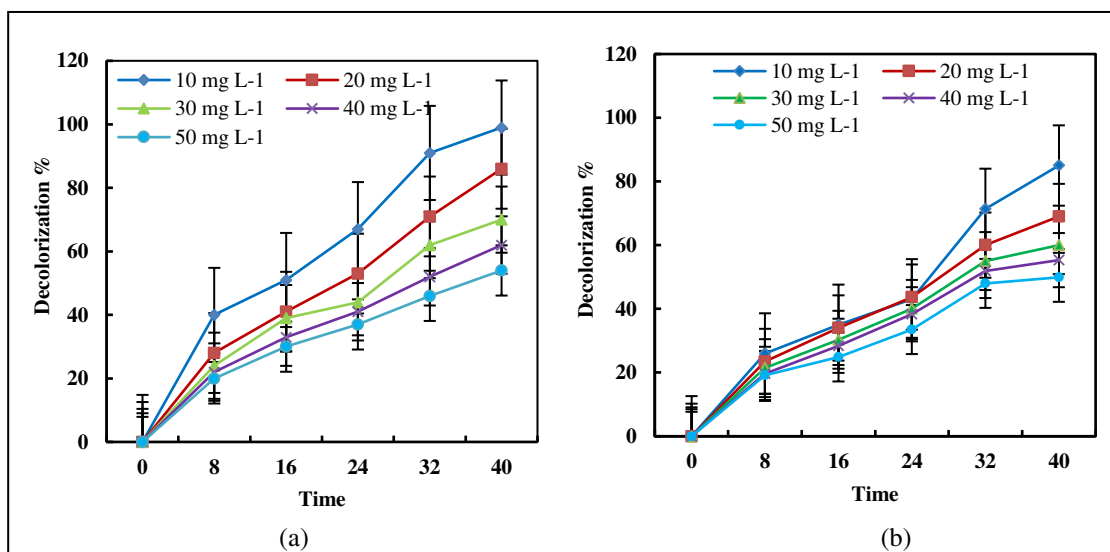


Fig. 2 Decolorization variation of (a) Methylene blue and (b) Congo Red dye by *T. ammi* with time

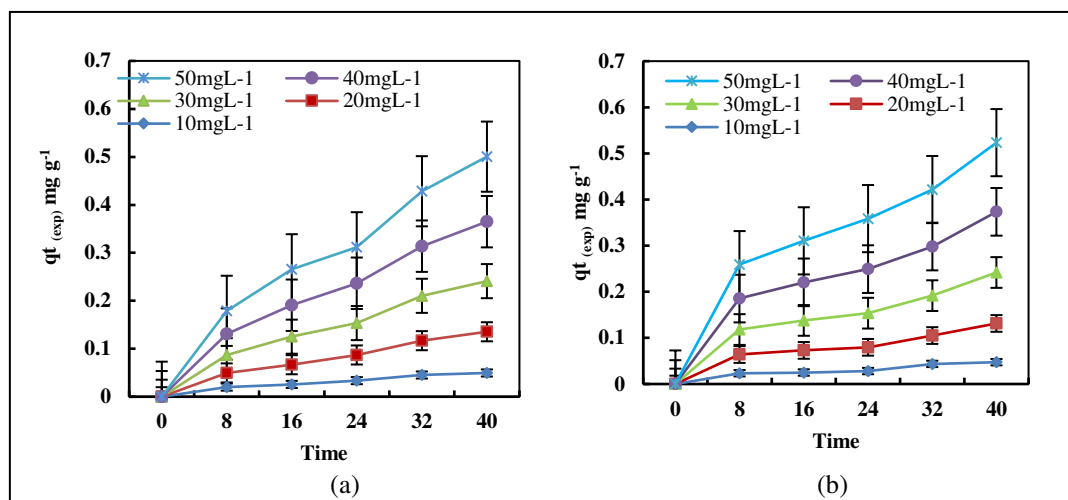


Fig. 3. Adsorption capacity of *T. ammi* (a) Methylene blue (b) Congo red dye as a function of time

Similar work was reported for removal of MB dye by using *L. minor* plant (Imron et al. 2019) and *Chara vulgaris* for the decolorization of the CR dye (Mahajan and Kaushal 2020). The maximum amount of MB and CR dye found to be adsorbed is 2.345 mg g⁻¹ and 2.345 mg g⁻¹ in phytoremediation experiment with 50 mg L⁻¹ concentration. Initially the rate of adsorption may be high due to proximity of the dye molecules with roots of *T. ammi* plant. However, it has been observed that the phytoremediation capacity of *T. ammi* to decolorise MB and CR dyes solution decreases with further increase in concentration.

This may be due to because of high concentration of dye molecules in solution immediately blocks the pores on the root surface and rate of decolorization decreases. Similar results were reported in research study of *Glandularia pulchella* for remediation of Green HE4B (sulphonated azo dye). The reduction in decolorization capacity might be toxic on plant system due to the influence of enhancing concentration which hinders the metabolism of plant, and thus, reduces the metabolic activity of the plant system (Kabra et al. 2011). The fast adsorption at initial phase of experiment is caused by the high amount of dye particles in the solution and large number of unoccupied sites available on plant which behaves as driving force for remediation process, while on last phase the driving force reduces. The decrease in the driving force could be due to saturation of the active sites available on adsorbent surface as a plant root surface.

The pH values of dyes solution have great effect on phytoremediation process for removing the dyes in their aqueous phase. In the current study, pH effect on MB and CR dyes remediation by *T. ammi* was projected by performing experiment in the pH range 3 to 10 along with 20 mg L⁻¹ MB and CR dyes solutions. It was examined that both at high and low pH, decolorization ability of plant get effected. The maximum decolorization 95.7% is obtained at pH 8 in case of MB dye by using *T. ammi* while with CR dye, maximum decolorization 97.3% was observed at value of pH 5 (Fig. 4).

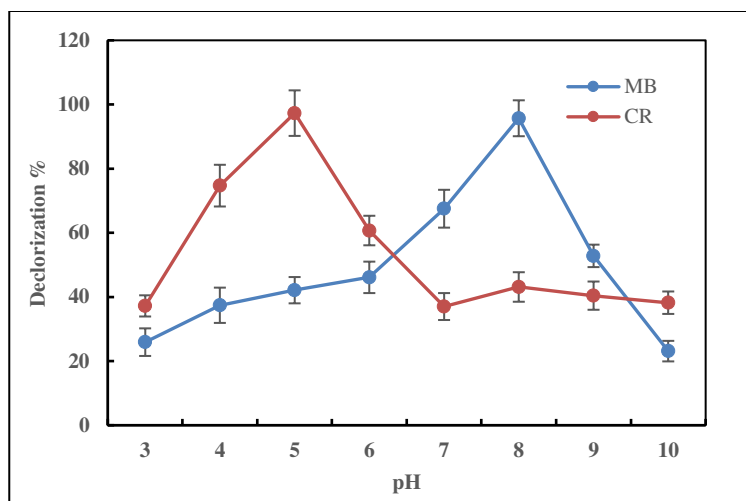


Fig. 4 Effect of pH on decolorization pattern of *T. ammi* for MB and CR

The pH of dye solution might be having large impact on the functional groups of dyes and the plant surface. The pH value above 8, there was a sharp reduction in the decolorization of MB and decrease in decolorization was observed at above pH 5 in CR. Earlier also the similar effect of pH was documented in studies to remediate the acidic dyes in their aqueous state (Khataee et al. 2012). Therefore, the optimum value of pH for removal of MB and CR by *T. ammi* is at pH value 8 and 5 respectively.

