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Green remediation of textile dyes containing wastewaters by *Ipomoea hederifolia* L.

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Abstract

Wild plants and tissue cultures of *Ipomoea hederifolia* decolorize Scarlet RR (SRR) dye at a concentration of 50 mg L^{-1} up to 96 and 90% within 60 and 96 h, respectively. Significant induction in the enzyme activities of Lignin peroxidase, laccase, 2, 6-dichlorophenol indophenol reductase, superoxide dismutase, catalase and tyrosinase were found in the plant roots and shoots during decolorization. *I. hederifolia* was also able to treat a dye mixture and a real textile effluent efficiently with a reduction in American Dye Manufacturers Institute (ADMI) value (color removal) up to 85 and 88%, BOD up to 65 and 63% and COD up to 62 and 68%, respectively. The detailed anatomical studies of the stem and root cells of *I. hederifolia* during uptake and degradation was carried out showing a stepwise and mechanistic degradation of the model dye SRR. Products formed after dye degradation were analyzed by UV-Vis spectroscopy, FTIR, HPLC and HPTLC; which confirmed the phytotransformation of SRR, dye mixture and textile effluent. A possible pathway for the phytotransformation of SRR was proposed based on GC-MS analysis which confirmed the formation of different metabolites with lower molecular weights. The phytotoxicity study revealed the non toxic nature of the formed products.

Keywords: *Ipomoea hederifolia* Linn.; Decolorization; Phytoremediation; Scarlet RR; Textile effluent; Anatomy

1. Introduction

The industrial developments are always associated with a cost in terms of pollution of air, water bodies and soil by a number of toxic compounds. Fast growing textile industry is one of the sources to release toxic chemical compounds in the form of dyes. Approximately 10-15% of the synthetic dyes having carcinogenic and mutagenic effects are released during the dying process, ultimately causing threat to all life forms.[1] The valuable water bodies needed for irrigation, drinking and other household and industrial purposes are being polluted because of the textile dyes released through effluent. Inclusion of textile dyes to water reservoirs alters the vital parameters of water bodies by influencing the levels of chemical oxygen demand (COD), biological oxygen demand (BOD), total organic carbon (TOC), total dissolved solids (TDS), total suspended solids (TSS), pH and color.[2] Therefore, addressing the issue of these environmental contaminants becomes very important and needs attention from environmentalists.[3] This, at the end, results in development of odour and deterioration of overall water quality in the reservoir. The ability of textile dyes to remain recalcitrant to degradation in water and soil can be ascribed to their complex chemical structure. The persistence of toxic compounds in aquatic and soil environments may lead to accumulation in phytoplanktons, fishes and vegetables.[4] In developing countries, the textile dyes are not only the part of textile industry effluent but also add to the municipal sewages.[3]

A number of physicochemical methods such as adsorption, coagulation, flocculation, filtration, photodegradation and chemical oxidation are available for dealing with the pollution created by textile dyes. These methods are associated with high cost, low efficiency, secondary pollution problems and inability to treat a wide array of dyes having structural diversity.[2] Though, these methods are employed to remove the colour; their capacity to reduce the toxicity is still a matter of major concern. These facts certainly demand the development of an efficient, cost effective and eco-friendly technology for the removal of

these dyes from environment. Use of microorganisms for purifying dye contaminated water has extensively been studied and demonstrated but is still associated with some limitations as far as *in situ* administration of pollutants is concerned.[5]

Use of plants viz. phytoremediation to decolorize and detoxify the textile dyes in the laboratory as well as at the actual site of contamination appears as an attractive option.[6] Phytoremediation appears to be an ideal and potential approach for the treatment of pollutants; it is more admired because of its solar driven nature, cost effectiveness, aesthetic advantages and long-standing applicability as it can be directly employed at the polluted sites compared to other expensive methods of treatments.[7] Some wild plants like *Phragmites australis*, *Blumea malcolmii*, *Typhonium flagelliforme, Rheum rabarbarum* (rhubarb) and *R. hydrolapatum* have been proposed for textile dye removal.[6,8-11] Recently, common garden plants such as *Aster amellus*, *Glandularia pulchella*, *Portulaca grandiflora*, *Petunia grandiflora*, *Zinnia angustifolia* and *Tagetes patula* have been shown to have a great potential of textile dye degradation [12-18] Though, the use of nursery grown garden plants appears attractive, their use for the cleanup of actually contaminated site has yet to be demonstrated.

This work deals with the decolorization and degradation of a commonly used disperse textile dye Scarlet RR (SRR), a dye mixture and a real textile effluent by *Ipomea hederefolia* Linn. This plant was procured from the actual site of dye disposal site, Ichalkaranji, India. Therefore was thought to have the ability to grow in high dye concentrations and thus was better adapted to these dye stress conditions. The plant also possesses characteristics like dense root system, ability to grow in marshy places and non-edible nature. Moreover, the seeds of *I. hederefolia* can germinate in the soil contaminated with dyes which makes it a favourable candidate plant for phytoremediation of textile dyes. This work also explores the removal and degradation pattern by the plant at anatomical level and reveals the histological dye degradation process by *I. hederefolia.*

2. Materials and methods

2.1. Chemicals

2, 2 Azino-bis (3-ethylbenzothiazoline) 6-sulphonic acid (ABTS) and riboflavin were available from Sigma Aldrich (St Louis, MO, USA). Nicotinamide adenine dinucleotide (di-sodium salt), *n*-propanol, 2, 6-dichlorophenol indophenol (DCIP), veratryl alcohol and catechol were purchased from Sisco Research Laboratories, Mumbai, Maharashtra, India. Tartaric acid was available from BDH chemicals (Mumbai, Maharashtra, India). The MS Basal Medium was purchased from HiMedia Laboratories, Pvt. Ltd., India. SRR, Navy Blue HE2R, Green HE4B, Blue 2 RNL, Direct Red 5B, Reactive Red 2, Red HE3B, Remazol Red, Remazol Black B dyes and effluent were obtained from Mahesh, dye processors and Common Effluent Treatment Plant, Ichalkaranji, Maharashtra, India, respectively. All the chemicals used were of the highest purity available and an analytical grade. The seeds of *Sorghum vulgare, Phaseolus mungo and Vigna radiata* purchased from local grain market and the seeds of *I. hederifolia* for tissue culture were purchased from Naamdhari Seeds Pvt. Ltd., India.

2.2. Potential of plants *I. hederifolia* for dye decolorization

The plants of *I. hederifolia* were uprooted carefully and their roots were carefully washed with flowing water and exposed to 100 mL solutions of Navy Blue HE2R, Green HE4B, Blue 2 R, Direct Red 5B, Reactive Red 2, Red HE3B and SRR at a concentration of 50 mg L^{-1} independently. Based on the decolorization performance and longevity in *I. hederifolia* was selected for further studies.

