Current Trends in Biotechnology and Pharmacy
Vol. 15 (3) 299-314, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI:10.5530/ctbp.2021.3.1

Phytotoxicity of Tetracycline and Amoxicillin on *Vigna radiata* and its Remediation Potential in Hydroponic System

Ekta Bhatt[1], Pammi Gauba[1*]
1.Department of Biotechnology, Jaypee Institute of Information Technology
A-10, Sector-62, NOIDA-201309
Corresponding author: pammi.gauba@jiit.ac.in

Abstract

The current study aimed to evaluate the phytotoxicity of tetracycline and amoxicillin on *Vigna radiata* and its remediation potential in hydroponic system. Germinated seeds of *Vigna radiata* were planted with varying concentrations of tetracycline and amoxicillin (150 – 500 mg L^{-1}) in triplicates (n=3) for three weeks. Toxicity biomarkers, i.e. changes in plant biomass, photosynthetic pigment, phenol, flavonoid content and antioxidative enzymes were estimated after completion of 3 weeks. The results illustrated that high tetracycline concentration (500 mg L^{-1}) in hoagland media resulted decrease in total chlorophyll content (3.045- 2.252 mg total chlorophyll/g tissue) while in case of amoxicillin, chlorophyll content was increased (5.18 - 7.309 mg total chlorophyll/g tissue). Carotenoid, Total flavonoid and phenolic content, were also significantly ($p < 0.05$) reduced due to toxicity of these antibiotics. Antioxidant enzyme like catalase showed 7.22 % degradation in their activity with highest concentration (500 mg kg^{-1}) of tetracycline and 27.3 % degradation was found in case of amoxicillin. Glutathione peroxidase activity was also decreased in both of the cases. Subsequently, the *Vigna radiata* showed 63% remediation potential with tetracycline and 93 % in case of amoxicillin. Hence, overall results indicate that, the phytoremediation rate shown by *Vigna radiata* is very promising and these antibiotics also showed its toxicological impact on plants.

Keywords Tetracycline, Amoxicillin, Flavonoid, phenolic content, Phytoremediation and Vigna radiata.

Introduction

The use of antibiotics is regularly rising in humans and animals since the last century. Antibiotics are generally used to prevent diseases in human and animals and its use is also rising in animals to promote growth, and improve productivity. The antibiotics are not only used as medicine, but also in animal food as a growth promoter. Generally, antibiotics are weakly absorbed in human and animals, and excreted in active form in faeces and urine, which then enters the environment via wastewater and manure. As per reported data, consumption of antibiotics has increased from 21.1 to 34.8 (65%) billion in 2000-2015 period globally [1]. Antibiotics reach aquatic environments via various pathways i.e. domestic wastewater, hospital waste and also from water treatment plants. Wastewater is generally used to irrigate agriculture land; hence antibiotics are accumulated in soil also [2]. Concentration of antibiotics in surface water ranges between 319-3630 μg L^{-1} [3]. In hospital effluents, concentration range between 0.26 - 39.13 μg L^{-1} have been reported in USA. Ahmad et al., (2012) [4] It is reported that, antibiotic con-

Vol. 15 (3) 299-314, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI:10.5530/ctbp.2021.3.1

sumed 11.3–15.7 DDDs per 1,000 inhabitants per day (expressed in defined daily doses) in between 2000 – 2015 [1]. As per reported data, in 2000, consumption of antibiotics was led by France, New Zealand, Spain, Hong Kong, and the United States. Similarly, in 2015 also the main consumers of antibiotics were the United States, France, and Italy. In low- and middle-income countries the leading consumers were India, China, and Pakistan. 2000 to 2015, showed that antibiotic consumption increased from 3.2 to 6.5 billion DDDs (103%) in India, from 2.3 to 4.2 billion DDDs (79%) in China, and from 0.8 to 1.3 billion DDDs (65%) in Pakistan. Global antibiotic consumption in animals was reported to be 63,200 tons in 2010, which is more than human consumption in the same period [5].

