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Assessing Phytoremediation Potential of Basil for Ciprofloxacin

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Over the past years, the prevalence of antibiotics in water and soil has become a significant environmental hazard that requires immediate attention. The purpose of this research is to examine the *Ocimum basilicum* (Basil) potential to remediate the antibiotic ciprofloxacin (CIP) and to determine its toxic effects on plants. A study in a greenhouse was conducted to eliminate ciprofloxacin from the soil. For four weeks, plants were grown with varying concentrations of ciprofloxacin (50 to 300 $mg{\cdot}kg^{-1}$) in triplicates. To analyze the ciprofloxacin uptake in Basil, remediation rates, translocation factor, and toxicity measures such as fresh & dry biomass, shoot & root lengths, change in chlorophyll, flavonoid, carotenoid, proline, phenol, and catalase content were assessed. With the help of HPTLC (high-performance thin layer chromatography) technique, accumulation of ciprofloxacin in root and shoot were analyzed. The result showed that at 100 and 200 $mg{\cdot}kg^{-1}$ of ciprofloxacin concentration, the selected plant showed maximum remediation of 93.81% & 92% respectively. Total chlorophyll, carotenoid, flavonoid, phenol, and catalase content, were higher at 100-200 $mg{\cdot}kg^{-1}$. Such increase is observed to manage ciprofloxacin-induced stress in plants. These levels later decreased at higher concentrations due to toxicity of ciprofloxacin. Therefore, this study suggests that *O. basilicum* is a promising plant species with high remediation rate and also confirmed that phytoremediation has a significant capability as a sustainable & eco-friendly approach for the effective removal of CIP from soil.

Keywords: Antibiotics, HPTLC, Basil, Remediation, Translocation

Introduction

In recent years, antibiotics are increasingly regarded as major pollutant of concern on a global scale. As they are most common prescribed medications to treat or prevent bacterial infections. Therefore, antibiotic use has expanded in both the human and animal health care sectors, which has resulted an increase in generation of pharmaceutical waste.[1] Due to its inappropriate disposal, antibiotic resistance which is one of several public health problem is getting worse with time. Antibiotics consumed by humans and animals for disease prevention and to enhance meat and milk production are excreted in an undigested form into the environment.[2] Antibiotics can enter the environment through urine and feces or indirectly through the application of animal manure. Manure is utilized in organic farming as a source of nutrients, from where the active antibiotic residues are discharged into soil bodies. According to various reports, huge amount of antibiotics was detected in vegetables which could be linked to presence of antibiotics in animal manure utilized as a soil nutrition source.[3] Sludge-fertilized soil may cause food crops to absorb antibiotics, which could have an impact on health of humans and animals. Also, the discharge of antibiotics into the ecosystem from sources, such as waste water treatment plants and pharmaceutical waste, results in major environmental concerns, such as ecological risks and impact on health of living organisms. Due to the widespread usage of antibiotics over the past several decades, many pathogenic bacteria have developed resistance to antibiotics and easily enter the food chain. This has led to development of incurable infections among living organisms and also causes damage to the nervous system.[4] As a result, on January 23, 2020, the Ministry of Environment, Forest and Climate Change, India issued regulations for concentrations of antibiotics found in waste released into rivers and other environmental channels by pharmaceutical manufacturers.[5,6] According to several studies[7–10] fluoroquinolone antibiotics have been discovered in main Indian rivers like the Yamuna, Ganga, and Kaveri, which are a key source of drinking water. Among all Fluoroquinolones (FQ), Ciprofloxacin (CIP) is a commonly prescribed second-generation broad-spectrum antibiotic. It treats many bacterial infections such as urinary tract,

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respiratory tract, chest (pneumonia), bone and skin etc. It has 385.82 g/mol of molecular weight and according to the pH- solubility profile, ciprofloxacin has two isoelectric constants of pKa_1 = 5.76–6.09 (strongly acidic) and pKa_2 = 8.62 (strongly basic).[11] High quantities of FQs antibiotics in soil have been recorded, up to 0.45 $mg{\cdot}kg^{-1}$.[12,13] The surface soil concentrations of CIP in China varied from 0.10 to 288 $\mu g{\cdot}kg^{-1}$.[14] Similarly in the farm soil of Southern China growing vegetables, 42 $\mu g{\cdot}kg^{-1}$ of CIP was detected.[15]

There are numerous physico-chemical remediation techniques for antibiotic removal from soil. These traditional methods have a number of drawbacks, including high-cost expenditure, production of harmful byproducts, less economical, labour intensive, and not a green approach.[4] Whereas, a new sustainable and eco-friendly way of decontaminating soil and water from multiple pollutants in the environment is phytoremediation. Phytoremediation is thought to be more effective, economical, and environment friendly than other remediation techniques.[16] The basic concept behind phytoremediation is that plants may absorb harmful elements from the environment and transform them into less harmful forms or accumulate in the plant parts. Plant-based remediation is more environment friendly than other techniques since it has less adverse effect on the soil physical and biological properties. *Ocimum basilicum* L. (Basil), a member of the Lamiaceae family and genus *Osmium* L., is a popular industrial and medicinal plant.[17] *O. basilicum* is widely known for its ability to produce antioxidant enzymes under stress, which aids in the degradation of organic contaminants in plants. Basil was chosen for this study due to its strong root structure, economic feasibility, and potential to produce antioxidants. Bhatt *et al*.[18] performed similar study utilizing *O. basilicum* to remove amoxicillin from soil. According to our best knowledge, this is the first research on the toxicological effect of CIP on plants and its elimination using *O. basilicum*.