3.2 PRGR Studies

The relative growth rate of *T. ammi* was observed after 40 h of exposure time and shifted the plants in soil culture medium and there kept for 120 h. PRGR results are depicted in Table 1. The PRGR value of *T. ammi* is found to be minimum in 50 and 40 mgL⁻¹ CR dye water is 0.008 and 0.006 respectively after 40 h of direct exposure of dye solution. Thus, it has been concluded that high concentration of both the dyes MB and CR had a negative effect on PRGR in comparison to compared with the control after 40 h of exposure. Similar observations have been noticed on exposure of *L. minor* with MB different dye concentration (Reema et al. 2011). From these results, it has been concluded that *T. ammi* plant has potency to tolerate the toxicity of MB and CR dyes in low concentration.

In present research studies, phytotoxicity of treated water was also checked with PRGR. For this purpose, the treated plants were shifted to soil culture medium after direct exposure of 40 h and watered with treated dye water for next 120 h. It has been observed that PRGR value of control plant (0.15/h) was found to aligned with plants watered with treated 10, 20 and 30 mgL⁻¹ CR and MB dye solution (Table 1). These results clearly indicate that *T. ammi* plant showed good adaptability toward both the dyes MB and CR dye solution and even after treatment plants can rejuvenate themselves with treated wastewater.

Table 1. Plant Relative growth rate of *T.ammi* in response to MB and CR dye

Concentration of Dye solution	PRGR/h (%) after treatment with MB		PRGR/h (%) after treatment with CR	
	40 h	120 h	40 h	120 h
10 mg L ⁻¹	0.04 ±0.0003	0.20 ±0.0002	0.08 ±0.0001	0.25 ±0.0003
20 mg L ⁻¹	0.09±0.0001	0.10 ±0.0003	0.002 ±0.0003	0.37 ±0.0004
30 mg L ⁻¹	0.001±0.0002	0.13 ±0.0003	0.003 ±0.0001	0.14±0.0045
40 mg L ⁻¹	0.004±0.0002	0.01±0.0005	0.006 ±0.0002	0.03±0.0003
50 mg L ⁻¹	0.005 ± 0.0001	0.03 ±0.0002	0.008 ±0.0001	0.08 ±0.0001

*Values are average of three experiments ± SE

3.3 Kinetic and equilibrium studies

The kinetic studies of phytoremediation for different dyes in aqueous phase in living forms were suggested by classical kinetic models and limiting methods to understand the mechanism of transfer of mass during phytoadsorption (Mahajan and Kaushal 2019, 2020, Kaushal and Mahajan 2021, Imron et al. 2021). Therefore, the phytoremediation kinetics study of MB and CR dyes with the help of *T. ammi* was studied with pseudo-first order, pseudo-second order, and Elovich models at room temperature. The kinetic graphs of various models at different concentrations of MB and CR dyes are shown in Fig. 5-7. The constants of kinetics and other used parameters determined from kinetic plots for MB and CR dye are presented in Table 2 and 3 respectively.

Fig 5a-b depicts the pseudo-first order straight-line graph with negative value of slope when $\log (q_e - q_t)$ against time (t) had plotted at various concentrations for *T. ammi*. Fig. 6a- b depicted a linearized plot with the positive slop when t/q_t plotted versus “t” for pseudo-second order kinetic model and k_2 and $q_{e(\text{theor})}$ values are predicted with the help of intercept and slope respectively for different concentration for both the dyes. The Elovich constants α and β has been determined by the slope and intercept, of the plot between q_t vs $\ln t$ depicted in Fig. 7a- b. From Table 2 and 3, it has been clearly depicted that R^2_1 values lies either above 0.90 or near to it for most of concentrations of both the dyes in case of pseudo-first order in comparison to pseudo-second order and Elovich model. Moreover, $q_{e(\text{theor})}$ values are for *T. ammi* are found to be more consistent to $q_{e(\text{exp})}$ for pseudo-first order in case of both of dyes MB and CR while for pseudo-second order and Elovich model $q_{e(\text{theor})}$ values are far away from $q_{e(\text{exp})}$. Also, the values of Δq_{t1} (%) in comparison to Δq_{t2} (%) and Δq_{t3} (%) are lower which indicate toward the acceptability of the data for pseudo-first order for both the dyes. Hence, the value of experimental data of the phytoremediation of MB and CR dyes by *T. ammi* showed better compliance with pseudo-first order. It has been also observed that when there is increase in the initial dye concentration then the rate constant (k_1 and k_2) value decreases gradually for MB and CR dye removal by *T. ammi* (Table 2 and 3). There is probability of competition in dye molecules to acquire available adsorbent sites on surface of plant. Hence, there is decrease in electrostatic interaction on increasing the initial dye concentration and the reduction in the dye interaction towards the plant surface. Therefore, chemisorption mechanism may restrict by the rate of the adsorption of MB and CR dyes by *T. ammi* (Sánchez-Galván et al. 2008).

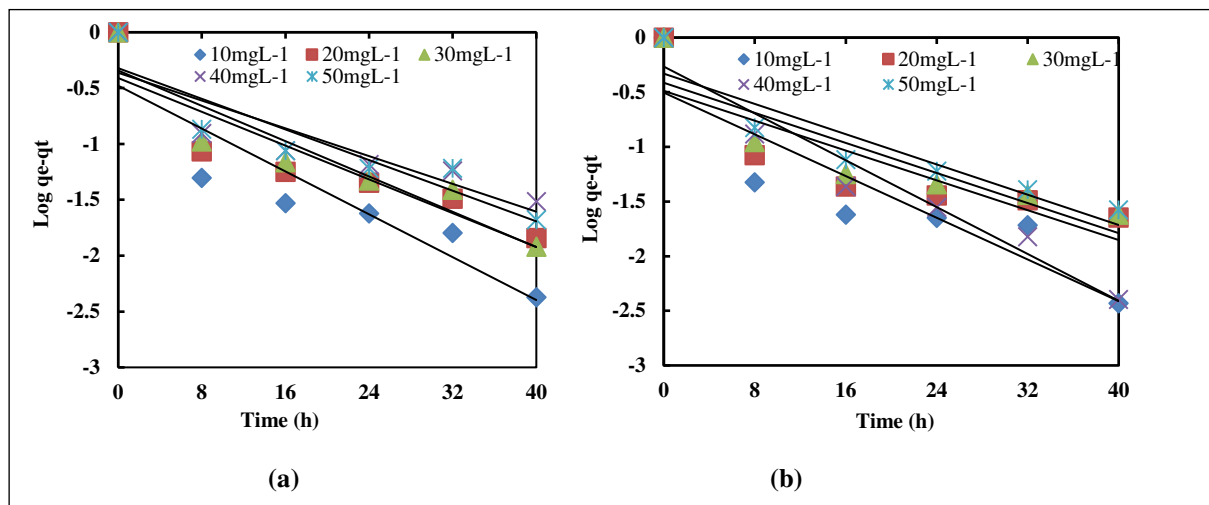


Fig. 5 Pseudo first order kinetic model for (a) Methylene blue (b) Congo red dye
phytoremediation by *T. ammi*

