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A Sustainable approach for Phytoremediation of Amoxicillin using *Ocimum basilicum*

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Abstract

The current study aims to evaluate the remediation potential of *Ocimum basillicum* for amoxicillin and its toxicological impact on plants. *Ocimum basillicum* was grown with 200 mg kg^{-1} to 600 mg kg^{-1} amoxicillin in soil. After 4 weeks of analysis, its content in root, shoot and soil was analyzed by HPTLC. *Ocimum basillicum* showed 48.3 % remediation capability with 200 mg kg^{-1} of amoxicillin. Secondary metabolites analysis was also performed by GC-MS for toxicity analysis. linalool oxide, menthol acetate, para- methoxy cinnamic aldehyde and caryophyllene oxide completely degraded in plants due to toxicity caused by amoxicillin. Current study also assessed the toxic effect on photosynthetic pigments along with antioxidant enzymes of plants, where decreased level of catalase (41.09–29.88 $Umin^{-1}$ g^{-1}) and glutathaione peroxidase (43.76–40.58 $Umin^{-1}$ g^{-1}) was observed. *Ocimum basilicum* is reported to have ability to remediate heavy metals from the soil, therefore this study investigates its ability to remediate antibiotic. Hence, overall results indicate that, the phytoremediation rate shown by *Ocimum basilicum* is very promising and amoxicillin also showed its toxicological impact on plants.

Keywords: Amoxicillin, Phytoremediation, Green leaf volatiles, Antibiotics, GC-MS, HPTLC.

Introduction

Antibiotics reach the environment from agriculture, pharmaceutical waste and also from wastewater treatment plants. It is reported that antibiotics are widely used in human and veterinary medicine for the health treatment. There use is also rising in animal food for their growth promotion. It is reported that, use of antibiotics increased about 65% during the time period 2000 – 2015 and 36% increase was reported during 2015–2016 (1). Total use of 5 million kg of antibiotics is reported globally (2), from this 3.5 million kg used for therapeutic purposes while remaining 1.5 million kg used in animal food as a growth promoter (3). USA and some European countries are principal users of antibiotics in animals food and U.S. Food and Drug Administration (FDA) reported that 14.6 million kg. of antibiotics were used in animals in 2012 in USA (4). Antibiotics reach the environment indirectly via animal manure when it is used in agriculture as a nutrient source and directly when un-metabolized antibiotics are excreted from the animal body via feces and urine into the environment (5). Presence of these compounds in environment may lead to serious environmental problems as well cause an ecological risk. The antibiotics released into agricultural lands are uptaken by the food crops (6). The consumption of these crops contaminated with antibiotics may cause risk to human health. This has became a matter of great concern (6).

High concentrations of amoxicillin (AMX) up to 1670 ng L^{-1} and 900 ng L^{-1} has been found in surface water and hospital effluents (7). It is also reported that, from 28 mg L^{-1} to 82.7 mg L^{-1} concentrations of amoxicillin was detected in waste water while up to 48 ng L^{-1} reported in the surface waters of North Rhine-Westphalia region as well (8). In Kumamoto city, Japan about 100–3,000 ng L^{-1}(9), 280 – 6,940 ng L^{-1} of amoxicillin concentrations range was reported in Australia (10). Moreover, in Brazil alone amoxicillin 6 billions units of amoxicillin has been used between 2007–2011 (11). Thus, we can say that presence of antibiotics is an emerging environmental concern and hence need to monitor the toxic effect and work towards removal of these substances from environment has increased.

Amoxicillin (AMX) is a modified form of ampicillin having β-lactam structure and penicillin class antibiotic. Amoxicillin is reported to effective against Gram-positive and Gram-negative bacteria and used to treat respiratory, gastrointestinal, urinary and dermal bacterial infections (12). Molecular weight of amoxicillin is 365.4 g mol^{-1}, log KOW value 0.87, Pka values are 2.8, 7.2 and water solubility is 3430 mg l^{-1} respectively (13). Amoxicillin is usually sold in the market as Allmox, Amoxil, Amoxipen, Amoxyn, Trimox, Bactox etc.

Phytoremediation is an emerging, eco-friendly and sustainable removal method to decontaminate soil and water polluted with various environmental pollutants. The main theory behind phytoremediation is that plants uptake contaminants from environment and convert them into less toxic forms. *Ocimum basilicum L.* belongs to the genus of *Osmium L.* and family lamiaceae and it is widely used as medicinal and industrial plant. *Ocimum basilicum* is well known for its antioxidant enzymes production capability in stress condition which helps to

degrade organic pollutants in plant. Remediation through plants is a more sustainable and feasible approach compared to any other methods because it not only remediate but improves the quality of soil. Zahedifar et al., (2019) [14] used *Ocimum basilicum* for removal of heavy metals and plant showed high tolerance against heavy metal but impact of amoxicillin on plants and its remediation is not reported yet. To our best knowledge, this is the first study for removal of amoxicillin using *Ocimum basilicum* and toxicological impact of amoxicillin on plants.

Material and methods

Chemicals and reagents

The ultra-pure grade amoxicillin tridydrate with purity up to 99% ($C_{16}H_{19}N_3O_5S$) (CAS NO. 26787-78-0) purchased from Hi-Media. Methanol (Merk india) was used in plant extraction. All stocks and working solutions used in experiments were prepared in distilled water. The pH of amoxicillin dilution was kept between 6.5 - 7.3 measured by PHM95 pH Meter (Fisher Scientific).