Tetracycline and amoxicillin are the most widely used antibiotics for human and animal health, therefore their massive presence is expected in environment. It has been reported that more than 2,500 tons of tetracycline were used annually in Europe in veterinary alone [2]. Concentration of tetracycline in soil ranges between 86–199 **µ**g/kg while up to 6.8 mg/L concentration of tetracycline was reported in water [6]. Moreover, concentration of oxytetracycline up to 305 **µ**g/kg was reported in soil while up to 0.067 **µ**g/L of oxytetracycline reported in water [7]. Concentration of chlortetracycline in water ranges between 0.03 - 0.69 **µ**g/L [8]. Moreover, concentrations of chlortetracycline, oxytetracycline and tetracycline up to 764,000 **µgkg**$^{-1}$, 354, 000 µgkg^{-1} and 98000 µgkg^{-1} was also reported in soil [9].

As compared to tetracycline, concentration of amoxicillin (β-lactams) detected is less in soil and water due to hydrolysis nature of amoxicillin though amoxicillin reported one of the highly consumed antibiotics for human and veterinary medicine. Concentrations of amoxicillin in soil and water data is very limited. As per reported data, amoxicillin concentrations in wastewater ranges between 28 mg/L - 82.7 mg/L while in hospital effluents up to 35,500 ng/L concentration of amoxicillin was reported [10]. Amoxicillin concentration up to 6940 ng/l in wastewater treatment plant was also reported [11]. Tremendous overuse of antibiotics has led to antibiotic pollution that has become a pressing environmental crisis. Therefore, this emerging environmental concern, warns researchers to monitor the toxic effect and work towards the removal of these substances from environment.

Tetracycline (TC) is broad-spectrum polyketide antibiotic and has a hydronaphthacene based structure produced from *Streptomyces aureofaciens.* Tetracycline used to treat infections of the urinary tract, respiratory tract, and intestines. TC is hydrophilic in nature and its aqueous solubility is 1700 and 1000 mg/L, respectively [12]. Molecular weight of TC is 444.4 g mol^{-1} and has a three Pka value 3.3, 7.7 and 9.7. Tetracycline log Kow values are -2.2 to -1.3, which represents its hydrophilic nature. Amoxicillin (AMX) is a modified form of ampicillin has a β-lactam based structure and penicillin class antibiotic reported to effective against Gram-positive and Gram-negative bacteria and used to treat respiratory, gastrointestinal and bacterial infections. Molecular weight of amoxicillin is 365.4 g mol^{-1}, log KOW value of 0.87 and Pka value is 2.8, 7.2 respectively [12].

Vigna radiata L. (mung bean) is a leguminous pulse crop that belongs to the Fabaceae family. It plays a very important role in conservation of agriculture as it contains plant growth promoting bacteria/free living bacteria in its root part. These bacteria enhance the plant growth by producing hormones or by fixing nitrogen. *Vigna radiata L.* contains antioxidant enzymes like flavonoid and isoflavonoid etc. which help to degrade organic compounds taken up by the plant, thereby making it more feasible for phytoremediation. It is reported that, *Vigna radiata* has a potential to tolerate lead stress [13] and the toxic effect of paracetamol and ibuprofen on *Vigna radiata* is also reported [14]. The removal of TC by chickpea (*Cicer arietinum)* is reported by Makhijani et al. (2014) [15] whereas Opris et al. (2012) [16] showed the toxic effect of TC and AMX on wheat (*Triticum aestivum*) but to our

Vol. 15 (3) 299-314, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI:10.5530/ctbp.2021.3.1

best knowledge, phytotoxicity and remediation potential of *Vigna radiata* against TC and AMX has not been studied yet; therefore, this study aimed to assess the toxicity of tetracycline and amoxicillin in *Vigna radiata* and its remediation potential.