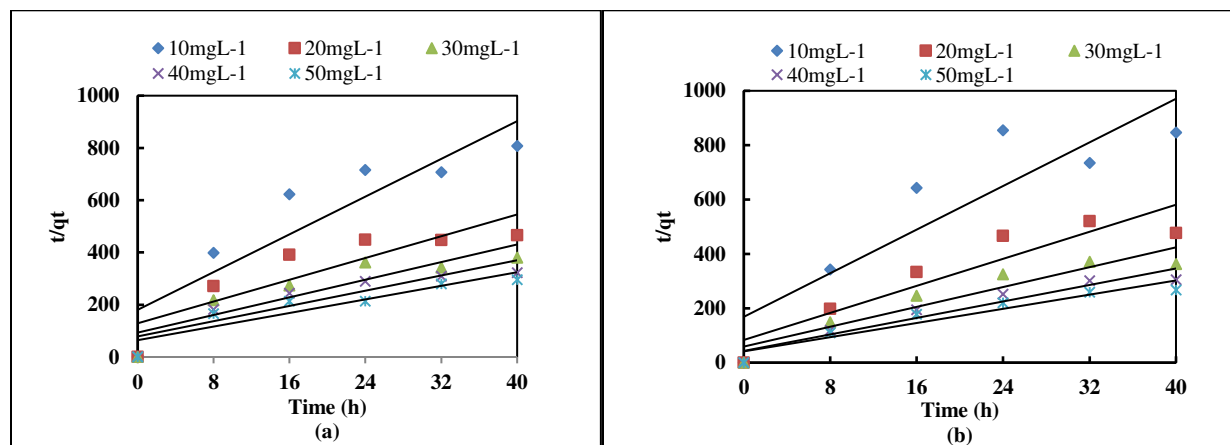


Fig. 6 Pseudo second order kinetic model for (a) Methylene blue (b) Congo red dye
phytoremediation by *T. ammi*

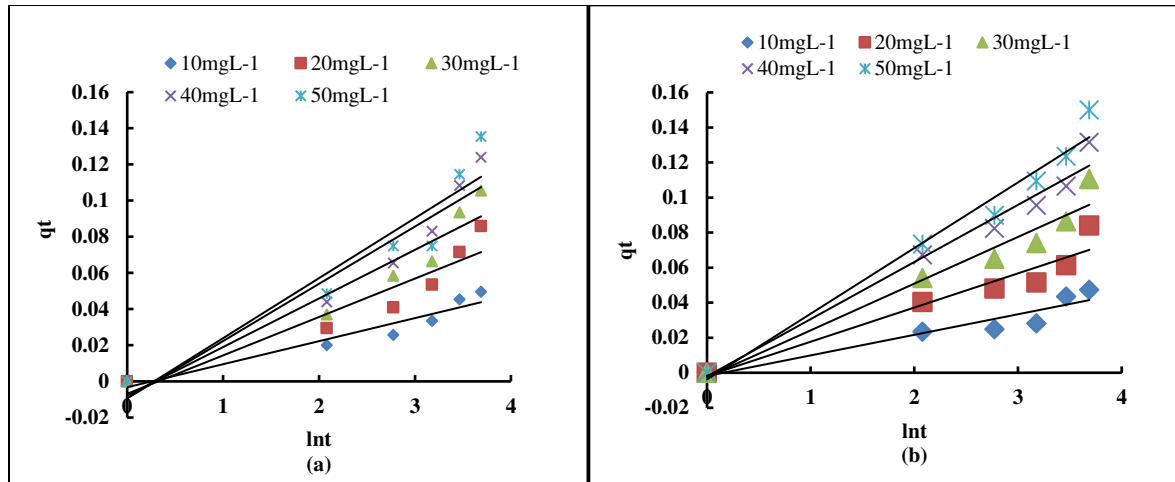


Fig.7 Elovinch equation kinetic model for (a) Methylene blue (b) Congo red dye
phytoremediation by *T. Ammi*

Table 2. Kinetic model constant values for phytoremediation of Methylene Blue dye by *T. ammi*.

Kinetic Model	Kinetic Parameters	Initial dye concentration mg L ⁻¹				
		10	20	30	40	50
Pseudo-First Order	$q_e \text{ (exp) (mg g}^{-1}\text{)}$	0.692	0.956	1.305	1.624	2.345
	K_1	0.130	0.152	0.126	0.173	0.242
	$q_{e(\text{theor})} \text{ (mg g}^{-1}\text{)}$	0.756	0.891	1.416	1.725	3.140
	χ^2_f	0.907	0.943	0.934	0.992	0.921
	$\Delta q_{t1} \text{ (%)}$	33.25	31.14	32.45	30.32	29.65
Pseudo-Second Order	$q_{e2} \text{ (theor) (mg g}^{-1}\text{)}$	0.586	0.779	0.902	1.332	1.644
	K_2	11.5	5.57	4.93	4.33	4.08
	R^2	0.844	0.880	0.937	0.915	0.933
	$\Delta q_{t2} \text{ (%)}$	2.85	2.14	1.85	0.94	0.87
Elovinch Equation	$q_{e3} \text{ (theor) (mg g}^{-1}\text{)}$	0.729	1.313	1.613	2.654	1.952
	α	0.005	0.008	0.011	0.014	0.014
	β	53.1	29.41	23.98	14.47	19.84
	R_3^2	0.912	0.909	0.929	0.837	0.854
	$\Delta q_{t3} \text{ (%)}$	16.55	16.02	14.43	14.21	14.14

In Table 2, the value of constant β has been decreased by the enhancement in the dye concentration which shows that the site of adsorption for MB and CR dye was low in value in the *T. ammi* surface and therefore, MB and CR uptake dyes by plant surface might occur through functional group interaction (Kaushal and Mahajan 2021). Additionally,

the constant value α_{EI} decreased by the increase in initial dye concentration, which indicated that more than one mechanism which drives the phytoremediation process.