Plants Growth conditions in green house

The concentration range of amoxicillin in this study has been selected after studying various experiments and the concentration of amoxicillin reported in the environment. A concentration of amoxicillin in an environment ranging from 1 µg/L to 200 mg/L reported by Hillis et al., (2011)[7] and Litskas et al., (2018).[6] Therefore, the lower concentration was 200 mg kg^{-1} selected in this study. Higher concentrations of 400 and 600 mg kg^{-1} were also taken to understand remediation potential of plant against high antibiotic exposure. Three weeks old saplings of *Ocimum basilicum* were planted with various concentrations of amoxicillin and grown for 4 weeks in 1 kg of soil placed in porous pots (15 cm diameter 14 cm deep). These pots were designated as A1, A2 and A3 respectively. The working solutions of amoxicillin (200 mg kg^{-1} - 600 mg kg^{-1}) were prepared from 1000 ml of stock solution and poured uniformaly into soil around the root at first day. These plants were grown in greenhouse under controlled conditions at 36^0C temperature the 12:12 hr light: dark. Two control pots were also set: first, negative control (no antibiotics + with plant) and second, positive control (with 600 mg kg^{-1} antibiotic + no plant). The negative control was prepared to compare the growth of plants with and without amoxicillin and positive control was prepared to assess the degradation (photodegradation and hydrolysis) of amoxicillin in soil. Three individual sets (n = 3) were established to account for error and experiment was repeated 3 times and data were calculated. All reduction data calculated by the % formula given below;

---------- (1)

$$\% \text{ Reduction} = \frac{A-B}{A} X\ 100$$

Where A is the initial parameter of the experiment and B is final.

Toxicity assessment

The Biochemical analysis was done after 4W of experiments to examine toxicity impact of amoxicillin in plants. Present study revealed the induction of phytotoxic effects of various concentrations of amoxicillin by the variation in root, shoot length and weight of plants. To evaluate the phytotoxic effect of amoxicillin on plant growth, root, shoot length and weight of plants was measured. Antibiotic effects on photosynthetic pigments such as: Chlorophyll a, b and total Chl, Carotenoid, was tested to collect information towards antibiotic exposure on plants. Total Flavonoid content, antioxident enzyme (Catalase and Glutathione peroxidase) and secondary metabolite estimation was also carried out to monitor the toxicity of amoxicillin present in soil.

Root, shoot length and biomass analysis

All plant samples were uprooted and washed with distilled water at the end of 4 weeks and root and shoot length were analyzed by using the standard centimeter scale. They were thenair dried at 34 °C and fresh and dry weight was taken.

Secondary metabolite analysis in plants

Extraction was done using Soxhlet apparatus and Rota evaporator with 5g dry plant, 200 ml of 80 % methanol and the extract was collected in the distillation flask. Secondary metabolites were analyzed by method described by Redfern, et al., (2014).[15] The GC-MS used auto system XL GC fitted with Equity-5 (60 m x 0.32 mm and film thickness 0.25 µm) fused silica capillary column coupled with Perkin Elmer turbo mass fitted with Equity- 5 capillary column. The extract was analyzed with column temperature at 290°C and a rate of 3^oC/min. Hydrogen was used as a carrier gas at 10 psi column head pressure, the injector temperature was 280^oC and detector (FID) temperature was 290°C. The column temperature was programmed at 300°C at a rate of 3°C /min using helium as carrier gas. MS were taken at 70 eV with a mass range of 40-450 amu with 220^oC injector temperature and 0.5 µl extract volume were used for analysis. Secondary metabolites were identified by comparison of their mass spectra with those obtained from authentic compounds from the NIST/NBS,Wiley 575 libraries.

Chlorophyll and Carotenoid estimation

The chlorophyll (Chl) content was estimated using the method of Arnon (16). 1 gm of plant shoot was homogenized with 10 ml of 80% (v/v) acetone followed by centrifugation at 5000 rpm for 10 minutes. Optical density of supernatant was measured at 645 and 663 nm.Chl a and Chl b and total Chl was estimated using the formula:

$$\text{Chl a} = \{\text{abs}(663)\ X\ 12.7\} - \{\text{abs}(645)\ X2.69\}$$

$$\text{Chl b} = \{\text{abs}(645)\ X\ 22.9\} - \{\text{abs}(663)\ X\ 4.68\}$$

$$\text{Total Chl} = \{\text{abs}\ (645) * 20.2\} - \{\text{abs}\ (663) * 8.02\ \}$$

For carotenoid estimation absorbance was measured at 480 and 510 nm and results were analyzed for estimation of change in carotenoid content with the increasing amoxicillin concentrations.