2.3. *In vitro* culture of *I. hederifolia* and its decolourization potential

Role of bacteria in the decolorization was doubted upon the first exposure to dye and therefore; *in vitro* grown cultures of *I. hederifolia* were obtained by seed culture method.

Seeds of *I. hederifolia* were collected from the plants found at the actual site of contamination, surface sterilized and cultured on MS basal medium as per the method described by.[5] The half-strength MS medium was discovered suitable for 90% of seed germination. One week old seedling with profuse roots was used for phytoremediation studies. The tissue culture plants of *I. hederifolia* also showed degradation of SRR, mixture of textile dyes and real textile effluent taken for the study which is explained ahead.

2.4. Decolorization studies

Decolorization experiments were initially carried out with the wild plants in the dye SRR and the dye mixture containing Green HE4B, SRR, Remazol Black B, Remazol Red and Navy Blue HE2R. The structures and names of all the dyes used in this study are shown in the appendix. The experiments were carried out in 250 mL Erlenmeyer flasks containing 100 mL of 50 mg L^{-1} of dye solution in plain distilled water. As the bacterial contamination was doubted during decolorization, the experiments were independently performed with the tissue culturally grown plantlets of *I. hederifolia* exposing them to 15 mL of dye solution of in test tubes. Absorbance of dye solution was recorded in an interval of 6 h each by removing 1 mL of solution. This solution was centrifuged at 4561 *g* for 10 min to remove any solid matter if present and the absorbance of the clear solution were measured at the wavelength of 510 nm. Decolorization percentage was calculated as follows:

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

Abiotic controls contained the respective dye solutions which were devoid of plants whereas plants in distilled water were kept as biotic controls.

2.5. Characterization of dye mixture and textile effluent

SRR, textile effluent and mixture of dyes were characterized for American Dye Manufacturers Institute (ADMI) value,[3] hardness, BOD, COD and TOC before and after dye degradation.[19]

2.6. Anatomical studies of stem and roots during dye degradation

Transverse sections of stem and root were taken and mounted in glycerin after overlaying with cover slip and result were micro-photographed with a Zeiss Axio Imager 2 Upright Trinocular Microscope with attached camera at 100X magnification.

2.7. Photosynthetic pigments analysis

5 gm leaves of both exposed and unexposed plants were crushed in mortor and pestle which contains 4 ml/L liquor ammonia, while crushing a pinch of $MgCo_3$ powder was added. The extracts were centrifuged for 10 min at 2000 x g. Chlorophyll a, chlorophyll b and carotenoids were determined spectrophotometrically at 652, 645, and 663 nm.[20]

2.8. Preparation of cell free extract and enzyme assays

Roots and shoots of the plants of *I. hederifolia* were cut, weighed equally (2 g) were finely chopped and then suspended separately in 50 mM potassium phosphate buffer (pH 7.4). The chopped shoot tissue was then grind in a mortar and pestle followed by homogenization in a glass homogenizer and then centrifugation at 8481 *g* for 20 min. The cell free extract thus obtained was used as an enzyme source. The supernatant obtained after harvesting the plant roots and shoots was used as a source of extracellular enzymes after centrifugation. The cell free solution obtained after harvesting cells was used as a source of extracellular enzymes.[21]

Activities of the enzymes lignin peroxidase (LiP), veratryl alcohol oxidase, laccase, tyrosinase, DCIP reductase and azo reductase were determined spectrophotometrically at

room temperature in the case of control and test for plants. LiP activity was determined by monitoring the formation of propanaldehyde at 300 nm in a reaction mixture of 2.5 mL containing 100 mM n-propanol, 250 mM tartaric acid, and 10 mM H_2O_2.[22] Laccase activity was determined in a reaction mixture of 2 mL containing 10% ABTS in 0.1 M acetate buffer (pH 4.9) and increase in optical density was measured at 420 nm.[23] Tyrosinase activity was determined as described by an earlier report.[24] NADH-DCIP reductase was measured in cell-free extract as reported earlier by Salokhe and Govindwar.[25] Veratryl alcohol oxidase activity was determined by using veratryl alcohol as a substrate. The reaction mixture contained 1 mM veratryl alcohol, in 0.05 M citrate phosphate buffer (pH 3.0) and enzyme in a total volume of 2 mL, which was used for the determination of oxidase activity. Oxidation of the substrate at room temperature was monitored by an absorbance increase at 310 nm due to the formation of veratraldehyde. One unit of enzyme activity was measured as the amount of enzyme that releases 1μmol product min^{-1}.[26] Antioxidant enzyme status was assessed by spectrophotometric assays. Antioxidant enzymes that were analyzed include catalase and superoxide dismutase.[27]

All enzyme assays were performed out at 27^{o}C with reference blanks that contained all components except the enzyme. The protein contents of all the samples were determined using Lowry's method.[28] All enzyme assays were run in triplicate, average rates were calculated and one unit of enzyme activity was defined as a change in absorbance unit min^{-1} mg of $protein^{-1}$.

2.9. Analysis of the degradation products

Decolorization of all the dyes was monitored using UV-Vis spectroscopic analysis (Hitachi U-2800; Hitachi, Tokyo, Japan), using supernatants, whereas biotransformation was monitored using Gas Chromatography-Mass Spectroscopy (GC-MS), High Performance Liquid Chromatography (HPLC), High Performance Thin Layer

Chromatography (HPTLC) and Fourier Transformed Infrared Spectroscopy (FTIR). For the extraction of products, plants were removed from the distilled water containing decolorized dye, which was then centrifuged to remove any solid matter (e.g., root hairs,) if present. The decolorized and degraded solution was then mingled with equal volumes of ethyl acetate in a separating funnel and extraction of organic phase was done. The extract was then evaporated in in vacuum over anhydrous Na_2SO_4 and dried out. The solid residues obtained were dissolved in small volumes of HPLC grade methanol and sample used for analytical study.