Table 3. Kinetic model constant values for phytoremediation of Congo Red (CR) dye by *T. ammi*

Kinetic Model	Kinetic Parameters	Initial dye concentration mg L ⁻¹				
		10	20	30	40	50
Pseudo First Order	$q_{e(\text{exp})}$ (mg g ⁻¹)	0.473	0.845	1.110	1.431	2.135
	K_1	0.128	0.135	0.138	0.163	0.170
	$q_{e(\text{theor})}$ (mg g ⁻¹)	0.506	0.709	0.958	0.124	0.134
	R_1^2	0.931	0.909	0.950	0.967	0.978
	Δq_{t1} (%)	30.43	29.23	28.45	28.23	27.65
Pseudo Second Order	$q_{e2(\text{theor})}$ (mg g ⁻¹)	0.533	0.774	0.944	1.127	1.145
	K_2	13.73	9.30	6.90	5.96	4.96
	R_2^2	0.784	0.790	0.873	0.929	0.931
	Δq_{t2} (%)	2.74	2.12	1.94	0.97	0.79
Elovinch Equation	$q_{e3(\text{theor})}$ (mg g ⁻¹)	0.5874	0.9037	1.2351	1.4383	1.8139
	α	0.00702	0.01338	0.01824	0.0248	0.02484
	β	65.78	43.66	31.94	27.54	22.42
	R_3^2	0.747	0.745	0.839	0.896	0.914
	Δq_{t3} (%)	16.25	16.14	14.41	14.13	14.02

The equilibrium studies on phytoremediation were also work out by using the Langmuir and Freundlich adsorption isotherm. The monolayer adsorption is depicted by Langmuir isotherm with specific and uniform adsorption sites on the surface while Freundlich model is affected by the heterogeneous nature of surface sites (Hameed et al. 2007). The evaluation of regression coefficient (R^2) and other various constant values had been predicted by linear regression plot in Table 4. The value of R^2 Langmuir adsorption isotherm was found to be 0.987 and 0.983 for MB and CR and the equilibrium constant R_L value has been found to be decreases with increase in concentration. R_L value at 50 mg L⁻¹ was determined to be 0.0197 for CR and the value for R_L is 0.0198 MB dye concentration, indicated that MB and CR dye adsorption by *T. ammi* is highly favorable in high concentration due to bonding between functional groups present on *T. ammi* root cells and dye molecules (Baek et al. 2010). The little bit better fitting of Freundlich model ($R^2=0.999$) during the adsorption for CR dye than for MB ($R^2 = 0.990$) on *T. ammi* which suggested the heterogenous nature of *T. ammi* surface (Table 4). As the value $1/n$ of Freundlich isotherm obtained to be lower than 1, hence it has been predicting the surface with heterogeneity (Hameed et al. 2007). The value of 'n' determines the type of process either homogenous or heterogenous. As the value of n equal to unity or below the unity the process will be chemical and if the value obtained more than unity the process will be physical and favorable (Kumar et al. 2020). The $1/n$ value for

of MB adsorption on the surface of *T. ammi* has been obtained to be 0.1784, which determine the valuable adsorption of MB dye on the *T. ammi* surface (Fytianos et al. 2000) and for the CR dye value of $1/n$ was obtained as 0.3175 which is supportive adsorption at surface of *T. ammi*.

Table 4. Langmuir and Freundlich constants for Phytoremediation of Methylene Blue (MB) and Congo Red (CR)

Equilibrium Model	Constants	MB	CR
Langmuir Isotherm	Q_0 (mg g ⁻¹)	0.138	0.162
	b (mg g ⁻¹)	0.700	0.388
	R^2	0.987	0.983
Freundlich isotherm	$1/n$	0.1784	0.3175
	K_F	0.0745	0.0576
	R^2	0.990	0.999

3.4 Surface Characterisation of *T. ammi* roots

From the above discussion, it has been clearly indicated that plants roots surface plays an important role in dye removal from solution. Hence, it is very important to detect the morphology of *T. ammi* root surface and characterize the functional group present on *T. ammi* roots. Therefore, SEM analysis has been done to confirm the surface adsorption of dye by roots of *T. ammi* during phytoremediation process. SEM of *T. ammi* roots is recorded for both before and after the phytoremediation experiment with MB and CR and results are presented in form SEM micrographs (Fig. 8). Fig. 8a, indicates the smooth surface having prominent pores in the fresh roots of *T. ammi* before phytoremediation process while Fig. 8 b and c the dye loaded surface of *T. ammi* roots. It has been clearly depicted from Fig. 8 b and c shows that surface of *T. ammi* roots is covered with MB and CR dyes, respectively. The SEM images of *T. ammi* roots showed prominent colored marks which could be assumed as indication of adsorption of dyes by *T. ammi* roots acts as the adsorbent for removal of dyes from its solution during phytoremediation process (Qamar et al. 2020).

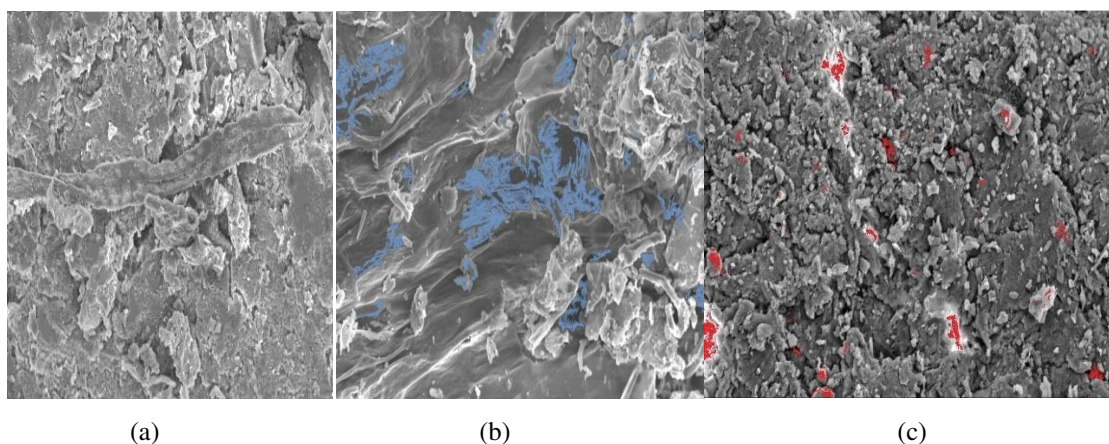


Fig. 8 SEM of *T. ammi* roots (a) Before phytoremediation (b) After phytoremediation of MB (c) After phytoremediation of CR dye

FTIR analysis was done to study the interaction of dye molecules with root surface of *T. ammi* roots. FTIR spectra of roots of *T. ammi* plant was taken before and after phyto remediation of both MB and CR dyes. The comparative interpretation of results of FTIR spectra was presented in Table 5.