Estimation of catalase

0.5 gm plant leaves were homogenized using mortar pestle with 5 ml of 100 mM potassium phosphate buffer (pH 7.0) at 0°C and it was then centrifuged at 15000 rpm for 20 min. 1.2 ml hydrogen peroxide (150 mM) and 1.5 ml of phosphate buffer (100 mM) were added along with 300 µl of plant extract and absorbance was noted at 240 nm (17). Phosphate buffer was taken as a blank. Three readings were taken in every minute for every set.

$$\text{Catalase unit activity} = \frac{\text{Change in abs/min} \times \text{Total volume}}{\text{Extinction cofficient} \times \text{volume of samples}}$$

Where; *Extinction coefficient= $6.93 \times 10^{-3} mM^{-1}cm^{-1}$

Estimation of Glutathione peroxidase

The plant samples were centrifuged and the supernatant was collected for the assessment of glutathione peroxidase activity (18). 0.5 ml of plant extract was added to a reaction mixture consisting of 2 ml of tris buffer (1M, pH7.0), 0.1 ml of sodium azide (10 mM), 0.2 ml of EDTA (15 mM), 0.2 ml of glutathione (6.3 mM) and 0.1ml of hydrogen peroxide (150 mM). Reaction mixture was incubated at 37°C for 10 minutes along with a tube containing all the reagents except sample. Reaction was stopped by addition of 10 % TCA (Trichloroacetic acid solution) and absorbance was noted at 436 nm.

Remediation assessment

Remediation of amoxicillin was calculated in present study to check the remediation potential of *Ocimumbasilicum*. Phytoremediation potential of the plant calculated by the amoxicillin present in root and shoot, and remaining concentration of antibiotics in soil.

Quantification of amoxicillin in plants

All plant samples were air dried at 36 – 40°C for 4 days and separated into root and shoot. 10 mg of dry root and shoot were macerated in 100 mL of 80 % methanol for 2 days at room temperature in falcon tubes, and then shaken at 2000 rpm for 20 min (19). The extraction process was done 3 times and the extracts were applied to HPTLC (High performance thin layer chromatography) plates for quantification study.

Standard preparation

For standard preparation, stock solution of amoxicillin having 0.5 mg/ml (W/V) of concentration was prepared in pure methanol and 1.0 µl applied on aluminum silica gel plates (Merck HPTLC plates) and R_f (Retention factor) was calculated. In mobile phase plate were developed with the mixture of Ethyl Acetae : Water : Acetic acid (6:2:2 v/v/v) . Silica gel 60F 254 RP (size 100.0 mm × 100.0 mm, thickness 0.2 mm, length: 8.0mm) and aluminum backed RP-18W plates (size 10 cm × 10 cm, thickness 0.25 mm) was used in this experiment. Server vision CATS-server-PH, Version 2.5.18262.1 software was used. TLC plate was coated with 10% aqueous EDTA for amoxicillin bands visualization. These were placed into a heating plate (110°C) until all solvent evaporated completely. Further investigations were done using UV in deuterium lamp in ultraviolet region. After experiment, quantification of amoxicillin was done on the basis of R_f value. It was 0.33 in HPTLC for amoxicillin.

Quantification of amoxicillin in soil

5.0 g of soil samples were collected from control (setup without plant) and experimental setup (200 mg kg^{-1} to 600 mg kg^{1}). Soil samples were crushed andthen air dried at 36-40°C for 2 days. After that 0.1g of air dried soil samples was mixed with 10 ml of 100 % methanol in volumetric flask. After mixing, 0.5 µl of samples were applied in silica gel plate and retention factor was calculated for quantification of amoxicillin in soil. Moreover, Cation exchange capacity was measured by the displacement of cations with ammonium acetate (20), Organic carbon was assessed by Walkley and Black (1934)[21] method, nitrogen and potassium were assessed by method of Subbiah and Asija (1956)[22] and phosphorous was evaluated by the molybdo-phosphate blue method (23).

Statistical analysis

All experiments was performed in triplicate (n=3). The mean and standard error (SE) of plant growth and amoxicillin concentration in plants and soil for all samples were calculated and mean values of all samples were presented. One way ANOVA was performed by excel to test the significant difference of the means of the control and treated variables (all samples) at 95 % confidence of intervals. The t-test ($p < 0.05$) was also performed to see the significant difference between antibiotic content in control and treated soils and plants.

Results and Discussion

Root and Shoot length of plant

It has been found that, increasing concentrations of antibiotic in soil significantly affect the growth of plants and thus, plants not continued to grow effectively. Presence of amoxicillin showed toxic effect on root length it showed a decrease of 13.26 %, 14.39 % and 18.1 % in A1, A2 and A3. Similarly, shoot length also decreased by 11.3 % in A1, 9.67 % in A2 and 12.63 % in A3 plant (Figure 1). These finding confirm that root length is one of the most sensitive indicators of phytotoxicity.

It is reported that, in presence of amoxicillin (concentration of 10,000 µg/L) significant decrease was found in root length of *Daucus carot* (7). Root activity

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decreases with high antibiotic concentrations, because antibiotics influenced folic acid synthesis and catalyzed ATP-dependent DNA super coiling to prevent the growth of new cells inplant roots (24).

Fresh weight (FW) and dry weight (DW) of root and shoot

The fresh and dry weight of root and shoot of plants was analysed. This was found to be affected in all sets (A1 – A3) due to amoxicillin toxicity. Presence of concentration of amoxicillin (600 mg kg^{-1}) showed maximum toxic effect on FW of root in A3 and it was seen to reduce by 21.07 % while in case of A1 and A2 only 2.91 % and 2.81 % reduction was found (Figure 2). The decrease in the FW of root was in the order: B > A1 > A2 > A3 and root DW was B > A1 > A2 > A3. The FW and DW of root was not significantly different in all the samples as compared to blank.

Furthermore, the highest FW of shoot was in A1 and due to amoxicillin toxicity FW of shoot reduced by 3.9 %, 14.3% in A2 and A3. Highest DW of shoot order was in B > A1 > A2 > A3. Hillis et al., (2011)[7] also explored the toxic effect of amoxicillin in which 1 mg/kg of amoxicillin in soil showed toxic effect on plant weight (7). The FW of shoot showed not significant difference but in case of DW significant difference was found.