Identification of the metabolites produced was carried out using (GC-MS). GC-MS analysis of the metabolites was carried out using a Shimadzu 2010 MS Engine, equipped with integrated gas chromatograph with a HP1 column (60 m long and 0.25 mm). Helium was used as carrier gas at a flow rate of 1 mL min^{-1}. The injector temperature was maintained by oven at 80^{o}C for 2 min. The temperature was increased up to 200 ^{o}C with the rate of 10 ^{o}C min^{-1} and then raised up to 280 ^{o}C with 20 ^{o}C min^{-1}. The compounds were identified on the basis of mass spectra and using database of the National Institute of Structure and Technology (NIST) library. HPLC analysis was carried out (Waters model no. 2690; Waters Corp., Milford, MA) on C18 column (symmetry, 4.6 mm x 250 mm) by using methanol with flow rate of 1 mL min^{-1} for 10 min and UV detector at 254 nm. HPTLC analysis was carried out by using HPTLC system (CAMAG, Switzerland). Samples of dye SRR, dye mixture and its biodegradation metabolites (dissolved in HPLC-grade methanol) were loaded on precoated HPTLC plates (Silica gel 60F 254, Merck, Germany), by using nitrogen as a spraying gas and TLC sample loading instrument (CAMAG LINOMAT 5). The bands (12 mm) were applied at 10 mm from the lower edge of the plate with first application position at 20 mm from left edge of the plate and 5 mm apart from each other. HPTLC plate was kept in pre saturated twin-trough chamber (10×20 cm) for the development of dyes and ethyl acetate extracted

metabolites (products); with 10 mL of standardized developing solvent system of toluene to methanol (8:2). After development, the plate was observed in UV chamber (CAMAG) and scanned at 254 nm with slit dimension 5×0.45 mm by using TLC scanner (CAMAG). The results were generated by using HPTLC software WinCATS 1.4.4.6337. The phytotransformed dye SRR was characterized by FTIR (Agilent Cary 630 FTIR spectrometer) and compared with the control sample. The FTIR analysis was conducted in the mid-IR region of 400-4000 cm^{-1}. The samples were mixed with spectroscopically pure KBr in the ratio of 5:95, pellets were put on the sample analyzer, and the analyses were carried out.

2.10. Phytotoxicity study

SRR solution at 2000 ppm concentration in distilled water was prepared and applied for the toxicity testing on seeds of *Sorghum vulgare, Phaseolus mungo and Vigna radiata* at room temperature. Similarly, treated and untreated dye mixture and effluent were used for phytotoxicity tests. The mentioned three crop plants are commonly cultivated in India.5 mL distilled water was used as a control. The seeds were put in 5 mL solutions of dye and degraded metabolites separately at room temperature. The seed germination percentage was computed and shoot length (plumule) and root length (radicle) were measured after 8 days.

2.11. Statistical analysis

Data was analyzed by one-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparisons test. Readings were considered significant when P was ≤ 0.05.

3. Results and discussion

3.1. Decolorization of dyes by *I. hederifolia* L.

The plants of *I. hederifolia* from contaminated site showed different decolorization potentials for various dyes. *I. hederifolia* decolorized Navy Blue HE2R up to 96%, Green

HE4B up to 90%, Blue 2RNL up to 89%, Direct Red 5B upto 87%, Reactive Red 2 up to 68%, Orange HE2R up to 56% and SRR up to 96% within 60 h, respectively. *I. hederifolia* showed versatility in decolorization of the dyes and showed the potential to be used for textile dyes treatment. Tissue cultures of *I. hederifolia* also gave 90% decolorization of SRR within 96 h showing the direct role of this plant without any bacterial involvement. UV–Vis scan (800–400 nm) of medium supernatants withdrawn after 96 h of exposure of *I. hederifolia* cultures to the dye SRR indicated its decolorization and decrease in concentration (Fig. 1). The tissue culture is recognized as an efficient tool for phytoremediation studies (Doran et al., 2009).[29] Phytoremediation of textile dyes have earlier been shown by tissue cultures of *P. grandiflora, Z. angustifolia, Blumea malcolmii* and *Sesuvium portulacastrum*.[5,6,10,13,30]

3.2. Characterization of dye mixture and effluent before and after treatment

Textile dyes containing effluents are known to have high BOD and COD. Simulated dye mixture and textile effluent was also decolorize efficiently by *I. hederifolia* and it gave ADMI removal of 85 and 88%, BOD removal of 65 and 63% and COD removal of 62 and 68%, respectively. Effluents and mixture of dyes do not have true colors; therefore their color is measured by the tristimulus method which measures the color value independent of the hues.[12] It is highly desired to have a phytoremediator which is able to treat actual textile effluents. Earlier, *Phragmaites australis*, *Aster amellus*, *Glandularia pulchella*, *Portulaca grandiflora*, *Petunia grandiflora*, *Zinnia angustifolia* and *Tagetes patula* have been shown to treat the textile effluents up to harmless levels.[11-15] *I. hederifolia* is a natural inhabitant of the dye effluent contaminated soils and therefore it can be proposed for further on field phytoremediation trials.

3.3. Anatomical studies of stem and roots during dye degradation

Anatomical study of stem for understanding the histochemistry, movement and metabolism of SRR was carried out. The control plant stem (Fig. 2a) showed no coloration of any cell and they appeared to be normal and undisturbed. After 6 h of SRR dye exposure (Fig. 2b) was found to be accumulated in the epidermal cells. As the exposure continued the accumulation increased, reaching up to cortical cells at 12h (Fig. 2c). Further, at 24 h (Fig. 2d) the accumulation increased in the cortex along with disappearance of dye around epidermal layer, showing degradation and metabolism of SRR. Phytotransformation in epidermal as well as cortical cells after 48 h (Fig. 2e) clearly revealed an increased dye removal. The exposed plants were transferred to normal tap water and the stem section regains the clear cortex with some distortion in epidermal cells having a small amount of dye in it (Fig. 2f).

The meristematic cells of *I.hederifolia* were observed under microscope, the control plant root tip cells (Fig. 3a) showed normal cuboidal cells but after dye exposure the cells were observed to be elongated in shape (Fig. 3b). This distortion in morphology could have occurred because of the dye stress.

3.4. Photosynthetic pigments

The phototosynthetic pigments are considered to be one of the factor sensisitive to stress and the energy requirement to the cells. After 60 h, the chlorophylls (a, b) and carotenoids concentrations were estimated. Surprisingly, the chlorophylls and carotenoids concentration was found to be increased in the plants exposed to SRR compared to those with control plants which were kept in normal tap water. *I. hederifolia* is a C_4 plant which under abiotic stress like presence of dye might have synthesized more chlorophylls and carotenoids leading to increase their concentration in the exposed plants, similar kind of results were reported previously where temperature played role as a abiotic stress in which the decrease in

photosynthetic pigments were seen in case of C_3 plants and increase in pigments concentration was observed in case of C_4 plants.[31]