Materials and Methods

Chemicals and reagents

The ultra-pure grade tetracycline ($C_{22}H_{24}N_2O_8$) (CAS NO. 64-75-5) and amoxicillin ($C_{16}H_{19}N_3O_5S$) (CAS NO.26787-78-0) was purchased from Hi-Media. Working solutions were prepared in distilled water. Antibiotics purity up to 99 % was used in analytical work during the study. The pH of tetracycline and amoxicillin dilution was kept between 6.5 - 7.3 and measured by PHM95 pH Meter (Fisher Scientific).

Growth conditions of Vigna radiata in laboratory scale

Seeds of *Vigna radiata* (Mung beans) were surface sterilized with 0.1% w/v aqueous mercuric chloride for 5 min to avoid fungal growth and kept for germination on moistened Whatman filter paper in petri plates in growth room for 1 week. Two individual set (n=3) were prepared for tetracycline and amoxicillin at a same day in plant tissue culture lab (PTCL). Four germinated seeds of *Vigna radiata* were grown *in vitro* in Hoagland media (pH 6.5) with various concentrations of TC and AMX in culture tubes for three weeks (3W). Autoclaved (121ºC, 20 minutes, 15 psi) sponge pieces were used to provide support and test tubes covered with cotton plugs. The stock solutions of antibiotics were prepared at a concentration of 1000 mg L^{-1} and working dilutions were made by this. The concentration ranges of both the antibiotics used in this study has been selected after studying various experiments and the concentrations reported in the environment. As per these reports we have selected concentration range from 150 mg L^{-1} to 500 mg L^{-1} [9] [17]. Two control sets were also prepared: first, negative control (no antibiotics + with plant) and second, positive control (with 500 mg kg^{-1} antibiotic + no plant). The negative control was set to compare the growth of plants with and without antibiotics and positive control was prepared to assess the degradation (photodegradation and hydrolysis) of antibiotics in nutrient media. All TC sample were labeled as B, C, T1, T2, T3, T4 and T5 whereas AMX setup labeled as B, C, A1, A2, A3, A4 and A5 respectively. All the sets containing seeds and nutrient media were covered with aluminium foil and placed in the Plant Tissue Culture Lab (PTCL), where the temperature was maintained at 24.8 ºC and photoperiod, 14 h light/10-hrs dark phase and light intensity was, 10,000 lux. Three individual sets (n = 3) were established to account for error and experiment was repeated 4 times.

Toxicity assessment of Vigna radiata in exposure to tetracycline and amoxicillin

After three weeks of experiment two parameters was estimated (i) Toxicity (ii) Remediation.

After 3W roots and shoots of *Vigna radiata* were separated and air dried for 2 days and length of root and shoot was measured. Fresh and dry weight of root and shoot was also recorded after drying in a hot air oven at 65± 2°C for 1 h. The photosynthetic pigment i.e. Chl a, Chl b and total Chl was estimated by Arnon method. (1949) [18] and total carotenoid was assessed by methods describe by Lichtenthaler et al. (1987) [19]. This was done for both tetracycline and amoxicillin treated plants. The activities of catalase (CAT) and glutathione peroxidase (GPX) were measured by methods of Caverzan et al. (2016) [20] and Miao et al. (2006) [21] respectively. Total phenolic content (TPC) and total flavonoid content (TFC) was analyzed according to method developed by Chang et al. (2002) [22]. All data of treated plant compared by the blank sample of plant and percentage of reduction was calculated. Reduction rate was calculated by using following Eq. [23].

$$\% \text{ Removal} = \frac{\text{Initial concentration of antibiotics - Final concentrations of antibiotics}}{\text{(Initial concentrations of antibiotics)}} \times 100$$

Vol. 15 (3) 299-314, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI:10.5530/ctbp.2021.3.1

Remediation analysis

Remediation rate of tetracycline and amoxicillin was calculated to check the potential of *Vigna radiata*. Standard was prepared with HPTLC (High performance thin layer chromatography) for tetracycline and amoxicillin. After standard preparation, phytoremediation potential of plant calculated by the amoxicillin and tetracycline present in root and shoot, and remaining concentration of antibiotics in Hoagland media.