Secondary metabolites of Ocimum basilicum

Secondary metabolites are the group of phenolic compounds, which help in removing free radicals and inhibit hydrolytic and oxidative enzymes (25). 20-27 compounds were found in *Ocimum basilicum* grown with amoxicillin (A1 – A3) while 27 were found in blank (Table 1). The compounds like Levomenthol (14.32 %), Germacrene-B (77.53 %), Alpha-Cubenene (0.18 %) and Delta-cadinene (0.05 %) showed highest percentage in blank and due to amoxicillin toxicity it degraded in A1, A2 and A3. Similarly, Iso menthone also showed its highest percentage (0.62 %) in blank and due to toxicity of amoxicillin it degraded in A1, A2 and A3. Bicyclo Germacrene completely degraded in A1, A3 while it was 0.9 % and 0.8 % in blank and A2. Similar observation has been found in Beta – sesquiphellandrene, where it was also completely degraded in A1 and A3 while 0.04 % and 0.05 % in blank and A2.

Some compounds showed increase in presence of amoxycillin. Linalool oxide was increased in A3 (0.66 %) while 0.06 % was present in blank. Similarly, menthol acetate also increased from 0.04 % to 0.06 % (B – A3), Para - methoxycinnamic aldehyde increased from 0.11 % to 0.86 % and caryophyllene oxide increased from 0.08 % to 0.52 %. It is reported that green leaf volatiles are sensitive indicators of antibiotic stress in which Opris et al., (2012)[26] also found the enhancement in green leaf volatile at 1.5 mg L^{-1} concentration of amoxicillin during their research work. Beta-Ocimene also increased in A1

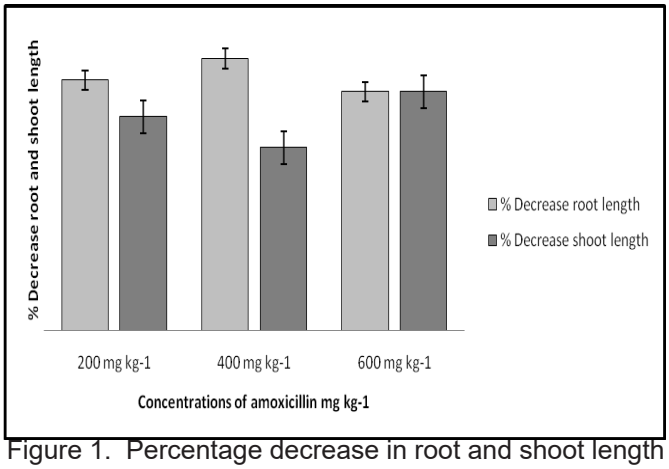


Figure 1. Percentage decrease in root and shoot length of plant. Statistical analysis separately done for % decrease root and shoot length (n=3).

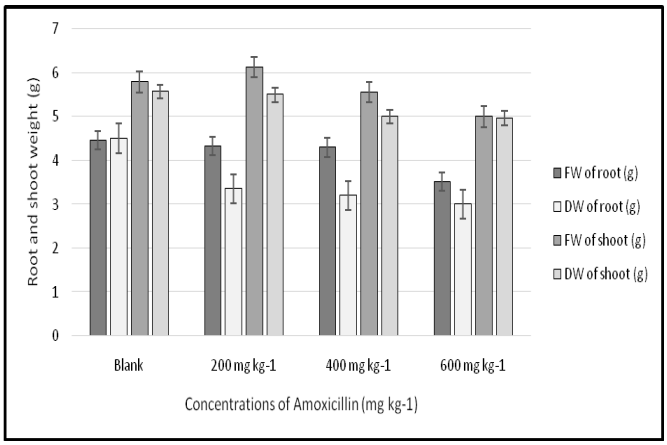


Figure 2. Effect of amoxicillin on shoot fresh weight (FW), shoot dry weight (DW), root fresh weight (FW), root dry weight (DW) after four weeks.

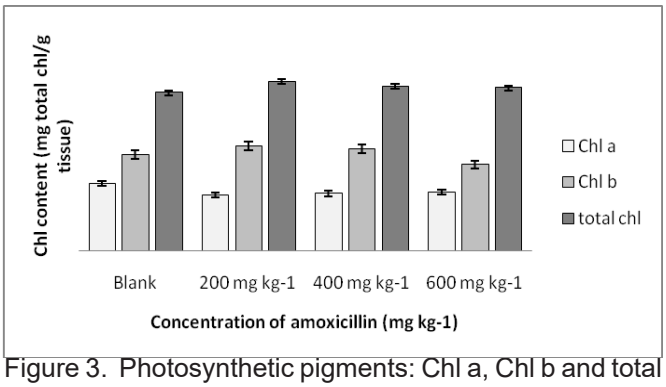


Figure 3. Photosynthetic pigments: Chl a, Chl b and total Chl (mean ± SD, n=3) in Ocimum basilicum exposed to different concentration of amoxicillin in soil.