3.5. Enzymes induction studies during textile dye degradation process

Enzymatic treatments possess no risk of biological contamination and do not put any harmful impact on the ecosystems. In order to have an additional insight into the decolorization mechanism, the activities of Lignin peroxidase, veratryl alcohol oxidase, tyrosinase, laccase, 2, 6-dichlorophenol indophenol reductase and azo reductase were assayed. Some organic compounds can be precisely degraded and completely mineralized by plant and microbial enzymes.[32] In this study, the root cells of *in vitro* grown *I. hederefolia* gave significant inductions in the enzyme activities of LiP (628%), veratryl alcohol oxidase (146%), DCIP reductase (59%), laccase (31%), tyrosinase (23%), catalase (76%) and SOD (77%). While in case of stem cells, significant inductions in the enzyme activities of LiP (204%), veratryl alcohol oxidase (33%), DCIP reductase (41%), laccase (214%), tyrosinase (183%), catalase (271%) and SOD (209%) were seen (Table 2). Earlier, *I. palmata* and *Saccharum spontaneum* and *Phragmites australis* have been used for dye decolorization showing the involvement of peroxidase enzymes.[33,34] *B. malcolmii, A. amellus, Zinnia angustifolia, P.grandiflora* and *G. pulchella* also showed their active role of veratryl alcohol oxidase, tyrosinase, LiP, laccase and DCIP reductase during the decolorization of Direct Red 5B, Remazol Red, Brilliant Blue R and Green HE4B.[6,12-15,21] Induction in the activities of lignin peroxidase, laccase, NADH-DCIP reductase, veratryl alcohol oxidase and tyrosinase shows their direct or indirect role in degradation of SRR showing their role in degradation.

3.6. Analysis of metabolites

The FTIR spectrum of untreated sample of SRR (Fig. 4a) showed the peaks at 3094.8 and 2904.8 cm^{-1} showed C-H stretching, a peak at 3517.3 cm^{-1} represented O-H stretching, at 2454.9 cm^{-1}showed NH^{+} stretching, at 2247.6 cm^{-1} revealed C≡N stretching, at 1598.5

showed NO_2 stretching, at 1517.0 cm^{-1} represented N=O as in aromatic nitro compounds, the peak at 1043.1 cm^{-1} showed the sulfonated nature of SRR, the peaks at 808.7, 969.74 and 682.82 cm^{-1} showed C-H deformation, whereas 620.62 showed C-Cl stretching. The treated sample of SRR (Fig. 4b) showed different peaks at 2947.8, 2835.1, 2798.5 cm^{-1}showed C-H stretching and 1115.6,1021.1 cm^{-1} indicated presence of C-OH stretching. Similarly, the untreated mixture of dyes (Fig. 4c) showed peaks at 3425.7, 2327.6 cm^{-1} revealing the N-H stretching, a peak at 1497.7 cm^{-1} showed C=C and C=N stretching, the peak at 1544.2 showed N=O stretching, at 1408, 1109.4 cm^{-1} stood for C-H deformation, a peak at 1371.4 cm^{-1}revealed the S=O stretching, a peak at 1324.9 cm^{-1} showed NO_2 stretching, the peaks at 1181.8 and 909.7 cm^{-1}showed C-OH stretching, the peak at 735.3 represented the aromaticity of the compounds, whereas, a single peak at 664.8 cm^{-1} showed C-Cl stretching. The treated sample of dye mixture (Fig. 4d) showed peaks at 3233.2 cm^{-1} representing N=O stretching, at 2957.6 cm^{-1} showing C-H stretching, the peaks at 1445.2, 1303.5 and 918.2 cm^{-1}showed C-H deformation, a peak at 1227.2 cm^{-1}showed S=O stretching, and a peak at 695.2 cm^{-1} showed C-Cl stretching .The FTIR spectrum of the untreated effluent sample (Fig. 4e) showed the peaks at 3442.8 $cm^{-1}$for N-H stretching, at 2927.1 cm^{-1}, at 2850.8 for C-H stretching, 1635.6 cm^{-1}showing C=C stretching, at 1591.6 cm^{-1} showing N-H deformation , at 1495.3 cm^{-1} showing N=O stretching, the peaks at 1340.4 and 1132 cm^{-1} showed S=O stretching, the peaks at 1221.9, 844.5 and 735.6 represented C-H deformation, a peak at 1015.5 cm^{-1} showed C-OH stretching and a peak at 637.9 revealed the C-Cl stretching. On the other hand, the FTIR spectrum of the treated effluent sample (Fig. 4f) showed different peaks at 3417.8 cm^{-1}showing N-H stretching, the peaks at 2922.7 and 2852.1 cm^{-1} showed C-H stretching, a peak at 1613.7 cm^{-1} showed C=O stretching, a peak at 1420.6 cm^{-1} showed S=O stretching. The differential FTIR spectra obtained before and after the treatment of SSR, dye mixture and real

textile effluent supported the transformation of the dyes to different products. This also reveals the phytotransformation potential of *I. hederifolia*.

HPLC analysis of the untreated SRR (Fig. 5a) showed the peak at retention time of 3.12 min whereas this peak was disappeared after treatment (Fig. 5b) with appearance of new peaks at retention of 2.10, 2.51,3.03,3.31,3.58 and 4.50 min. HPLC analysis of mixture of dyes (Fig. 5c) showed the peaks at retention time of 1.90, 2.43, 2.59, 2.90, 3.28 and 5.69 min, while treated dye mixture (Fig. 5d) showed the peas at a retention time of 2.00, 2.11, 2.57, 3.33, 3.80 and 4.50 min. Similarly, HPLC spectrum of untreated effluent (Fig. 5e) showed distinct peaks at retention time of 3.05, 3.39, 3.68, 4.28, 4.55 and 6.32 min. However, treated effluent (Fig. 5f) showed the peaks at retention time of 3.14, 3.52 and 3.62 min. The differential HPLC spectra obtained in untreated and treated samples of SRR, mixture of dyes and textile effluent confirms the phytodegradation of dyes.

HPTLC analysis (Fig. 6) of the untreated SRR (Lane 1) showed three peaks at an R_f value 0.85, with a major band at 0.76 and third band at 0.53 with an absorbance of 0.4, 0.2 and 0.9 AU. In lane 2 (treated SRR) only a diminished peak was observed at 0.85 $R_f$with an absorbance of 0.2 AU confirming the biodegradation of SRR. Lane 3 (untreated dye mixture) showed a distinguished appearance of 5 bands at the $R_f$of 0.87, 0.67, 0.38, 0.20 and 0.16 with an absorbance of 138.4, 95.5, 29.5, 13.9, 97.6. Lane 4 (treated dye mixture) shows a mere presence of dye at an R_f of 0.67 and 0.2 having an absorbance of 23.1 and 16.8 AU. Untreated textile effluent (Lane 5) shows 5 bands at an $R_f$of 0.54, 0.41, 0.29, 0.21 and 0.12 with an absorbance of 29.8, 76.4, 494.2, 279.0 and 14.7 whereas treated effluent showed removal of most of the peaks and only three peaks were seen at an $R_f$of 0.96, 0.42 and 0.30 with an absorbance of 69.5, 18.3 and 82.7 AU.