Standard preparation

Standard were prepared separately for TC and AMX. Stock solution of TC having 0.3 mg/ml was prepared with 80 % methanol. Similarly, AMX stock solution was prepared with 0.2 mg/ml with 80 % methanol. Aluminum plates (Merck HPTLC) backed silica gel 60F254 (size 200.0 mm × 100.0 mm, thickness 0.2 mm, Length: 8.0mm) Catalogue no. 5554 was used in. In mobile phase amoxicillin dilutions were prepared with ethyl acetate: water: acetic acid (6:2:2 V/V/V) and for tetracycline, Dichloromethane: methanol: water (5.9:3.5:0.6 V/V/V) were used. Server vision LABSERVER, Version 2.5.18262.1 software was used. TLC plate was coated with 10% aqueous EDTA for TC and AMX bands visualization in plates (silica gel and RP-18W layer). 1.0 µl of antibiotics solutions were applied on HPTLC plate and placed into a heating plate (110°C) until all solvent evaporated completely. Further investigations were done using UV in deuterium lamp in ultraviolet region. The linear regression determined by UV-absorption where wave length range was 190 to 400 nm. After experiment, quantification of antibiotics was done on the basis of R_f value.

Estimation of tetracycline and amoxicillin in Vigna radiata by HPTLC

The *Vigna radiata* root and shoot samples were air dried for 2 days and then macerated in 20 ml of 80 % acetone in falcon tubes, and centrifuged at 2000 rpm for 20 min. The extraction process was done 4 times for each sample and TC and AMX content in root and shoot was separately analyzed with HPTLC (High performance thin layer chromatography). The remaining medium in the culture tube was also collected and stored at −20 °C to check further the residual content of TC and AMX.

Statistical analysis

All experiments were performed in triplicate (n=3). The mean and standard error (SE) in plant growth and antibiotics concentration in plants and media 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 effect in control and plants.

Result and Discussions

Change in biomass and length of Vigna radiata

Presence of amoxicillin in the hydroponic media did not affect the growth of plants at low concentrations and, plants continued to grow while in case of tetracycline growth of plants were affected. The maximum root and shoot length were found in the blank (in tetracycline and amoxicillin set). In toxicity analysis, root showed about 12.5 – 50 % reduction (from B – T5) and in shoot length about 9.09 - 36 % reduction was observed in TC plant samples (Fig.1). In case of amoxicillin about 6.6 - 55 % (B - A5) reduction was recorded in root length and in shoot length 7.69 – 38.4 % reduction was observed due to toxicity of amoxicillin (Fig.2). On comparing both setup (TC and AMX) highest reduction was observed in root and shoot length of TC treated *V. radiata* than AMX. After 3W of experiment it was observed that, Amoxicillin treated plants grew very well with well-developed roots and shoots while in tetracycline treated plant growth was slightly less. Similar observation has been found by Brain et al. (2004) [24] where they found tetracycline was more toxic then amoxicillin in *L. gibba* because tetracycline binds irreversibly to the 30S subunit of ribosomes, blocking the binding of aminoacyl transfer to DNA and then inhibit the protein synthesis. Sharma et al. (2017) [14] also observed the effect of paracetamol and ibuprofen on root, shoot length of *Vigna radiata.* Statistically, not significant difference was found in root length (p=0.615) but in case of shoot length significant difference (p=0.03) was observed in both of the cases (TC and AMX).