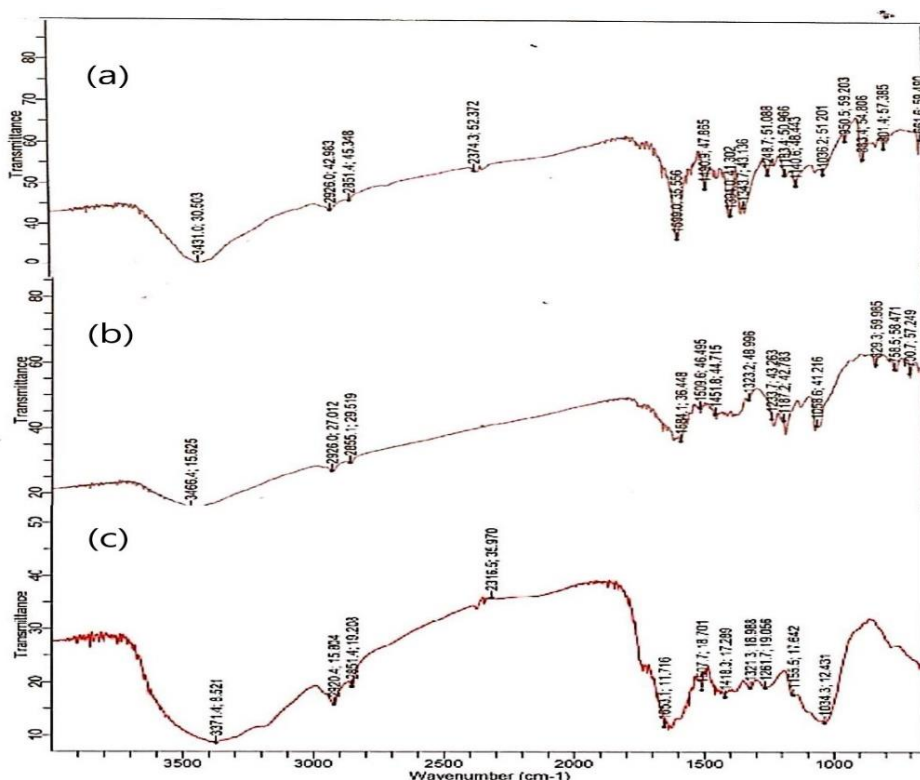


Fig 9. IR spectra of *T. ammi* roots before and after MB dye adsorption

The FTIR spectra (Fig. 9) depicts the presence of functional groups such as phenolic and carboxyl group which is indicated in roots of *T. ammi* and these results agree with the previous spectra reported for *T. ammi* extract (Qamar et al. 2020). Mathew et al. 2008 also confirmed the presence of Thymol (2-isopropyl-5-methyl phenol) as main active constituent of *T. ammi* plant extract. *T. ammi* contains O-H functional groups, and C-O functional groups. These functional groups are involved and played role in phyto remediation mechanism of dyes which are basic structure of cellulose, polysaccharide, and carboxylic acid (Munajad et al. 2018, Reyes-Ledezma et al. 2020). It has been inferred in Fig. 9 that most of the FTIR peaks changes its position after the adsorption of dye molecules due to interaction among functional group of dye and root. For instance, the FTIR spectrum for *T. ammi* exhibits a strong broad band at 3431 cm^{-1} assigned to the O-H stretching vibration of phenolic group which on adsorption of MB and CR dye changes its position at 3364.4 & 3371.4 cm^{-1} respectively and gets more broader due to extensive intermolecular H-bonding (Mahajan and Kaushal 2019). Similarly, peaks appear due to carbonyl group stretching 1248.7 & 1036.2 cm^{-1} shifts their position and it has also been observed that the intensities of these peaks changed from strong to medium or weak. The new peaks appearing at 2926.0 to 2851.4 and 2920.4 cm^{-1} describe C≡C stretching, C=C=C stretching, C=C stretching, C-N stretching, after exposure to MB and CR respectively.

Table 5. Comparative analysis of FTIR peaks of *T. ammi* roots before and after adsorption of Methylene Blue and Congo Red dye

S. No.	Peak position (cm ⁻¹) in FTIR spectra of <i>T. ammi</i> roots			Functional group assigned	Intensity
	Before dye adsorption	After MB adsorption	After CR adsorption		
1	3431.0	3466.4	3371.4	O-H (hydroxyl group stretch)	Strong, broad
2	2926.0-2851.4	2926.0-2855.1	2920.4-2851.4	-CH ₂ - (methylene group stretching)	Medium
3	2374.3	----	2316.5	O=C=O (carboxyl group stretch)	Medium
4	1699.0	----	-----	-C=O (Non-conjugated stretch)	Strong
5	----	----	1653.1	N-H bending (amide interaction)	Medium, broad
6	---	1584.1	-----	-C=O (Conjugated stretch)	Medium
7	---	1509.6	1507.7	C-C stretching (aromatic group)	Weak
8	1490.9	----	-----	C=C stretching (aromatic)	Medium
9		1451.8	1418.3	C-O stretching	Weak
10	1343.7	1323.2	1321.3	O-H bending (phenol)	Medium, Weak, weak
11	1248.7	1233.7	1261.7	C-O (carbonyl)	Medium, Medium, Weak
12	1183.4	1187.2	1156.5	C-O stretch (alcohol)	Weak
13	1036.2	1058.6	1034.3	C-N stretching	Weak, medium, medium
14	950.5	----		=C-H (alkene)	Medium

It was indicated that there is an interaction among functional groups of *T. ammi* and functional groups of MB and the chemical reaction involved in the adsorption process (Imron et al. 2021). These results strongly recommended that functional group interaction plays an important role in adsorption of dyes from their solution by *T. ammi* roots. The feeble peaks appear at 1584, 1509 & 1451 cm⁻¹ (after MB adsorption) and 1653, 1507 & 1458 cm⁻¹ (after adsorption of CR dye) confirmed the phytoadsorption of dye molecules. Based on these result and discussions of SEM and FTIR studies, a hypothetical mechanism is also proposed for phytoadsorption of dyes during phytoremediation process of *T. ammi* plant (Fig. 10). The roots of the plant *T. ammi* play an important role in removal of dyes from solution. The adsorption of MB and CR dye by plant root has been confirmed through SEM studies of roots of *T. ammi* (Fig. 8). It has been clearly inferred the presence of phenolic and carboxyl functional groups in roots of *T. ammi* from FTIR results. Kinetic and equilibrium studies also indicate the adsorption of dye occur on surface through chemical interaction of functional groups. Hence, it can be assumed that dye molecules get adsorbed through intermolecular

H-bonding, electrostatic and aromatic $\pi - \pi$ interactions between functional group present on surface of roots of *T. ammi* and dye molecules as shown in Fig. 10. (Kaushal and Mahajan 2021).