GCMS analysis was performed to know the extracted metabolites of SRR. Six separate peaks were obtained. The pathway of degradation of SRR is predicted with the help

of metabolites obtained and analysis of induction in the activities of the enzymes in the roots of *I. hederifolia* Linn. SRR undergoes asymmetric cleavage by the action of LiP and form two intermediate quinolin-4(3*H*)-one and 2-(methylamino)-*N*-(4-nitro-1l^3-chlorinin-2-yl) acetamide. Ring of quinolin-4(3*H*)-one undergoes cleavage by the action of tyrosinase to form 2-ethenyl-2,5-dihydropyridin-4(3*H*)-one which further undergoes reduction and de-ethenylation to give 2,3,4,5-tetrahydropyridine. 2-(methylamino)-*N*-(4-nitro-1l^3-chlorinin-2-yl)acetamide undergoes oxidative cleavage by laccase and gives 2-amino-*N*-(4-hydroxy-1l^3-chlorinin-2-yl) acetamide which further upon deamination and reduction gives *N*-methyl-1l^3-chlorinin-2-amine (Fig. 5). Lignin peroxidase and veratryl alcohol oxidase are known to cleave the dye molecules asymmetrically.[12-16,30] The laccase cleaves the dyes by oxidative cleavage.[35] The differential enzymatic status also influences the degradation mechanism. At natural sites of dye contaminated soils, other factors such as soil microflora and environmental conditions along with plants may act on the dye molecule synergistically and mineralization of the dye molecules may occur. The *I. hederifolia* showed a great potential to degrade SRR dye to different metabolites.

3.7. Phytotoxicity Analysis

The textile dye SRR, mixture of dyes and effluent showed inhibition of germination of *Phaeseolus mungo* and *Sorghum vulgare* and *Vigna radiata* seeds. The untreated textile dye SRR, mixture of dyes and effluent, showed 60, 50 and 60% inhibition of *P. mungo* seed germination respectively, whereas *S. vulgare* seeds showed 60, 50, 60% inhibition of germination and for *V. radiata* germination inhibition percentage was found to be 50, 60 and 60%, respectively (Table 3). The plumule and radicle lengths also got decreased in the case of untreated textile dye SRR, effluent and dye mixture samples. The 8 days of exposure of the untreated textile dye SRR, textile effluent and dye mixture were highly toxic to the plantlets as the root and shoot lengths were also inhibited when compared to treated effluent and dye

mixture samples. The treated samples were found to be less toxic and showed lower germination inhibition percentages when compared to their untreated forms. A significant increase in seed germination percentage and, shoot and root lengths reveals the reduced toxicity of the metabolites formed after the treatment by *I. hederefolia.*

4. Conclusion

The phytoremediation of textile dyes has mainly been carried out by using common garden plants whose potential is yet to be tested at the in situ remediation purpose. This study explores the phytoremediation potential of *I. hederifolia*, a plant inhabitant of the actual dye disposal site. Its seeds germinate normally in high dye concentration and can complete the life cycle in high dye load. The plant could decolorize a real textile effluent sample and a dye mixture to meaningful levels. This study clearly revealed the dye removal by *I. hederifolia* at anatomical level. Studies to explore this plant in a constructed wetland system for textile effluent treatment at industrial scale are underway.

Acknowledgement

Niraj R. Rane, Avinash G. Gholave, Shrirang R. Yadav and Sanjay P. Govindwar wish to thank Department of Biotechnology (DBT), New Delhi, India for the funding under Interdisciplinary Program for Life Science (IPLS) project to Shivaji University, Kolhapur.

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Table 1

Characterization of textile effluent and mixture of dyes before and after their treatment

Parameter	**Untreated Effluent**	**Treated effluent**	**Untreated dye mixture**	**Treated dye mixture**
COD (mg L^{-1})	1840	580	1430	540
BOD (mg L^{-1})	1630	590	1250	430
ADMI units	962	108	678	96
Turbidity (NTU)	209	56	87	35

TDS (mg L^{-1})	3478	998	94	36
TSS (mg L^{-1})	496	408	28	17
Hardness	410	130	380	100
pH	10.2	7.9	8.1	7.5
Conductivity	3.80×10^{-3}	0.90×10^{-3}	2.80×10^{-3}	1.40×10^{-3}

Table 2. Enzyme activities in root of *I.hederifolia* plant control tissue at 0 h and after 60 h for SRR dye

Enzymes	***I. hederifolia* root cells**		***I. hederifolia* shoot cells**	
	Control	**Test**	**Control**	**Test**
Lignin peroxidase[a]	0.25 ± 0.01	1.82 ± 0.04†††	0.23 ± 0.65	0.70 ± 0.11††
Laccase[a]	13.86 ± 0.35	18.14 ± 0.28†	0.29 ± 0.95	0.91 ± 0.87†
DCIP Reductase[b]	256.02 ± 30.41	407.6 ± 1.45†††	350.55 ± 0.35	495.44 ± 0.16††
Tyrosinase[a]	40.24 ± 0.87	49.46 ± 0.53†	16.28 ± 0.33	46.14 ± 0.26†††
Azo reductase[c]	NA	NA	16.39 ± 0.057	19.27 ± 0.41†
Veratryl alcohol oxidase[a]	1.47 ± 0.13	3.62 ± 0.13††	3.66 ± 0.88	4.89 ± 0.16†
Catalase[d]	0.046 ± 0.76	0.081 ± 0.56†	0.35 ± 0.49	1.3 ± 0.90††
Superoxide dismutase[e]	2.15 ± 0.38	3.81 ± 0.84††	1.27 ± 0.66	3.92 ± 0.58††

Values are a mean of three experiments ± SEM. Significantly different from control (0h) at $^{\dagger}P< 0.05$, $^{\dagger\dagger}P< 0.01$ and $^{\dagger\dagger\dagger}P< 0.001$by one-way ANOVA with Tukey Kramer comparison test.