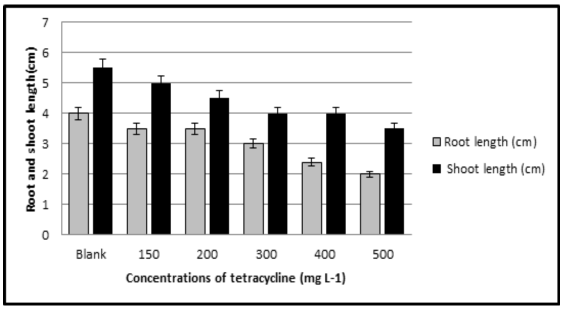

Figure. 1 Root and Shoot length of Vigna radiata exposed with the different concentration of Tetracycline

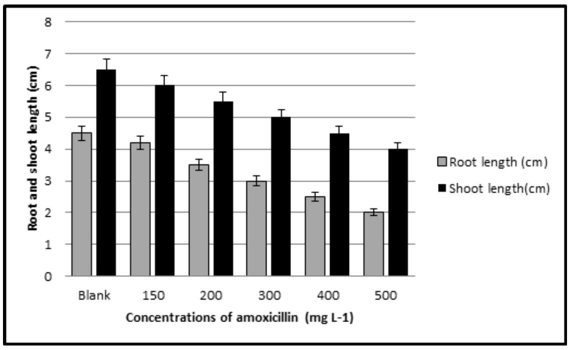

Figure. 2 Root and Shoot length exposed with the different concentration of Amoxicillin

In both the cases (TC and AMX) fresh weight of root and shoot of plants was found to be highest in blank which was decreased with increasing concentrations of antibiotics (150 mg L^{-1} – 500 mg L^{-1}). Similarly, dry weight of root and shoot was also highest in plants grown without antibiotics (blank) in both of the cases and dry weight of root and shoot was also decreased with increasing concentrations of antibiotics (Fig. 3).

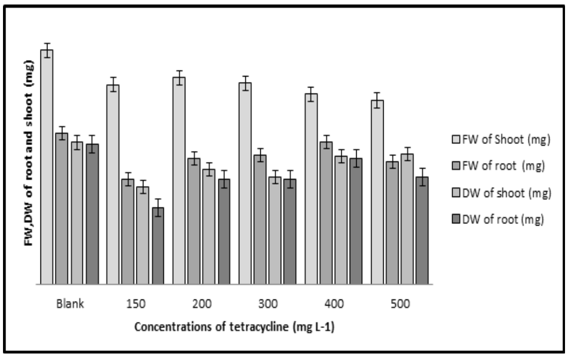

Figure. 3 FW (fresh weight) DW (dry weight) of root and shoot exposed with the different concentration of TC

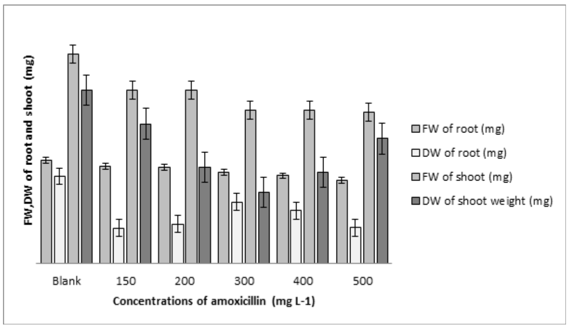

Figure. 4 FW (fresh weight) and DW (dry weight) of root and shoot exposed with the different concentration of AMX

Similar study conducted by the Gujrathi et al. (2005) [25] where they found wet weight of root part of *P. stratiotes* and *M. aquaticum* was decreased by tetracycline toxicity.

Phytochemical toxicity by tetracycline (TC) and amoxicillin (AMX) in Vigna radiata

The photosynthetic pigments are an indicator of toxicity in *Vigna radiata* which was affected by the antibiotic exposure (Fig. 5). In plants grown with TC and AMX, the total Chl content was found to be highest in TC plants as compared to AMX. In TC setup, highest total chlorophyll content was found in blank (7.085 mg total Chl/g tissue) and decreased by 3.25 %, 7.89 %, 8.92 %, 8.86 % and 11.19 % in T1, T2, T3, T4 and T5 as the TC concentrations increases. While, in case of AMX highest total Chl (8.118 mg total Chl/g tissue total Chl) was found

Vol. 15 (3) 299-314, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI:10.5530/ctbp.2021.3.1

in A2 (200 mg L^{-1}) (Fig.6). Tetracycline showed phytotoxic effects on the plants due to the translational inhibition of chloroplast (p)ppGpp synthase [26]. Opris et al. (2012) [16] also reported regarding the toxic effect of TC and AMX on chlorophyll content (Opris et al., 2012). The photosynthetic pigment was found to decrease with increasing concentrations of tetracycline and amoxicillin in *Vigna radiata* (Fig.6) and it might be due to chlorophyll destruction and reduction in chlorophyll synthesis and susceptibility of pigment protein complexes [27].