Material and Methods

Chemicals and Reagents

The ciprofloxacin hydrochloride monohydrate ($C_{17}H_{18}FN_3O_3{\bullet}HCl{\bullet}H_2O$) (CAS NO. 86393-32-0), which has a purity of up to 99%, was bought from Hi-Media. For plant extraction, HPLC grade methanol was utilized. CIP stock solution and working dilutions were made in 0.01 molar HCl along with autoclaved Milli-Q water with pH levels between 6.9 and 7.2, as determined by a PH 550 pH Meter (Cole-Parmer).

Conditions for Plant Growth

For the study, silt loam soil with a pH around 7 was selected. In porous plastic pots with 300g soil, three-week old Basil saplings were planted. The experiment was performed in triplicates. The plants were cultivated for 4 weeks in a greenhouse at 36 ± 1°C with a 12:12 hr. light: dark cycle and were watered daily. After studying the various reported CIP concentration in the environment, the concentration range of (50, 100, 200, 250, and 300 $mg{\cdot}kg^{-1}$) was selected for the study. These plant pots were labeled as P1, P2, P3, P4, and P5 respectively. Additionally, two control pots; a negative and a positive were set up.The negative control was intended to measure the growth of the plant in the absence of CIP, whereas the positive control was intended to measure the breakdown (photodegradation and hydrolysis) of CIP in soil.To account for error, three separate sets (n = 3) were prepared and the experiment was performed three times. Using the Eq. 1 mentioned below all the % reduction data was calculated[19];

$$\% \text{ Reduction} = \frac{A-B}{A} \times 100 \quad \ldots (1)$$

where, A represents the experiment's initial parameter and B final parameter.

Evaluation of Toxicity

After four weeks of experiment the toxicity impact of ciprofloxacin on plants was examined. The results of the study showed that due to phytotoxic effect of CIP changes in plant weight and length of root & shoot were observed. Root/shoot length, and plant weight measurements were made with the aim to evaluate the phytotoxic consequence of CIP on the development of plants. To collect data regarding antibiotic exposure on plants, the effects of CIP on photosynthetic pigments like chlorophyll a, b, and total chlorophyll, as well as carotenoids, were examined. To evaluate the toxicity of CIP present in soil, assessment of the total flavonoid, proline, phenolic and catalase content was also conducted.

Root and Shoot Length Analysis

After completion of four weeks, the individual plants were washed and cleaned. Later, the root and shoot length of each plant was determined using the standard centimeter scale.

Plant weight & Biomass Analysis

With the use of a weighing balance, the weight of the plants was determined. The fresh weight of cleaned root and shoot samples were measured immediately, and for dry weight the samples were air dried & weighed using analytical balance.

Chlorophyll Estimation

Following the approach outlined by Arnon *et al*.[20], the content of chlorophyll (Chl) was determined. To 1 g of leaves (cut into small pieces) 10 ml of 80% (V/V) acetone was added and homogenized to a smooth paste using a mortar and pestle that had been pre-cooled. The extract was centrifuged at 3000 revolutions per min. for 15–20 mins., and the final volume was made up to 25 ml with the help of 80% (V/V) acetone. Using Shimadzu 35 double beam spectrophotometer the absorbance of the obtained supernatant was taken at 645 nm and 663 nm in comparison to 80% acetone blank. The total chlorophyll content per g fresh weight was calculated with the help of Eq. 2:

$$\text{Chl a} = (12.7 \times \text{Abs}_{663}) - (2.69 \times \text{Abs}_{645})$$

$$\text{Chl b} = (22.9 \times \text{Abs}_{645}) - (4.68 \times \text{Abs}_{663})$$

$$\text{Total Chl} = (20.2 \times \text{Abs}_{645}) - (8.02 \times \text{Abs}_{663}) \quad \ldots (2)$$

Carotenoid Estimation

The carotenoid content was calculated using the approach explained by Lichtenthaler *et al.*[21] At 470 nm, the supernatant's absorbance was determined, and the results were examined to determine any changes in carotenoid content with varying CIP concentrations using Eq. 3;

$$\text{Carotenoid content} = \frac{1000 \times \text{Abs}470 - 2.27\ (\text{Chl.a}) - 81.4\ (\text{Chl.b})}{227} \quad \ldots (3)$$

Flavonoid Estimation

The analyses of total flavonoid content (TFC) were performed, using the procedure outlined by Masturi *et al*.[22] Firstly, the standard graph using quercetin was prepared and for the experiment 1g finely grinded powder of dried plant sample was mixed into 10 ml of ethanol.The prepared sample was kept for shaking at room temperature for 48 hrs. at 170–180 rpm. After completion of 48 hrs. the extract was separated. For final sample preparation, in 2 ml of plant extract 2 ml of 2% $AlCl_3$ and 2 ml of 120 Mm potassium acetate were mixed properly. The samples were then incubated at room temperature in dark for an hr. Finally, the sample's absorbance was taken at 440 nm using Shimadzu 35 double beam spectrophotometer. TFC was measured in both the treated plant and plant grown in absence of CIP (n = 3). Using Eq.4, total flavonoid content of extract was computed.

$$\text{TFC} = C \times \frac{V}{m} \quad \ldots (4)$$

where, C denotes the concentration of sample quantified by calibration curve (mg/L), V denotes the volume of solvent (ml) and m denotes the mass of sample (g).