[a]Activity in units min^{-1} mg^{-1}

[b]µg of DCIP reduced min^{-1} mg $protein^{-1}$

[c]µg of Azo dye reduced min^{-1} mg $protein^{-1}$

[d]50 % inhibition of the NBT photoreduction rate (U mg^{-1} protein)

[e]Nano moles of H_2O_2 Utilized (U mg^{-1} protein)

NA: No activity

Table 3. Chlorophyll and carotenoid content of *I.hederifolia* leaves before and after exposure to 50 mg/L with SRR over the period of 60 h

Sample	Chlorophyll a (ug/ml)	Chlorophyll b (ug/ml)	Total chlorophyll content (ug/ml)	Carotenoids (ug/ml)
Control Plant	10.979±0.68	7.162±0.89	18.141±0.76	13.412±0.66
Test Plant	17.952±0.87	11.755±1.12	29.707±1.06	18.262±0.90

Table 4. Phytotoxicity testing of disperse dye Scarlet RR, mixture of dyes and textile effluent and their respective degraded products

Parameters Studied	Water	Scarlet RR	Scarlet RR metabolites	Untreated dye mixture	Treated dye mixture	Untreated textile effluent	Treated textile effluent
			Phaseolus mungo				
Germination (%)	100	40	100	50	80	40	70
Plumule (cm)	9.71 ± 0.05	3.53 ± 0.06†	8.06 ± 0.03‡	2.83 ± 0.06†	3.53 ± 0.03‡	2.62 ± 0.02†	3.24 ± 0.04‡
Radicle (cm)	6.23 ± 0.03	2.42 ± 0.05†	3.60 ± 0.05‡	3.2 ± 0.05†	3.63 ± 0.02‡	2.86 ± 0.03†	3.15 ± 0.02‡
			Sorghum vulgare				
Germination (%)	100	40	90	50	90	40	70
Plumule (cm)	7.26 ± 0.08	4.13 ± 0.06†	5.81 ± 0.10‡	3.63 ± 0.08†	4.3 ± 0.05‡	3.5 ± 0.06†	4.33 ± 0.15‡
Radicle (cm)	5.94 ± 0.12	1.46 ± 0.04†	2.80 ± 0.03‡	2.3 ± 0.05†	2.8 ± 0.05‡	1.8 ± 0.01†	2.41 ± 0.02‡
			Vigna radiata				
Germination (%)	90	50	80	40	80	40	80
Plumule (cm)	5.93 ± 0.08	2.73 ± 0.06†	5.83 ± 0.06‡	2.53 ± 0.03†	5.73 ± 0.03‡	2.36 ± 0.08†	5.83 ± 0.06‡
Radicle (cm)	2.46 ± 0.06	1.02 ± 0.90†	2.36 ± 0.02‡	0.93 ± 0.90†	2.3 ± 0.06‡	0.73 ± 0.06†	1.13 ± 0.03‡

Values are a mean of three experiments ± SE. Root and shoot lengths of plants grown in textile dye SRR, textile effluent and mixture of dyes, respectively are significantly different from that of plants grown in distilled water by $^{\dagger}P < 0.001$. Root and shoot lengths of plants grown in the degraded treated SRR, textile effluent and mixture of dyes, respectively are also significantly different from that of plants grown in untreated SRR, textile effluent and mixture of dyes by $^{\ddagger}P < 0.001$.

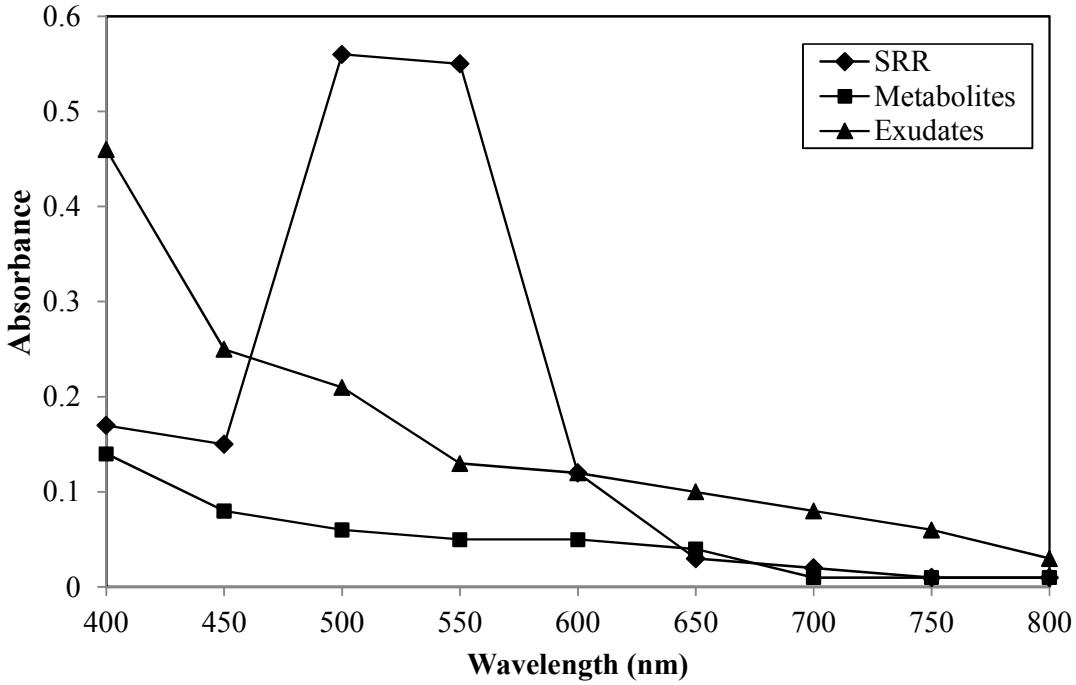

Fig. 1 UV–Vis spectrophotometric analysis of Scarlet RR, root exudates of *Ipomoea hederifolia* and the metabolites of the dye after treatment

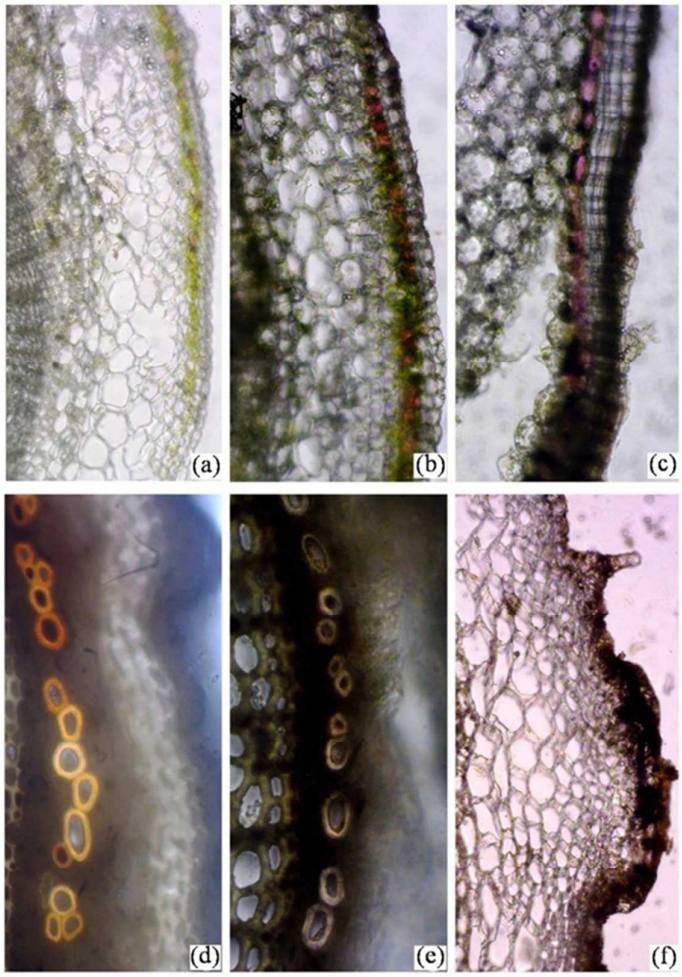

Fig. 2. Anatomy of stem of *I.hederifolia* a) control plant, SRR exposed plants b) at 6 h, c) 12 h, d) 24 h, e) 48 h and f) plants exposed to normal tap water after 48h of SRR exposure