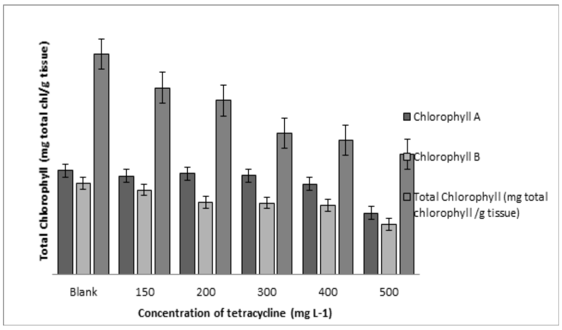

Figure. 5. The changes in photosynthetic pigments: Chl a, Chl b and total Chl, in Vigna radiata exposed to different
Concentration of tetracycline. Data presented in mean ± SD of three replicates (n = 3).

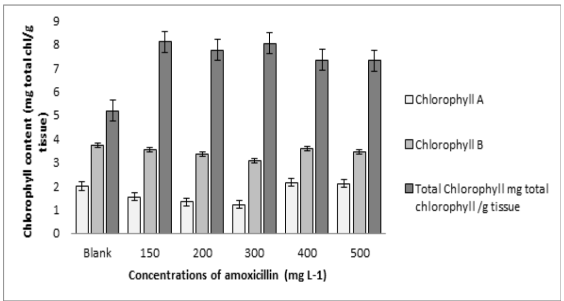

Figure. 6. The changes in photosynthetic pigments: Chl a, Chl b and total Chl, in Vigna radiata.
Data presented in mean ± SD of three replicates (n = 3).

Similar observation was found with Chl a and Chl b which was clearly decreased with the addition of TC. As compared to the blank, reduction percentage of Chl a was 2.25 % to 5.31 % (From T1 –T5) and in Chl b about 7.46 % to 44.9 % reduction was found. As compared to the blank, the maximum inhibition rates for chlorophyll a, chlorophyll b and total chlorophyll, found with highest (500 mg/L) antibiotic concentration. Previous studies suggested that, the toxic organic compounds can inhibit the activities of the aminole-vulinic acid which plays an important role in chlorophyll synthesis. In amoxicillin treated plants, Chl a and Chl b was decreased at the initial (150-300 mg L-1) while with the increasing concentration of AMX content of Chl a and Chl b increased (Fig. 6). In this study high concentrations of TC showed a toxic effect and as a result the plant leaves to turned light green in colour (chlorosis) and plant growth was decreased as compared to plants grown without tetracycline in day wise analysis in T4 and T5. While in AMX treated plant sam-

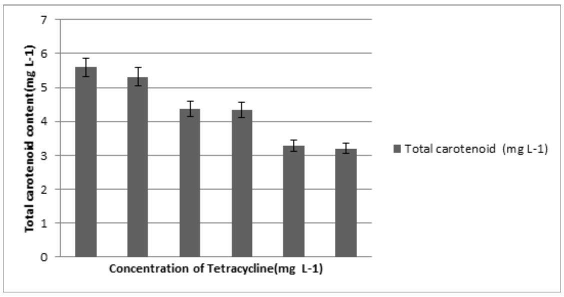

Figure. 7. Total carotenoid content in Vigna radiata exposed to different
concentration of tetracycline. Data presented in mean ± SD (n = 3).