Proline Estimation

The total proline content (TPC) analysis was conducted following the using the protocol outlined by Bates *et al.*[23] Firstly, the standard curve using proline was prepared. For the experiment fresh leaves weighing 0.5 g were crushed in 3% aqueous sulfosalicyclic acid (10 ml), and the resulting mixture was centrifuged for 10 mins. at 10,000 rpm. A reaction mixture consisting of sulfosalicyclic acid, glacial acid and acid ninhydrin in the ratio of 1:2:2 was prepared separately. Two ml of supernatant of plant extract was added to the reaction mixture. The solution was incubated at 96°C for 60 minutes and later was kept in ice to terminate the reaction. After adding 4 ml of toluene to the mixture, the samples' proline absorbance at 520 nm was measured. A standard curve was utilized to determine the proline concentration, and Eq. 5 was used to report the results on a fresh weight basis.

$$\text{Proline (µmol/g Fresh Weight)} = \frac{\text{µg proline per ml} \times \text{ml toulene}}{115.5} \times \frac{5}{\text{g sample}} \quad \ldots (5)$$

Phenolic Content Estimation

The approach outlined by Alara *et al.*[24] was used to determine the total phenol content. 0.2 ml Folin Ciocalteu reagent and 100 µl plant extract were combined and incubated at room temperature in the dark. 0.6 ml of Na_2CO_3(0.2 mM) solution was mixed with the plant extract and later incubated for 120 mins. At 765 nm, absorbance was taken. The phenolic content of the extract was determined using the standard graph for gallic acid and the results were presented in milligrams of gallic acid equivalents per gram of dried weight of sample (mg GAE·g^{-1} d.w.). Using Eq. 6, the total phenolic content was measured.

$$\text{Total Phenol Content} = C \times \frac{V}{m} \quad \ldots (6)$$

where, C denotes the concentration of sample quantified by calibration curve (mg/L), V denotes the volume of solvent (ml) and m denotes the mass of sample (g).

Catalase Unit Activity Estimation

Catalase activity was assessed with the help of protocol developed by Aebi *et al*.[25] The plant extract was prepared by homogenizing 0.5 g of plant leaves in 5 ml of 100 Mm potassium phosphate buffer with (pH=7.0) at 0°C. The prepared extract was centrifuged for 20 mins. at 15000 rpm at 4°C. A 300 µl of supernatant was mixed with 1.2 ml of hydrogen peroxide (H_2O_2) and 1.5 ml of potassium phosphate buffer (100 mM). By tracking the reduction in absorbance at 240 nm over time, the decomposition of H_2O_2 was determined. The CAT activity was calculated using Eq. 7.

$$\text{Unit Activity } \left(\frac{\frac{Units}{min}}{g}.F.W\right) = \frac{Change\ in \frac{Abs.}{min.} \times Total\ Volume\ (ml)}{Extinction\ coefficient \times Vol.of\ sample\ (ml)} \quad \ldots (7)$$

Assessment of Remediation Potential of Basil

In the current study, CIP remediation was estimated to evaluate *O. basilicum* potential for remediation. The CIP concentration in the root and shoots and the leftover antibiotic concentration in the soil were used to calculate the plant's potential for phytoremediation.

Quantification of Ciprofloxacin in Basil Roots and Shoots

In order to prepare the samples, the roots and shoots of the plant were kept for air drying (6–7 days) at 35–40°C. A 500 mg of dried samples were macerated in methanol, two drops of 0.01 mol HCl, and two drops of water. The solution was centrifuged at 3600 rpm for 2 mins. after being sonicated for 5 mins. Then prepared supernatant was heated in water bath for reducing its volume. A 10 µL aliquot of the resulting 1 ml solution was injected into the HPTLC apparatus, and the Rf value was computed.

Estimation of Plant Phytoremediation Efficiency

To determine if plant might be classified as accumulators, the Bioconcentration Factor (BCF) and the Translocation Factor (TF) were calculated using the Eqs. 8 & 9;

$$\text{BCF} = \frac{\text{Antibiotic concentration in plant}}{\text{Antibiotic concentration in soil}} \quad \ldots (8)$$

Translocation Factor is the transfer of antibiotic from roots to shoot

$$\text{TF} = \frac{\text{Antibiotic concentration in shoot}}{\text{Antibiotic concentration in root}} \quad \ldots (9)$$

The higher and better phytoremediation capacity of *O. basilicum* is indicated by TF>1. Whereas, TF value less than 1 shows poor remediation antibiotic toxicity and eventual death of the plant. This is because accumulation of antibiotics takes place in the root.[26,27]

Standard Preparation

For the standard, a stock solution of CIP (1000 ppm) was made in diluted 0.1 Molar HCl and methanol. Various CIP concentrations were then applied to an aluminum sheet silica gel plate. (Plate No. 60F254). UV-absorption with a 254 nm wavelength range was used to generate the linear regression.