and A2 as compared to plant grown without amoxicillin. Linalool was also increased in A1 (13.73 %), A2 (13.8 %) and A3 (13.23%) while it was only 0.54 % present in blank. Other metabolites such as L-Menthone increased in A3 (0.14 %) while it was 0.03 % present in blank. Similarly Estragole also increased from 0.16 % to 78.27 %, Z-Citral increased from 0.9 % to 0.56 %, Germacrene-D from 0.06 % to 0.34 %, Alpha-Copaene from 0.01 % to 0.04 %, Trans-Alpha-Bergamotene from 0.06 % to 0.8 %, Alpha – Humulene also from 0.05 %

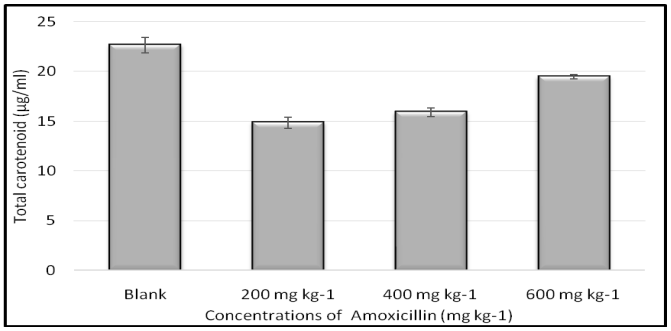


Figure 4. Carotenoid (mean ± SD, n=3) in Ocimum basilicum exposed to different concentration of amoxicillin in soil.

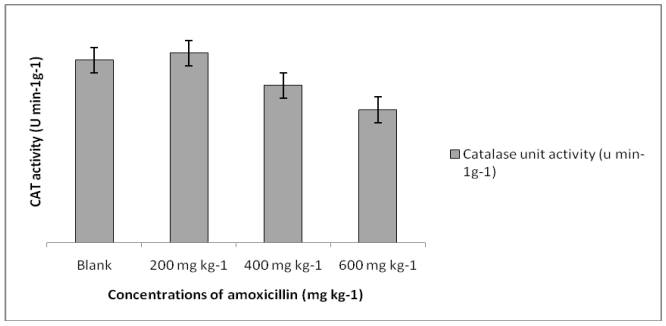


Figure 5. Catalase unit activity (mean ± SD, n=3) in Ocimum basilicum exposed to different concentration of amoxicillin in soil.

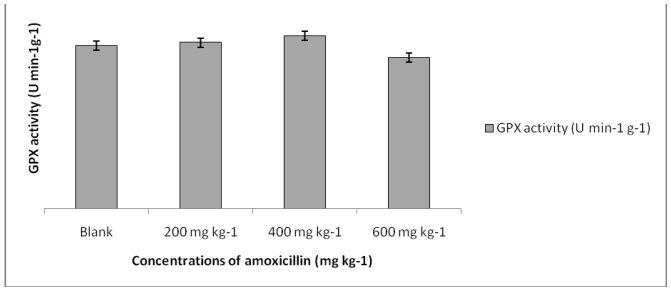


Figure 6. Glutathione peroxidase activity (mean ± SD, n=3) in Ocimum basilicum exposed to different concentration of amoxicillin in soil.

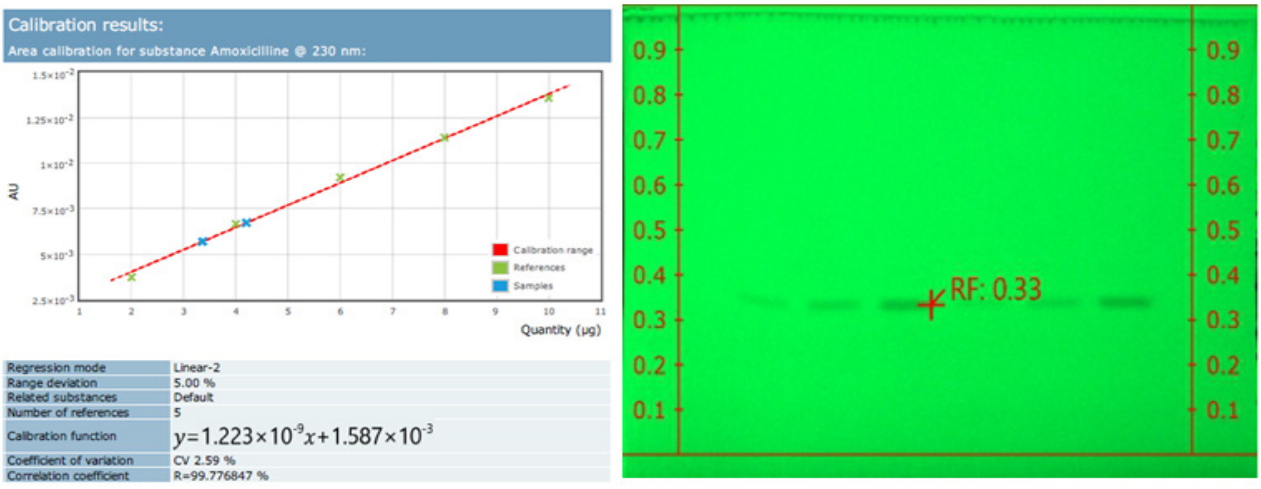
to 0.09 % and Cis-alpha-bisabolene increased from 0.11 % to 2.26 % from blank to A3. 1, 8-Cineole also slightly increased in A1, A2 and A3 as compared to blank sample of plant. It is reported that, Linalool, ocimene isomers, farnesene isomers and methanol are emitted as a defense mechanism (27). An emission of green leaf volatiles in the stressed plants is the result of destruction in free fatty acids in lipoxygenase pathway. Furthermore, It is also reported that Shikimic acid pathway, (28), and methylerythritol pathway are responsible for isoprene, monoterpenoids and sesquiterpenoids production under stress conditions (29-30).