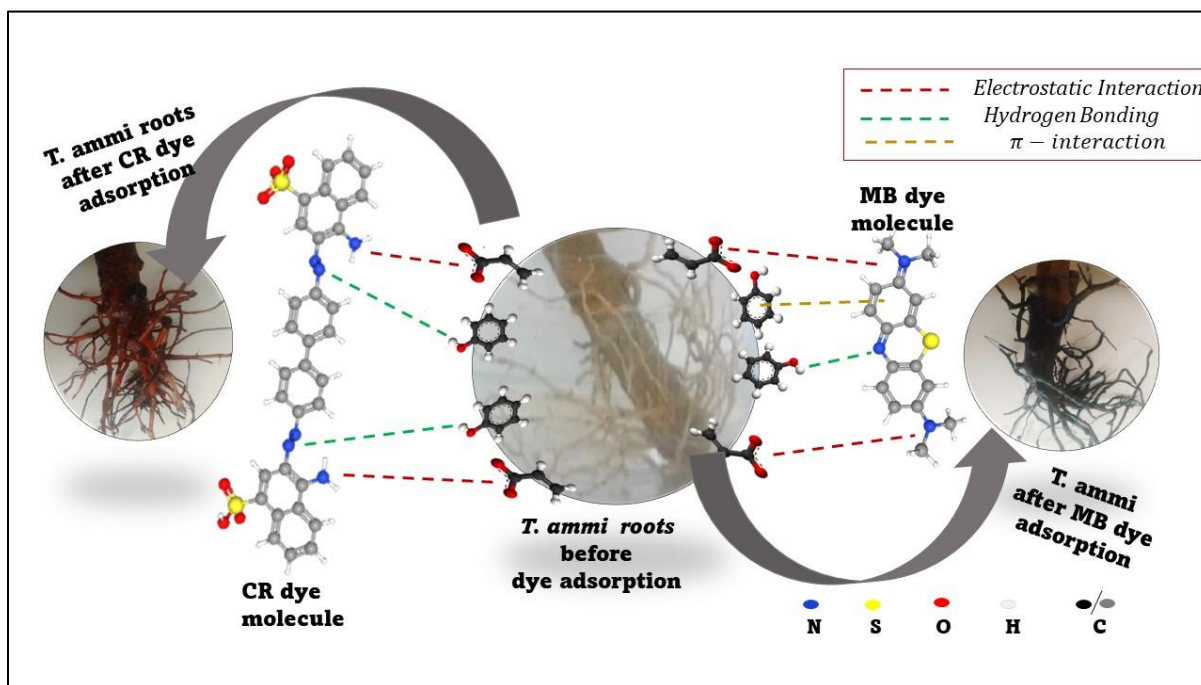


Fig. 10 Proposed Mechanism for Phytoremediation of Methylene Blue (MB) and Congo Red (CR) by *T. ammi* roots

Once the dyes get adsorbed on the surface of roots then get assimilated inside the plant root cell where it gets detoxified by the internal mechanism of plant cell (Kabra et al. 2013). As the growth of *T. ammi* was not restricted and even rejuvenate after adsorption of dyes which implies that *T. ammi* plant has potential of remediate structurally different types of dyes. Further, degradation pathway of MB and CR dye during phytoremediation of *T. ammi* plant was explored with help of HPLC and GC-MS analysis.

3.5 HPLC Analysis of dye degradation

The mechanism for decolorization the MB and CR dyes by the *T. ammi* in an upflow column was analyzed with different analytical techniques. The HPLC spectra for MB dye with control showed a single peak at a retention time 3.113 min⁻¹ (Fig. 11 a). After treatment of MB dye with *T. ammi* in an up-flow column, the dye control peak disappeared and new peaks with different retention times of 1.814, 2.503, 3.116, 3.330 min⁻¹ appeared represented in Fig.11 b (Zhang et al. 2021). The HPLC spectra for CR dyes with control showed a single peak at a retention time 3.148 min⁻¹ shown in Fig. 11 b. After treatment of the CR dye by means of *T. ammi* the new peaks with different retention times appeared at 2.642, 3.191, 4.589, 5.752, 7.509, 8.347, 11.225 min⁻¹ shown in Fig. 11 d (Victor et al. 2020). The disappearance of the control dye peak and appearance of several new peaks in the treated samples suggests the phytoremediation of both dyes as MB and CR into different metabolites.

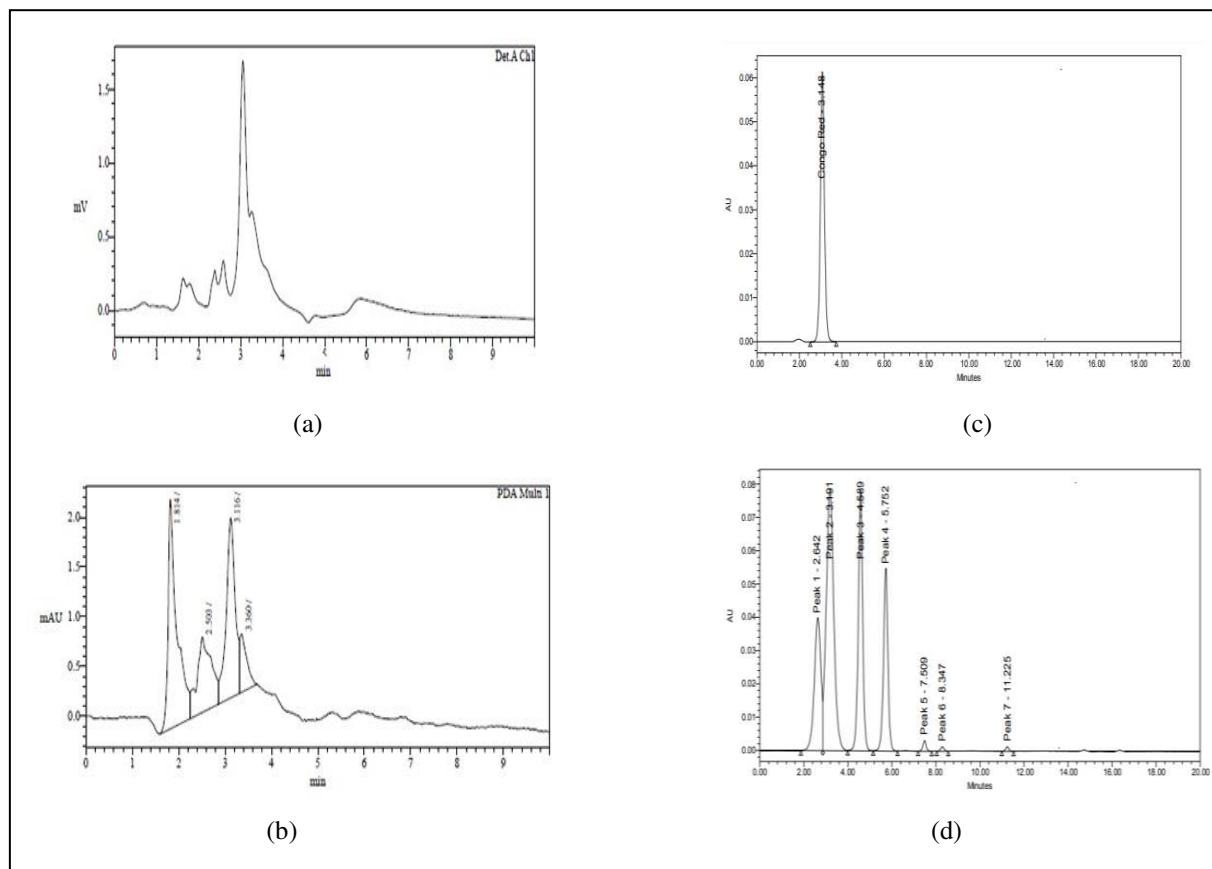


Fig 11 a) HPLC analysis of controlled MB b) Treated MB c) HPLC analysis of controlled CR d) Treated CR

3.6 GC- MS Analysis of dye degradation

The GC-MS analysis was used to determine the possible metabolites formed during phytoremediation of dyes and helpful in suggesting a pathway for the degradation of the MB and CR dye by *T. ammi* plant. Based on the results of GC-MS, the degradation pathway of MB and CR by *T. ammi* is presented in Fig. 12 and 13 respectively.