Quantification of Ciprofloxacin in Soil

By using the HPTLC technique, the remaining ciprofloxacin concentration in the control setup and the treated soil samples was analyzed. After a four-week period, soil sample of 5.0 g was collected from the pots at a depth of 0 to 15 cm, nearest to the root. After that, samples of soil were mixed crushed, and allowed to air dry at 36 to 40°C until reached a constant weight. A volumetric flask containing soil sample of 0.5 g was combined with 10 mL of pure methanol following manual shaking. After that, retention factor value was determined using 0.2 µL samples on HPTLC plates at varied concentrations.

Statistical Analysis

Every experiment was performed in triplicates. Using Analysis of variance (ANOVA) the experimental results were statistically assessed at the significance level of $p<0.05$.

Results & Discussions

Root and Shoot Length of Plant

In presence of CIP the plant length was affected, the maximum shoot length was observed in blank whereas maximum root length was observed at P2 i.e., plant grown at 100 mg·kg^{-1} of CIP concentration (Fig. 1). When compared to plants cultivated without CIP, the plant's shoot length decreased by approximately 10.5% at P1 concentration (50 mg·kg^{-1}) and 17% at P2 concentration (100 mg·kg^{-1}), and around 26–30% decrease at P3, P4, & P5 (200, 250,300 mg·kg^{-1}). Similarly, around 12 to 12.5% decrease in the plant's

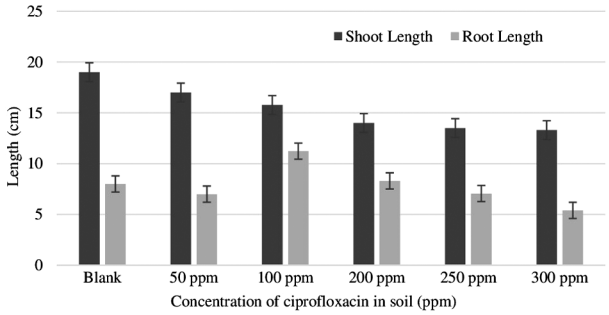

Fig. 1 — Shoot and root length (n = 3) of *O. basilicum* exposed to different concentration of ciprofloxacin in soil (P<0.05)

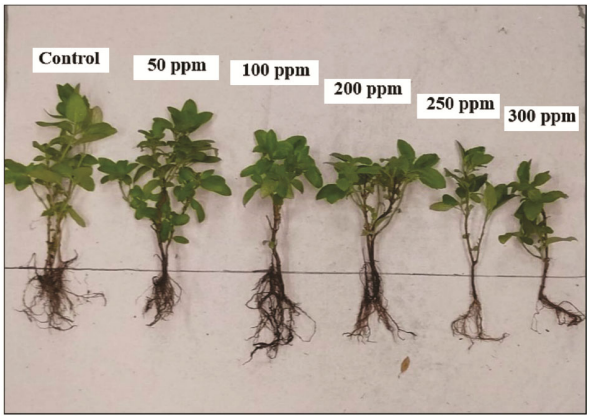

Fig. 2 — Changes in *O. basilicum* shoot and root length after remediation (4 weeks) at varying CIP concentrations

root length was noted at P1 and P4 followed by 32.5% decrease at P5 due to CIP toxicity. At P2 (100 mg·kg^{-1}) 40.4% and at P3 (200 mg·kg^{-1}) 3.75% increase in root length in comparison to blank was observed due to the hormetic response at lower concentrations.[28] Changes in the plant shoot & root length after 4 weeks at P1-P5, CIP concentrations are shown in Fig. 2. It is a dose dependent effect, at lower concentrations of antibiotic plant shows the increased growth and at high concentrations it has toxic effects. It has been noted that toxicity may be the cause of the apparent positive effect of increased growth, which would otherwise adversely affect the plant's growth, development and biological cycle.[28] This fact is also supported statistically using single factor ANOVA where value of p<0.05 shows that there is significant impact of increasing CIP concentration on root & shoot length of plant.

Analysis of Fresh and Dry Weight

The fresh and dried biomass of the root and shoot were measured in order to analyze the toxicity of CIP. The maximum FW & DW of shoot was observed in plant grown in the absence of CIP (Blank sample), whereas the highest FW & DW of root was noted at

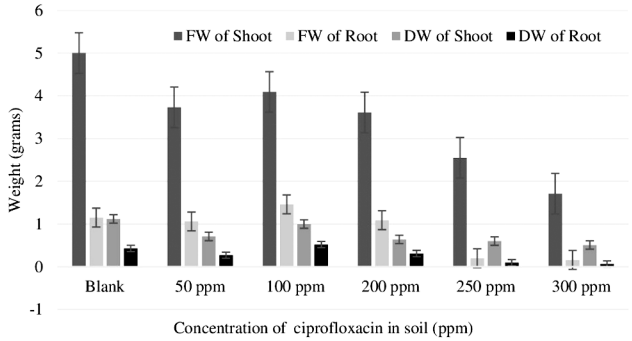

Fig. 3 — Fresh weight (FW), dry weight (DW) of root and shoot (n = 3) of *O. basilicum* exposed to different concentration of ciprofloxacin in soil (P<0.05)

P2 concentration (100 mg·kg^{-1}), as shown in Fig. 3. In comparison to all the applied concentrations i.e. (P1, P2, P3, P4, and P5) highest FW & DW was observed at P2 concentration (Fig. 2). As in presence of antibiotic plant shows hormetic response due to which the root & shoot length increases, which ultimately leads to increase in weight of the plant.[28] At P3, P4 & P5 concentration plant showed the decrease in root and shoot weight due to the increasing antibiotic toxicity with increasing concentration. Due to CIP toxicity 42.85%, 46.42%, and 54.46% reduction in DW of shoot was found and similarly 27.9%, 77.9%, and 84.65% reduction in DW of root was found at P3, P4, and P5 concentrations respectively. Statistical analysis showed significant decrease with P value less than 0.05 for both fresh and dry weight of theshoot and root.