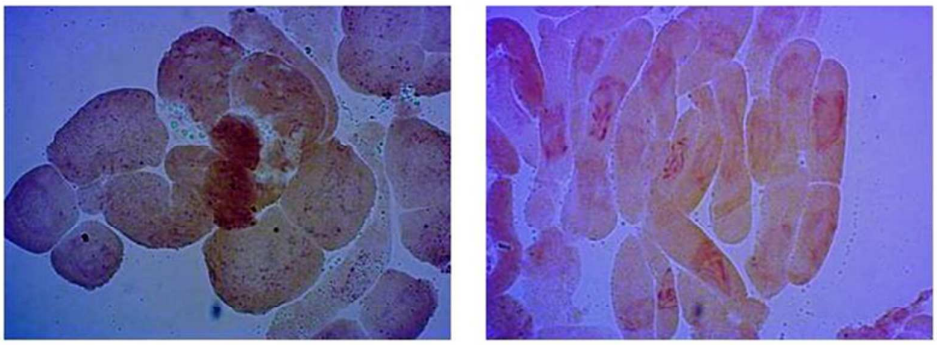

Fig. 3 Meristematic cells of root tips of *I .hederifolia* a) control plant (unexposed to Scarlet RR), b) plant exposed to Scarlet RR

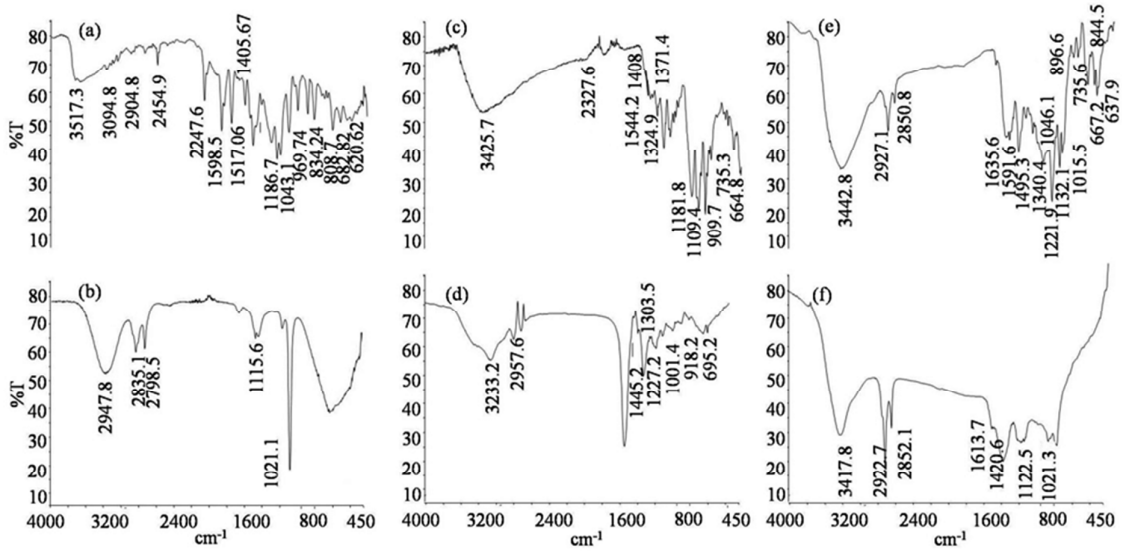

Fig. 4 FTIR analysis of a) Scarlet RR b) metabolites of Scarlet RR c) untreated dye mixture d) treated dye mixture e) untreated effluent and f) effluent after treatment

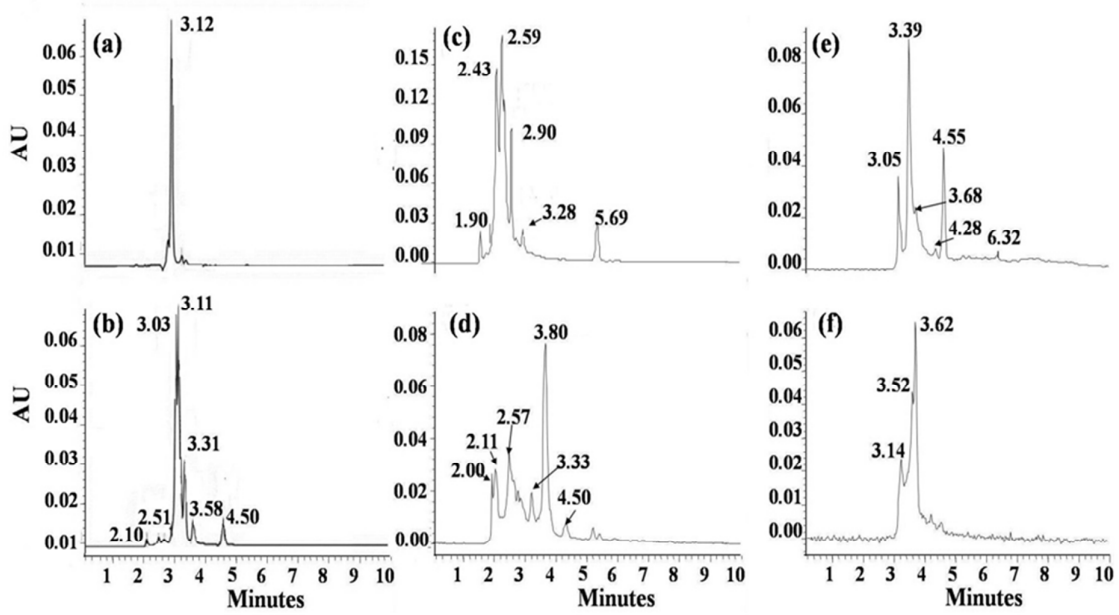

Fig. 5 HPLC analysis a) Scarlet RR b) metabolites of Scarlet RR c) untreated dye mixture d) treated dye mixture e) untreated effluent and f) effluent after treatment