The major peak shows at a retention time (RT) of 11.724 min which indicates the MB base compound (m/z at 285). After phytoremediation treatment by *T. ammi*, the major peak area of MB was decreased and the new peaks at RT of 15.302 which is showing compound, as intermediate product as identified with m/z 215.0 could be the 2-Amino-5-dimethylamino-benzenesulfonic acid which suggested that N-S heterocyclic compound of methylene blue were degraded (Wang et al. 2018 b). The m/z value of 173.0 and 189.0 correspond to the 4-Amino-benzenesulfonic acid, 2-Amino-5-hydroxy-benzenesulfonic acid and the m/z value predicted at 112.0 belong to p-Dihydroxybenzene was the oxidized resultant of benzene ring. The m/z value of 118.0 fit to the small molecular products of degradation, which corresponds to the succinic acid. The last small molecular products of degradation with m/z 46 and 18 obtained as CO_2 and H_2O molecules (Imron et al. 2021) which are giving the evidence that phytoremediation mechanism to decolorization the MB dye which activate through the *T. ammi* roots. There are the pathways that may be involving in

the degradation mechanism by plant roots through desulfurization and denitrification processes (Imron et al. 2021). There are different intermediate products were formed which were adsorbed on the surface of *T. ammi* roots and remaining compounds could be converted into CO₂ and H₂O molecules.

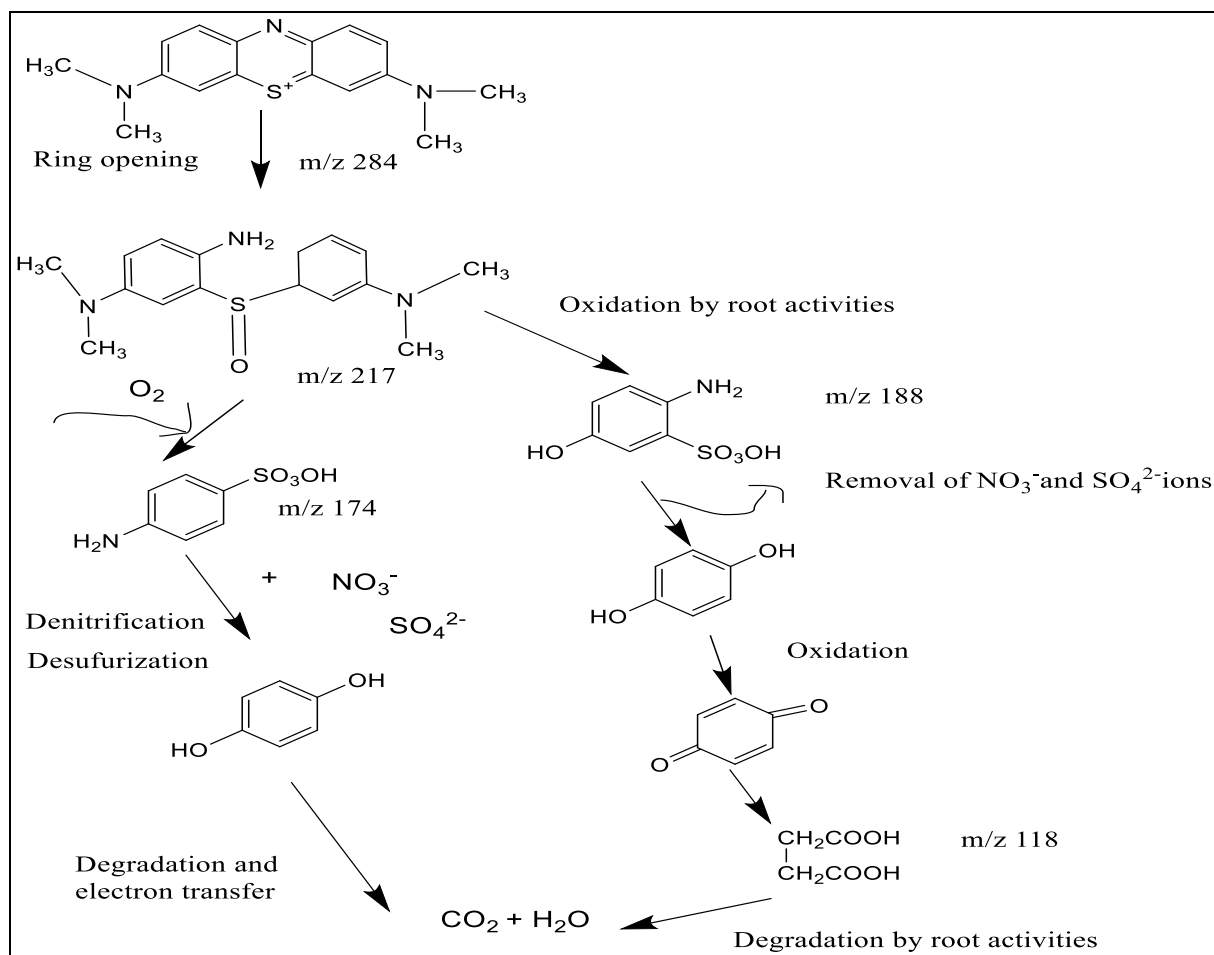


Fig. 12. Proposed mechanism of degradation of MB dye during Phytoremediation process of *T. ammi*