Chlorophyll Content in Response to Ciprofloxacin

Photosynthetic pigments like chlorophyll and carotenoids were incorporated as biomarkers of toxicity, serving as crucial indicators of primary production and the photosynthetic capacity of plants subjected to antibiotics. The results of chlorophyll analysis are depicted in Fig. 4. In this study, the maximum content of chlorophyll a, b and total chlorophyll was observed in P3 plant. Similar increase in chlorophyll content of basil at 200 mg·kg^{-1} of tetracycline concentration was observed and reported by Bhatt *et al*.[29] There was enhancement in total Chl. content in the plants grown at P1, P2, P3, and P4 concentrations in comparison to blank plant. Whereas for plant grown at P5 concentration the Chl. a, Chl. b and total Chl. was found to decrease in comparison to the blank plant. At P1 (5.87 mg·mL^{-1}) concentration slight increase in chlorophyll content was observed followed by increase in P2 (10.7 mg·mL^{-1}) and P3

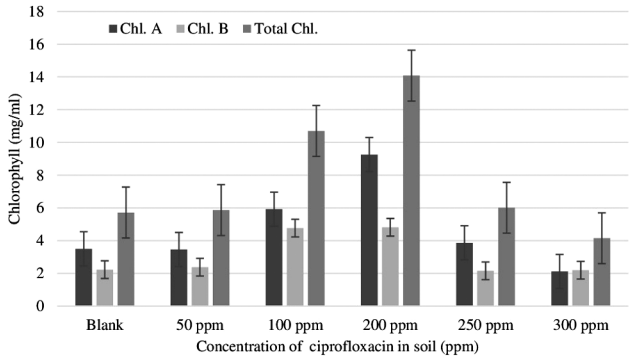

Fig. 4 — Photosynthetic pigments: Chl a, Chl b and total Chl (n = 3) in *O. basilicum* exposed to different concentrations of ciprofloxacin in soil (P<0.05)

Fig. 5 — Physical and morphological changes in *O. basilicum* leaves at highest ciprofloxacin concentration in soil

(14.08 mg·mL^{-1}). Due to the increased antibiotic toxicity the total chlorophyll content decreased in plants grown at P4 (6.01 mg·mL^{-1}) and P5 (4.15 mg·mL^{-1}) pot concentrations. According to statistical analysis, the overall Chl, Chl a, and Chl b content showed significant variation, between treated and untreated plants. With a p value below 0.05, the data was found to be of statistical significance. According to a number of research findings, low antibiotic doses may have an effect on the nucleic acids and proteins in plant cells, which in turn could impact the biosynthesis of chlorophyll. It suppressed chlorophyllase activity to delay chlorophyll degradation in cells, therefore increasing plant chlorophyll concentration as a result.[30]

Antibiotics at higher concentrations causes change in the overall morphology of leaf, which is the first obvious sign of antibiotic toxicity in plants.[30] Basil did not exhibit noticeable symptoms of chlorosis, but after completion of four weeks, it was discovered that leaves of plant grown at P5 showed wilting effect due to increased CIP concentration exposure as shown in Fig. 5. Also, the thickness of leaves was less at higher concentration in comparison to the lower concentrations. In a study *Pisum sativum* and *Lemna minor* grown in presence of tetracycline and ciprofloxacin respectively showed signs of antibiotic-induced leaf colour change, turning them light green or yellow.[31–33] In another study by Brain *et al.*[34] *Lemna gibba* showed significant phytotoxicity impact of fluoroquinolone, sulfonamide, and tetracycline classes of antibiotics.

Changes in Secondary Metabolite Content of Basil in response to Ciprofloxacin Exposure

Carotenoid Content in Response to Ciprofloxacin

In the study, highest carotenoid content was found to be at P2 (5.54 mg·mL^{-1}) followed by P3 (4.53 mg·mL^{-1}) in-order to inhibit or completely eliminate the elevated radicals of oxygen due to increased CIP toxicity.[35,36] Basically, Plants have an antioxidant called carotenoid that is lipid soluble and crucial in pigment-binding complexes. The presence of carotenoid aids in the organic pollutant's degradation in plants.[37] Carotenoid content of P1 (2.11 mg·mL^{-1}) was approximately equal to blank (2 mg·mL^{-1}). Due to increased antibiotic concentration level the content of carotenoid decreased at P4 and P5 in comparison to blank as illustrated in Fig. 6(a). With p<0.05, it was determined that the data were statistically significant. Hormesis refers to a biphasic dose-response to a stressor in which plant can benefit from adaptive responses when exposed to low to moderate doses of the stressor.[38] In this study, P2 & P3 (100 & 200 mg·kg^{-1}) of ciprofloxacin concentration may function as a mild stressor, inducing processes of adaptation that improve the synthesis of carotenoids and chlorophyll. The plant uses this hormetic reaction to help it deal with the stress that ciprofloxacin causes.[39]