No change was observed in the % of Alpha-Pinene, Beta-pinene and Beta-Bisabolene (A1 – A3). Caryophyllene also remains unchanged in A1 and A2 but slightly degraded in A3 (0.16 %). Whereas, Alpha-humulene remains unchanged in A1 and A2 and slightly increased in A3. Germacrene-D present 0.06 % in blank and no change was observed in its content in A1, A2 and A3.

Photosynthetic pigments in response to antibiotics

The chlorophyll content in the plants is considered as an important parameter used to measure the primary productivity, photosynthetic potentials and even phytotoxicity in plants (31). It is reported that photosynthetic electron transport rateand photosynthetic pigments like chlorophylls and carotenoids, are strongly reduced with amoxicillin (26) .

Current study showed that, the total chlorophyll content of the plants increased as the concentration of amoxicillin increased. Enhancement in total Chl was found in A1 to A3 as compared with the plant grown without amoxicillin (B) similarly Chl b content was also slightly increased in A1 and A2 as compared to B (10.772 mg total chl/g tissue) while in case of A3 Chl-b was slightly decreased (Figure 3). Chl-a content was also affeted in A1, A2 and A3 due to the toxicity of



[A] [B]

Figure 7. [A] X-Axis is the concentration of amoxicillin and Y-Axis denoted area under the peak. [B] HPTLC of amoxicillin on silica gel layers with pretreatment of 10 % EDTA with corresponding UV. Mention scale in a green plate was range taken for the retention factor.

Table 1. Metabolic Profiling of Organic Volatile Compounds affected by amoxicillin in Ocimumbasilicum

		R.T.				M.S.%				F.I.D.%		
Concentrations of amoxicillin	0 mg kg-1	200 mg kg-1	400 mg kg-1	600 mg kg-1	0 mg kg-1	200 mg kg-1	400 mg kg-1	600 mg kg-1	0 mg kg-1	200 mg kg-1	400 mg kg-1	600 mg kg-1
COMPOUNDS NAME												
ALPHA-PINENE	5.46	5.169	5.163	5.163	0.06	0.06	0.06	0.06	0.07	0.06	0.06	0.06
BETA-PINENE	6.427	6.17	6.164	6.17	0.02	0.06	0.06	0.06	0.02	0.09	0.08	0.09
1,8-CINEOLE	6.542	7.709	7.704	7.709	0.06	0.22	0.23	0.59	0.06	0.24	0.24	0.64
BETA-OCIMENE	6.696	8.298	8.293	NIL	0.02	0.14	0.14	NIL	0.02	0.17	0.19	NIL
LINALOOL OXIDE	8.121	NIL	NIL	9.151	0.06	NIL	NIL	0.66	0.05	NIL	NIL	0.77
LINALOOL	1.19	10.41	10.4	10.387	0.54	13.73	13.8	13.23	0.58	18.06	17.88	17.36
L-MENTHONE	8.796	12.601	12.596	12.579	0.03	0.06	0.07	0.14	0.02	0.08	0.08	0.16
ISOMENTHONE	9.712	NIL	13.139	13.088	0.62	NIL	0.09	0.06	0.63	NIL	0.1	0.06
LEVOMENTHOL	11.039	13.523	13.517	13.532	14.32	0.26	0.26	0.86	0.16	0.3	0.3	0.95
ESTRAGOLE	13.299	15.245	15.251	15.205	0.16	78.21	78.38	78.27	0.16	75.06	74.46	74.84
BICYCLO GERMACRENE	14.278	NIL	25.917	NIL	0.9	NIL	0.08	NIL	0.92	NIL	0.08	NIL
Z-CITRAL	14.278	18.283	18.278	18.278	0.9	0.89	0.93	0.56	0.92	1.12	1.19	0.64
GERMACRENE-D	15.359	25.482	25.482	25.482	0.06	0.34	0.34	0.34	0.05	0.3	0.31	0.31
GERMACRENE-B	16.08	25.607	NIL	NIL	77.53	0.12	NIL	NIL	74.69	0.1	NIL	NIL
MENTHOL ACETATE	17.591	NIL	NIL	19.33	0.04	NIL	NIL	0.06	0.31	NIL	NIL	0.05
ALPHA-CUBEBENE	17.677	22.448	21.442	NIL	0.18	0.02	0.02	NIL	0.21	0.02	0.02	NIL
ALPHA-COPAENE	17.751	22.312	22.312	22.317	0.01	0.33	0.34	0.04	0.14	0.25	0.24	0.03
CARYOPHYLLENE	17.677	23.673	23.668	23.673	0.18	0.55	0.55	0.16	0.21	0.48	0.47	0.16
TRANS-ALPHA-BERGAMOTENE	19.965	24.217	24.217	24.217	0.06	0.73	0.73	0.8	0.05	0.56	0.56	0.64
BETA-SESQUIPHELLANDRENE	22.889	NIL	24.429	NIL	0.04	NIL	0.05	NIL	0.03	NIL	0.05	NIL
ALPHA-HUMULENE	23.41	24.686	24.681	24.686	0.05	0.27	0.27	0.09	0.06	0.22	0.22	0.08
TRANS-BETA-FERNESENE	24.24	25.852	25.852	25.482	0.22	0.28	0.34	0.18	0.2	0.3	0.35	0.23
BETA-BISABOLENE	24.755	26.649	26.283	26.288	0.05	0.07	0.07	0.05	0.05	0.06	0.09	0.1
DELTA-CADINENE	24.955	27.175	26.649	26.649	0.05	0.04	0.03	0.03	0.05	0.03	0.04	0.03
CIS-ALPHA-BISABOLENE	25.247	27.175	27.175	27.17	0.11	2.29	2.29	1.26	0.1	1.63	1.61	0.94
PARA-METHOXY CiNNAMIC ALDEHYDE	25.356	NIL	NIL	27.667	0.11	NIL	NIL	0.86	0.15	NIL	NIL	0.5
CARYOPHYLLENE OXIDE	26.786	NIL	NIL	28.131	0.08	NIL	NIL	0.52	0.08	NIL	NIL	0.39
UNKNOWN					0.18	1.33	0.87	1.11	0.18	0.87	1.3	0.94