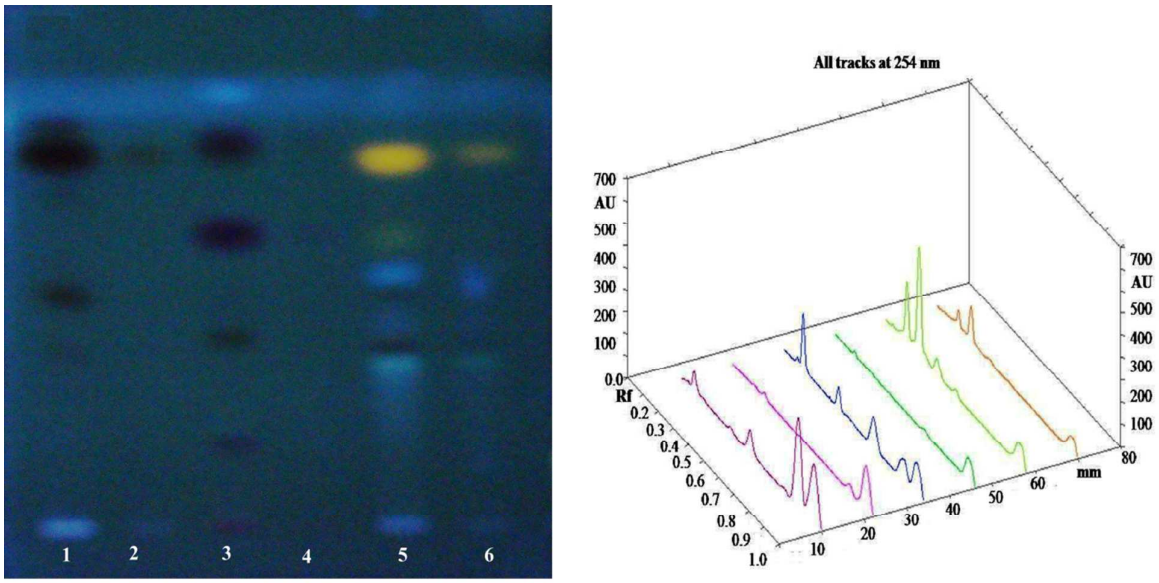

Fig. 6 HPTLC plate image with 3-D spectral scan at 254nm

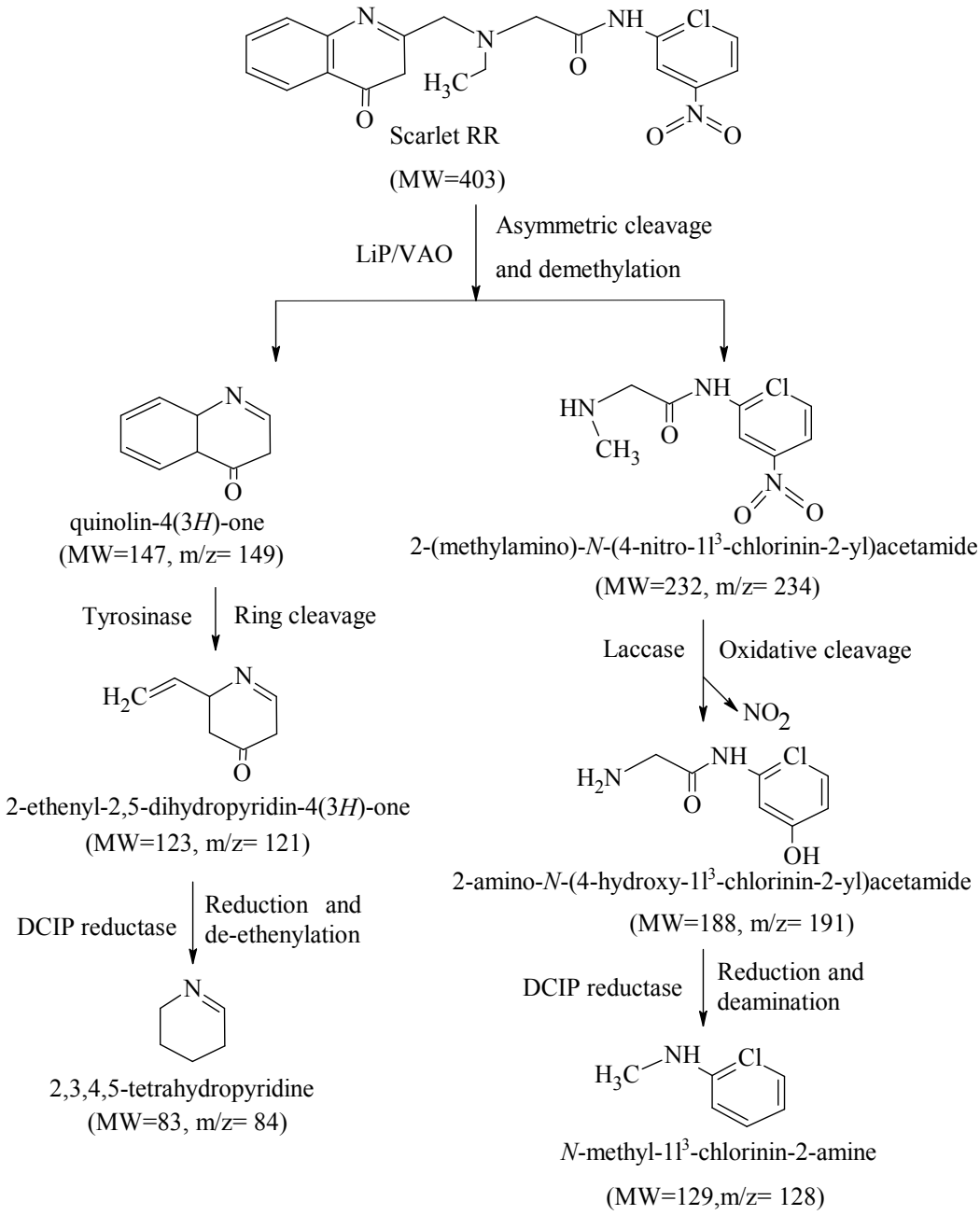

Fig. 7 Proposed pathway for degradation of Scarlet RR by *Ipomoea hederifolia* L.

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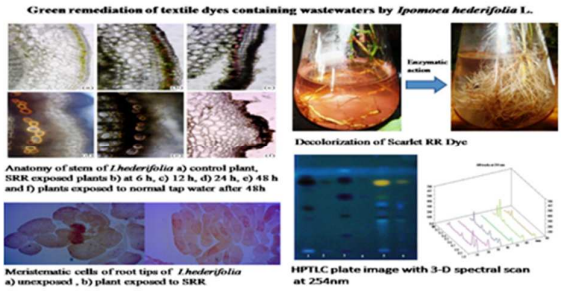

Ipomoea hederifolia **from dye disposal site was found to accumulate and simultaneously degrade toxic textile dyes**