Total Flavonoid Content (TFC) in Response to Ciprofloxacin

Flavonoid content was assessed to observe the toxicity in plants due to presence of CIP in soil. Since flavonoids are secondary metabolites, they are regarded as the secondary reactive oxygen species scavenging system in plants.[40] It was found that blank had the highest amount of TFC, rest all were less in comparison as demonstrated in Fig. 6(b). At P3 concentration, maximum TFC was recorded in order to deal with the stress caused by the increased CIP concentration. As plants generate Reactive Oxygen Species (ROS) when exposed to high ciprofloxacin concentration.[41] ROS damages cellular components like proteins and lipids. In-order to deal with such stress plants activate defense mechanism, which

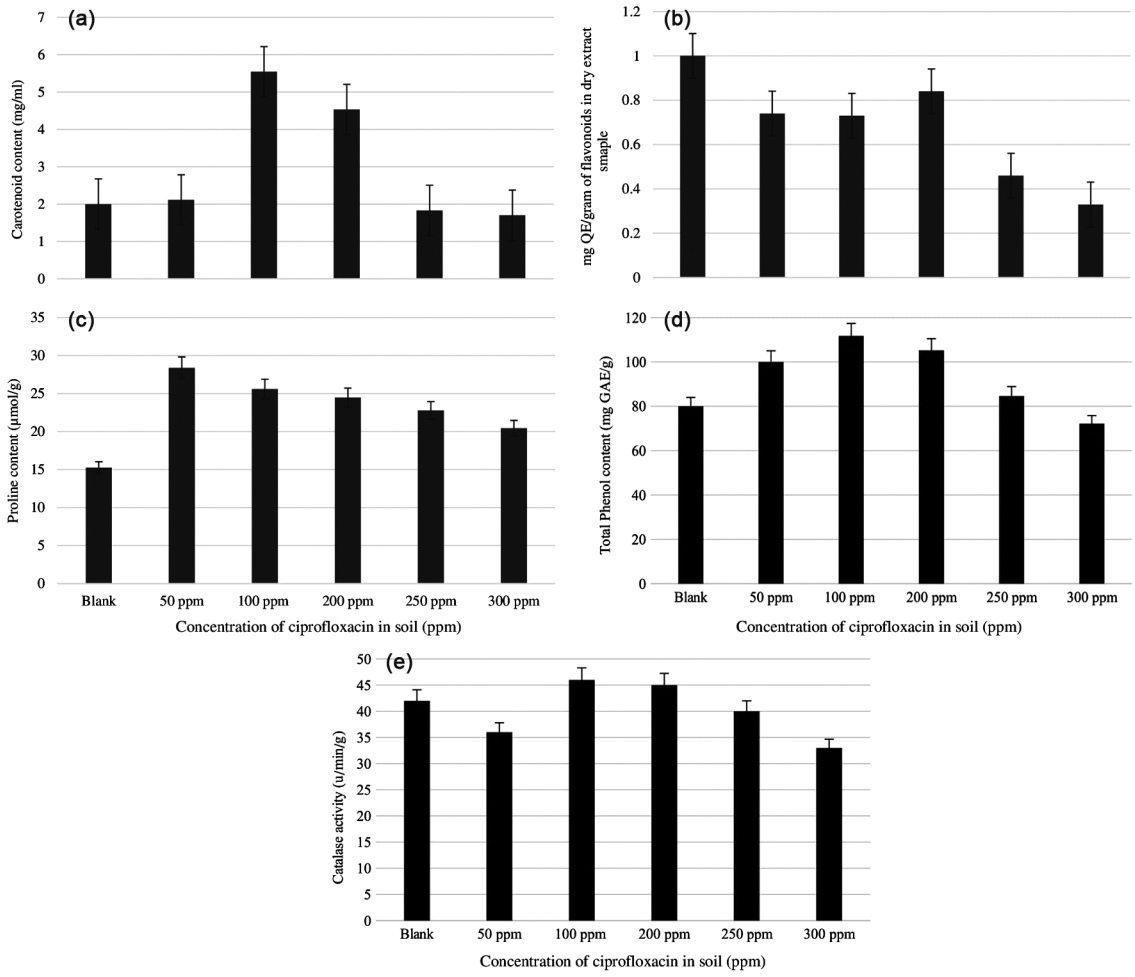

Fig. 6 — Exposure of *O. basilicum* to different concentrations of ciprofloxacin in soil (n = 3; P<0.05) and change in: (a) Total carotenoid content; (b) Total flavonoid content; (c) Total proline content; (d) Total phenolic content; (e) Total catalase content

includes synthesis of antioxidants like flavonoids.[42] Whereas, at P4 & P5 concentration lowest TFC was observed due to the toxicity and increase antibiotic stress on plants. With p<0.05, it was determined that the data were statistically significant.