amoxicillin. Statistical significant difference ($p < 0.05$) was seen in total Chl in between amoxicillin treated and untreated plant. *Ocimum basilicum* does not show any visible symptoms of chlorosis with lower concentrations of amoxicillin (200 mg kg^{-1}– 400 mg kg^{-1}), but with the higher concentration (600 mg kg^{-1}) plants showed the mortality, indicating a toxic effect of amoxicillin. These findings are similar to the study where penicillin's impact on the photosynthetic electron transport rate and change in chlorophyll content was seen (26). The toxic effect of amoxicillin on photosynthesis of *Synechocystis sp.* was also reported by Pan and Deng (2008).[32]

Carotenoid is lipid-soluble antioxidant found in plants which plays a very important role in pigment-binding complexes (33). They also helps to degrade organic pollutant in plants. Highest carotenoid content was found in blank (5.499 µg/ml) and degraded in A1 (2.514 µg g DW^{-1}) and A2 (2.934 µg g DW^{-1}) while slightly increased in (4.788 µg g DW^{-1}) A3. The carotenoid was significantly different ($p < 0.05$) in all the samples as compared to blank (Figure 4). Effect of amoxicillin on carotenoid also reported by Opris and Copaciu,(2012).[26]

Antioxidative enzymes in Ocimum basillicum

The antioxidative enzymes activity of *Ocimum basilicum* was assessed by measuring of catalase activity (CAT) after 4 weeks. The CAT is synthesized in mitochondria, glyoxysome and peroxisome of the plant cells and considered as one of the major enzymes among the stress-indicating metabolites (34). The CAT and other antioxidative enzymes work in a chain reaction as CAT and GPX enzymes reduce the H_2O_2 and that is disrupted by SOD in cells (35).

In the current study, maximum activity (42.7 U min^{-1} g^{-1}) of CAT was recorded in A1, relatively higher than blank setup (41.09 Umin^{-1} g^{-1}) and showed degradation by 35.4 U min^{-1} g^{-1} and 29.88 U min^{-1} g^{-1} in A2 and A3 plant samplesdue to amoxicillin toxicity. Assessment of the enzymatic activity in *Ocimum basilicum* exposed

Table 2. Accumulation of amoxicillin in roots and shoots (mg), Translocation factor (TF), Bioconcentration factor (BCF) and percentage remediation

Sample name (Concentrations of amoxicillin)	Initial amoxicillin content in soil (mg)	Accumulation in shoot (mg)	Accumulation in root (mg)	Amoxicillin content in soil (mg)	TF	BCF	% Remediation
Control (600 mg kg-1)	300			278.11± 271.9			7.33 %
A1 (200 mg kg-1)	100	31.522 ± 20.56	16.815 ± 13.56	Not detected	1.25	64.4	48.3 %
A2 (400 mg kg-1)	200	39.32 ± 34.23	53.94 ± 47.2	Not detected	0.73	58.25	46.63 %
A3 (600 mg kg-1)	300	74.58 ± 67.6	12.93 ± 9.5	Not detected	3.5	32.6	29.17 %

Data presented in mean (n=3) and ± SE.

Table 3. Difference in amoxicillin content

Sample name (Concentrations of amoxicillin)	Initial amoxicillin content in soil (mg)	Total amoxicillin accumulated in plant (mg)	Degraded amoxicillin content (mg)
Blank	0.00	0.00	0.00
A1(200 mg kg-1)	100	48.337	51.663
A2(400 mg kg-1)	200	93.26	106.74
A3(600 mg kg-1)	300	87.51	212.49

to amoxicillin, concluded that antibiotic-induced stress triggered the production of H_2O_2 in treated plant (200 mg- kg^{-1}) thus, catalase activity was affected (Figure 5).

The CAT activity was significantly different in all the samples. At the same time 46.44 $Umin^{-1}$ g^{-1} glutathione peroxidase (GPX) activity observed in A2 (400 mg kg^{-1}) which was relatively higher than untreated plant (43.76 U min^{-1} g^{-1}), A1 (44.66 U min^{-1} g^{-1}) and A3 (40.58U min^{-1} g^{-1}) (Figure 6). The GPX activity was not significantly different in all the samples as compared to blank.