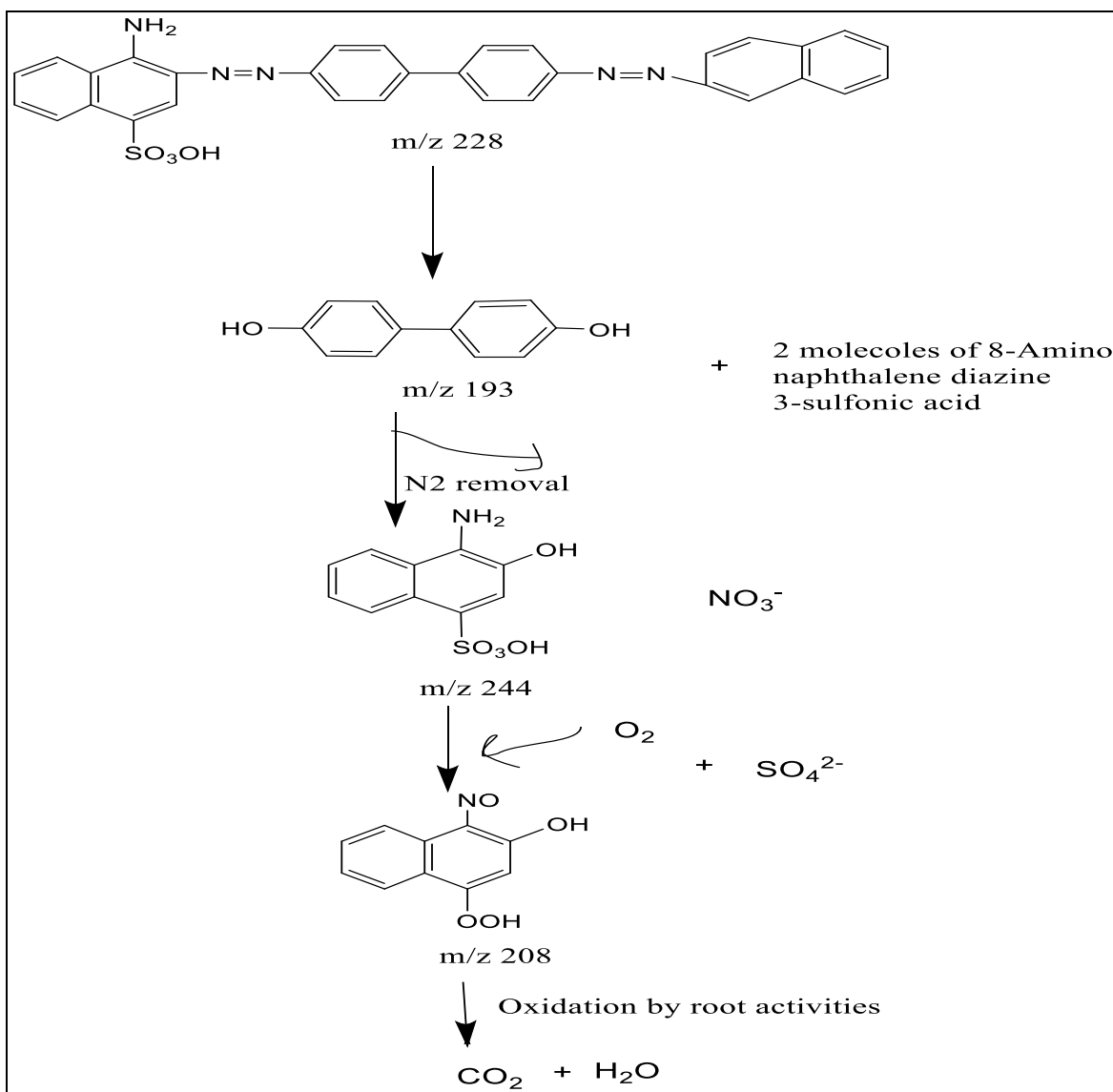


Fig. 13 Proposed mechanism of degradation of MB dye during Phytoremediation process of *T. ammi*

The GC-MS spectra of CR showed that control peak with retention time 17.786 having m/z value 228 of the dye disappeared with the time and the new peaks appeared. The first step was in phytoremediation of CR comprised the asymmetric cleavage of azo bond and formation of carbocation, this reaction intermediate was attacked by nucleophile OH⁻ ions (Chivukula and Renganathan 1995). After that there was a formation of p-dihydroxy-biphenyl and diazine intermediate having m/z 193 which leads to formation of the compound 8-amino naphthol 3-sulphonic acid with m/z having m/z 244 and another peak at 47 m/z predicted as amines (Telke et al. 2009, Victor et al. 2020). The compound 3-hydroperoxy 8-nitrosonaphthol with m/z 208 was formed by amine oxidation due to desulfurization activity of plant roots (Amitaia et al. 1998, Telke et al. 2009) which confirming that Congo red dye was cleaved into nontoxic hydroxyl and hydroperoxy metabolites instead of toxic aromatic amines. At last, 3-hydroperoxy 8-nitrosonaphthol degraded into the CO₂ and H₂O due to degradation and detoxification the roots interactions with dye. Thus, from the results of

GC-MS this is verified that the hazardous MB and CR dyes were degraded with the help of *T. ammi* roots during the phytoremediation process.

3.7 Cost effectiveness of the MB and CR removal through Phytoremediation

Phytoremediation is the cost-effective solar driven technology in contrast with any-other physical, chemical, and biological processes (Khandare and Govindwar 2015). This technique provides the neutral and resourceful application to the removal of harmful pollutants out of the surrounding environment (Dietz and Schnoor 2008). Some of the physical and chemical processes which are reported for the removal of MB and CR dyes, need large amount of money for their operations. The oxidation method using the various catalysts proved to be a fast and effective method for removal of MB and CR dyes, but it involves high energy and large amount of chemical for its processing and that is the major limitation of the process (Tavares et al. 2012). The description of the results in current research work, proved the capacity of *T. ammi* to degrade the dyes MB and CR in natural forms without any additional chemicals. Therefore, the textile industries could implement the cost-effective and eco-friendly technology of phytoremediation by using ornamental plant for the treatment of the dye loaded wastewater rather-than the using costly techniques for removal of the harmful dyes from the wastewater.

4. Conclusion

Phytoremediation studies were performed with *T. ammi* to remediate MB and CR dyes from the synthetic wastewater. The results of this research work indicated the capacity of *T. ammi* to treat the dyes wastewater for the water remediation methods. The various factors such as the influence of initial dye concentration, time and pH on the phytoremediation mechanism were observed. The decolorization potential of *T. ammi* toward MB and CR dye was decreased on increase in the initial dye concentration of these dye (10 to 50 mg L⁻¹) while adsorption capacity found to be increased. The maximum decolorization MB and CR dye solution was found 99 and 91% respectively. The pH of dye solution also found to influences the phytoremediation process as 95% decolorization of 20 mg L⁻¹ CR dye observed at pH 5 while 97% decolorization at pH 8 for MB. The maximum decolorization capacity by *T. ammi* explored its utilization for removal of the contaminants from the wastewater, especially dyes and can be grown in the dye contaminated area. The adsorption isotherms of Langmuir and Freundlich models investigate the adsorption pattern of MB and CR dyes on the *T. ammi* surface and Freundlich model found to be a little bit better fit for adsorption of both dyes MB ($R^2 \geq 0.990$) and CR ($R^2 \geq 0.990$) dye molecules on *T. ammi* root surface. The kinetic studies results supported the pseudo-first order kinetic model with the maximum correlation value $R^2 \geq 0.99$ and 0.97 for MB and CR respectively for phytoremediation by *T. ammi*. FTIR, SEM, HPLC and GC-MS studies confirmed that dye molecules get adsorbed on the plant roots through the functional group interaction. After phyto adsorption, dye get finally assimilated inside the root cells where it gets degraded into non-toxic compounds. Future research work scope will explore detoxification mechanism inside the plant cells using enzymatic system. Hence, the observed outcomes suggested that the *T. ammi* is an efficient ornamental plant for phytoremediation of noxious dyes from the environment and can be utilized as an effective phytoremediator which provide sustainable solution for wastewater treatment in present scenario of environmental safety crisis.

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