Total Proline Content (TPC) in Response to Ciprofloxacin

Proline functions as a stress protein as it accumulates under both abiotic & biotic stress conditions. It acts as a protective measure against cellular damage.[43] The regression equation y = 12.88x + 0.0654 was developed from the proline standard graph, yielding an R^2 value of 0.98. The TPC was highest at P1 (50 mg·kg^{-1}) concentration, followed by almost equal content at P2 and P3 concentrations (100 mg·kg^{-1}) and (200 mg·kg^{-1}) respectively as shown in Fig. 6(c). The initial increase in content may indicate an early stress response by the plant. However, higher CIP concentrations i.e. P4 (250 mg·kg^{-1}) & P5 (300 mg·kg^{-1}) could lead to stress mitigation, resulting in a subsequent decrease. A statistical significance level was determined for the data with p less than 0.05. In an investigation by Pawlowska *et al*.[44] barley leaves subjected to the drug naproxen showed an increased accumulation of proline. In another study, aquatic plant *Trapa bispinosa* also maintained high levels of proline in presence of tetracycline antibiotic.[45]

Total Phenolic Content (TPC) in Response to Ciprofloxacin

A standard curve for total phenolic content was created with gallic acid and the regression equation y = 0.0116x + 0.0609 and R^2 value of 0.988 was calculated. The results in Fig. 6(d) displays that the highest phenolic content was noted at P2 (100 mg·kg^{-1}) concentration and the content gradually decreased with increasing CIP concentration. It was observed that from P1 (50 mg·kg^{-1}) to P4 (250 mg·kg^{-1}) concentration the level

of proline was high in comparison to blank; whereas at P5 (300 mg·kg^{-1}) concentration the content was lowest in comparison to blank and other concentrations. Statistical analysis showed significant decrease with P value less than 0.05 for phenolic content. As increased CIP concentration can directly disturb the plant metabolism and synthesis of phenolic compounds. The presence of high level of phenolic compounds indicates that these compounds help in promoting the antioxidant activity.[46] It is stated that phenols during lipid peroxidation scavenge active oxygen species and halt the radical chain reaction, hence preventing oxidative damage.[46]

Catalase Activity (CAT) in Presence of Ciprofloxacin

Reactive Oxygen Species (ROS), produced as harmful by products of plant metabolism contributes as abiotic stressor for plant. Plants are able to regulate their ROS levels through the action of antioxidative enzymes like catalase (CAT).[45] Catalase is considered as one of the essential antioxidant enzymes that breaks down hydrogen peroxide (H_2O_2) into water and oxygen, thus protecting cells from oxidative damage.[48,49] As depicted in Fig. 6(e), at P2 and P3 concentrations i.e. (100 mg·kg^{-1}) & (200 mg·kg^{-1}), highest catalase activity was observed. The increased catalase activity helps the plant to cope up with the escalating stress levels due to antibiotic toxicity and maintain cellular homeostasis.[48] With $p<0.05$, it was determined that the data were statistically significant.

Bioaccumulation of Ciprofloxacin by Basil

Ciprofloxacin standard graph was prepared by applying different CIP concentrations to silica gel plate, and then analyzing the chromatograms under ultraviolet light. At UV range between 100–400 nm, CIP showed high absorption and highest peak at λ = 254 nm. After completion of 4 weeks the presence of CIP in the samples of plant was evaluated to determine *O. basilicum* potential for remediation (Fig. 7). As previously mentioned, silica gel plate was utilized for quantification research and the retention factor calculated was 0.63. The total amount of CIP in the root and shoot was assessed independently, and a greater amount was found in the shoot than the root, confirming the plant's potential for remediation. The mechanism which is commonly used by plant for bioaccumulation of contaminants is phytoextraction. It is also known as Phytoaccumulation. It is the most effective and broadly used technique for phytoremediation at the commercial level. Phytoextraction mechanism is mostly used to treat soil contaminated with both organic and inorganic

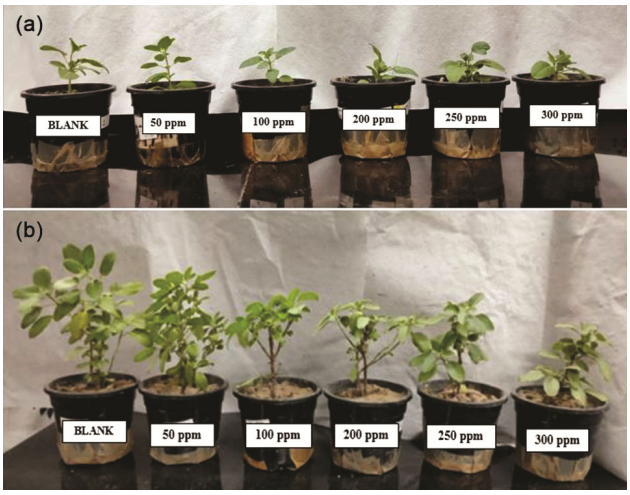

Fig. 7 — Comparison of *O. basilicum* growth (a) before and (b) after 4 weeks of remediation experiment at different concentrations of ciprofloxacin in soil

contaminants.[50] This mechanism involves the growth of tolerant plants in contaminated soil; the plant absorbs large amounts of contaminants from the soil and deplete it and translocate them to the aerial parts of the plant.[51] Phytoremediation techniques like phytoextraction are effective for the remediation of large-scale contaminated areas at the minimum cost.