Remediation of amoxicillin by Ocimum basilicum

Standard graph of amoxicillin prepared by applying various concentrations of amoxicillin in silica gel plate and then chromatograms were viewed under UV region. In UV region between 100- 400nm, amoxicillin shows high absorption and highest peak observed at λ = 230 nm. In standard graph, X-Axis is the concentrations of amoxicillin and Y-Axis denoted area under the peak (Figure 7) [A]. A linear relationship between the concentration of amoxicillin and peak areawas derived and correlation coefficient R^2 = 99.77 was achived. As mention above silica gel plate used for quantification study and mention scale in it used to calculate retention factor which was 0.33 calculated.

Total amoxicillin content in root and shoot was calculated separately and large amount was found in shoot as compared to root which represents remediation potential of plant. Trend of % remediation recorded was A1 > A2 > A3. Three replicates (n=3) were used for assessing remediation potential in control and treated plants and average values are presented.This study shows that *Ocimum basilicum* is a tolerant plant species and good hyperaccumulator towards amoxicillin. Several studies have reported that edible crops like *Brassica juncea, B. napus,* and *Zea mays* have shown the phytoremediation potential for antibiotics (36). Our study confirmes that, *Ocimum basilicum* shows its viability as phytoremediation crop even at the high concentration (600 mg kg^{-1}) in green house experiment. Our results are encouraging and further research will be validated by similar work at actual sites in near future. The physical and chemical properties of the soil used in this study was also tested before the experiment. The pH value of the soil (tested at a soil to water ratio of 2:1) was 6.5 ± 7.2. A particle size analysis showed that the texture of the soil used in this study was silty loam type. The cation exchange capacity (CEC) was 62± 56cmol+/kg. An analysis of soil fertility showsthat the soilused in this study having inorganic nitrogen 93.45 ± 85.22 mg/kg, available phosphate was 9.56 ± 21.76 mg/kg and exchangeable potassium 45.5 ± 34 mg/kg was calculated. Organic carbon 0.37 ± 0.09 g/kg was also calculated.

The difference in the amount of amoxicillin accumulated in the plant and present in soil could be attributed to its degradation in plants (values are in Table 3). It is reported that at initial level plant uptakes amoxicillin by gas exchange, aqueous channel and lipid channel uptake, etc. and then degrades them in plant tissues through mixed function oxidases (MFOs) and mono oxygenases mediated enzymatic degradation in plant cell processes (30). The antioxidants/secondary metabolites producing capacity of *Ocimum basilicum* also play a major role in degradation of organic pollutant in plant metabolism (phase (II)). According to green liver model amoxicillin can combine with enzyme/secondary metabolites and either completely degrade or convert

into transformation products which can comparatively less toxic to plants than the parent compound (37).

Translocation factor (TF) and bioconcentration factor (BCF) of amoxicillin in Ocimum basilicum

TF and BCF was calculated by method given by Michelini et al.(2012)[38] in which TF shows translocation of antibiotics from root to shoot while BCF showed total accumulation in plants, calcutalted as followes

$$\text{Translocation factor} = \text{C shoot} \therefore \text{C root} \quad \text{---------- (2)}$$

$$\text{Bioconcentration factor} = \text{C plant} \therefore \text{C soil} \quad \text{---------- (3)}$$

Where C represents concentration (mg kg^{-1}) of amoxicillin. The use of bioconcentration and translocation factors has proven to be effective method for identifying the ability of the plants for antibiotics uptake. Translocation of amoxicillin increasesas the concentration of amoxicillin increases similarly BCF values are also increases. Plants with TF value less than 1 accumulated amoxicillin more in roots and TF values > 1 represent accumulation more in shoots. Here, TF > 1 was observed in A1 (1.25) and A3 (3.5) set while in A2 translocation factor value did not exceed a value of 1. The value of BCF > 1 was found from 200 mg kg^{-1} to 600 mg kg^{-1} concentrations of amoxicillin in soil. On increasing the concentrationsof amoxicillin in soil led to increase the value of BCF and BCF > 1 also indicates the ability of the growing plants for amoxicillin accumulation. Higher TF and BCF values of amoxicillin found a greater ability of *Ocimum basilicum* to translocate and accumulation of amoxicillin. Hence, current study shows translocation from root to shoot by *Ocimum basilicum* took place easily and large amount of amoxicillin found in shoot and no amoxicillin found in soil showing that the plant has phytoremediation potential for antibiotic.

Conclusion

To the best of our knowledge, this is the first work reporting the ability of *Ocimum basilicum* to remediate amoxicillin. Many studies have reported the phytoremediation potential of *Ocimum basilicum* for heavy metals but no study has been reported so far for antibiotics. The current study confirmes, the use of *Ocimum basilicum* for antibiotics remediation is sustainable and environmental friendly. On increasing concentration of amoxicillin in soil, *Ocimum basilicum* showed high tolerance levels against amoxicillin. All remediation experimental setup of amoxicillin (A1-A3) in greenhouse showed significant remediation rate. Present study also reveled the induction of phytotoxic effects with high drug concentrations as signified by the variation in root, shoot length and weight. In this study it was seen that some compounds like Beta- ocimene, Bicyclo germacrene, Germacrene- B, Alpha- Cubebene, Beta- sesquiphellandrene were totally degraded in A3 (600 mg kg^{-1}) while some metabolites increased, showed dose specific impact. This study shows safe, economically feasible and eco-friendly approach for phytoremediation of antibiotics. This will help further research and establish the use of phytoremediation process for the removal of other pharmacuticals and antibiotics from the soil.

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