To determine if plants are capable of absorbing antibiotics, the usage of translocation and bio-concentration factors have demonstrated to be an efficient approach. The Bioaccumulation Factor (BCF) and Translocation Factor (TF) greater than 1.0 indicated Basil's potential for hyper-accumulation of CIP. The Translocation factor for all the concentrations was found to be greater than 1. It has been reported that plants with TF value lower than 1 accumulate antibiotic in roots whereas plants with TF value greater than one, antibiotic accumulates in stem and leaves. Numerous reports indicate that plant species with BCF>1 are hyperaccumulators, while those with BCF<1 are considered excluders. The assessment of *O. basilicum* for accumulation of CIP is illustrated in Table 1. In this study, the BCF values were near to 1 which suggests that *O. basilicum* can be a good hyperaccumulator for ciprofloxacin. The result revealed that high amount of CIP accumulated in the shoot and leaves of the plant. Thus, the current study demonstrates that *O. basilicum* efficiently translocated antibiotics from root to shoot, and that a significant amount of CIP was detected in the shoot while no CIP was detected in the soil, demonstrating the plant's potential for phytoremediation of antibiotics.

Table 1 — Ciprofloxacin accumulation in roots and shoot, translocation factors, bio-accumulation factors

Conc. of CIP ($mg \cdot kg^{-1}$)	Initial CIP amount in soil (mg)	After 4 weeks accumulation in shoot (mg)	After 4 weeks accumulation in root (mg)	Translocation Factor (TF)	Bioaccumulation factor (BCF)
P1 (50)	15	11.45	1.38	8.29	0.85
P2 (100)	30	26.29	1.98	13.27	0.94
P3 (200)	60	53.09	2.18	24.35	0.92
P4 (250)	75	56.15	3.79	11.45	0.79
P5 (300)	90	14.03	11.54	1.21	0.39

Table 2— Ciprofloxacin degradation in *O. basilicum*

Conc. of CIP ($mg \cdot kg^{-1}$)	Initial CIP amount in soil (mg)	Total CIP accumulated in plant (mg)	Degraded CIP content (mg)
P1 (50)	15	12.83	2.17
P2 (100)	30	28.27	1.73
P3 (200)	60	55.27	4.73
P4 (250)	75	59.94	15.06
P5 (300)	90	25.57	64.43

Remediation Potential of Basil for Ciprofloxacin

For evaluating remediation potential in control and treated plants, three replicates (n = 3) were set up and average values were recorded. The recorded trend of percentage remediation was P2>P3>P1>P4>P5. An increasing trend was observed in the percentage remediation up to the concentration of 100 $mg \cdot kg^{-1}$ as 93.81% and thereafter not a slight decline was seen Fig. 8. As at 200 $mg \cdot kg^{-1}$ concentration there was 92% remediation followed by 79% remediation at 250 $mg \cdot kg^{-1}$. According to this study, *O. basilicum* is a plant species that is tolerant of ciprofloxacin and an efficient CIP hyperaccumulator. Major amount of CIP was accumulated in *O. basilicum*. The initial concentration of CIP which was added in soil, accumulated in plants and degraded into transformation products is summarized in Table 2. According to reports, plants initially absorb CIP through gas exchange, aqueous and lipid channel absorption, among other mechanisms, and then degrade it through enzymatic degradation in plant tissues using mono-oxygenases and mixed function oxidases in plant as part of cell processes.[52,53] Basil's ability to produce secondary metabolites and antioxidants also contributes significantly to the phase II (degradation of organic pollutants) of plant metabolism. CIP can mix with enzymes and secondary metabolites, according to the green liver model, and either be totally destroyed or transformed into compounds that are relatively less harmful to plants than the parent compound.[54,55]

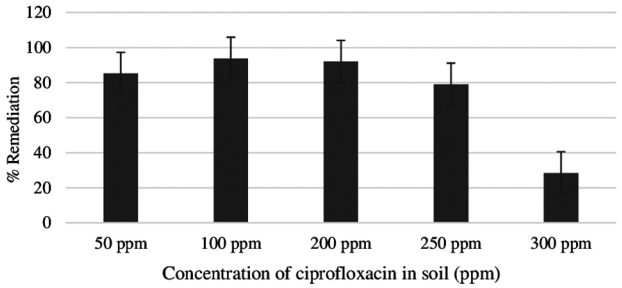

Fig. 8 — Percentage remediation (n = 3) in *O. basilicum* exposed to different concentrations of ciprofloxacin in soil

Conclusions

The current research is the first to prove that *O. basilicum* (basil) has a higher capacity for ciprofloxacin (CIP) phytoremediation. *O. basilicum* displayed high levels of CIP tolerance in response upto 300 $mg \cdot kg^{-1}$ concentration in soil. Highest percentage remediation was observed at P2 concentration i.e., 100 $mg \cdot kg^{-1}$. Plants exposed to ciprofloxacin at 100 and 200 $mg \cdot kg^{-1}$ concentrations experienced a complex stress response, which includes increased production of chlorophyll, carotenoids, flavonoids, proline, phenol, and catalase as defensive adaptations. However, because ciprofloxacin interferes with cellular functions and nutrient intake, this increased stress tolerance is associated with a decline in biomass and growth. These findings emphasise how plants respond to environmental pollutants in a dual way by balancing protective mechanisms against growth inhibition. This research presents a safe, cost-effective, and environment friendly method for antibiotics phytoremediation.

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