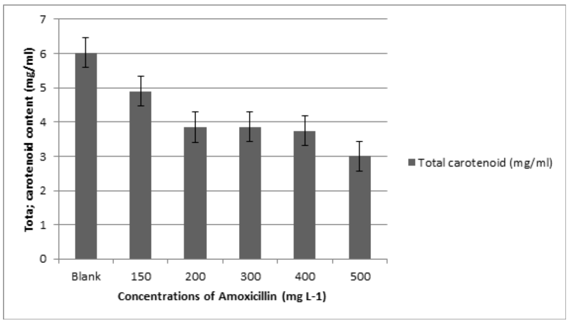

Figure. 8 Total carotenoid content in Vigna radiata exposed to different
concentration amoxicillin. Data presented in mean ± SD (n = 3).

Vol. 15 (3) 299-314, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI:10.5530/ctbp.2021.3.1

ples chlorosis was not found though the growth of *Vigna radiata* was decreased. Statistically significant difference was found in total Chl in tetracycline and amoxicillin (p=.032) but in case of Chl a and Chl b not significant difference was observed (p=.086).

High Carotenoid content was found in tetracycline treated plants as compared to amoxicillin. In tetracycline and amoxicillin setup, highest total carotenoid content was observed in blank plant samples which were clearly decreased with increasing concentrations of these antibiotics. The Carotenoid reduction trend in TC plants was B > T1 > T3 > T2 > T4 > T5 and 3.6 % - 41 % reduction on carotenoid content was found (B - T5) similarly in AMX plants reduction trend was B > A1 >A3 > A4 > A2 > A5 and 18 % - 50 % reduction was found (B - A5) (Fig.7) (Fig.8). In amoxicillin sample slight increase was found in 300 mg/L and 400 mg/L as compared to 200 mg/L set and it is reported that, carotenoid act as an inhibitor of singlet oxygen, and peroxy radicals to eliminate ROS, and this inhibition increase the carotenoid content in A3 and A4 as compared to A2 sample of plant. The study by Di Baccio et al. (2017) [28] where they also observed increase in carotenoid content in duckweed in the presence of pharmaceuticals because of their antioxidant action but to our knowledge effect of these antibiotics (TA and AMX) on carotenoid content of *Vigna radiata* is not reported yet.

Antioxidant activity of Vigna radiata against TC and AMX

Reactive oxygen species (ROS) is an abiotic stress and toxic product of plant metabolism. Plants have a capability to maintain the level of ROS by antioxidative enzymes such as catalase (CAT), and glutathione peroxidase (GPX) [29]. The CAT is produced in mitochondria, glyoxysome and peroxisome of the plant cells and considered as one of the important enzymes for ROS removal in plant metabolism [30].

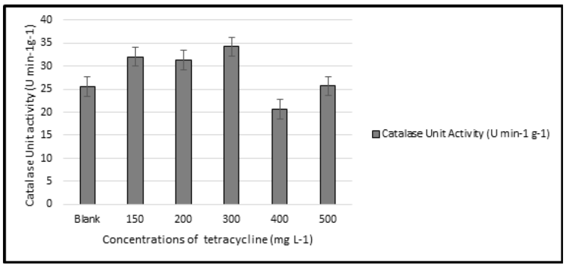

Figure. 9 The changes in the Catalase unit activity (CAT) in Vigna radiata.
Data presented in mean ± SD (n = 3).

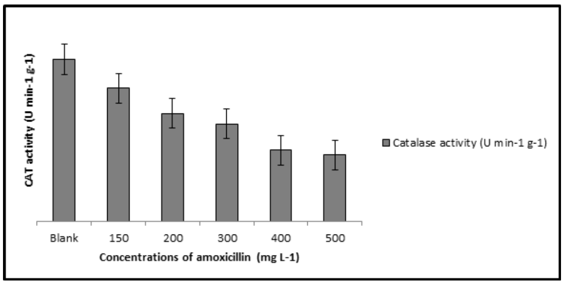

Figure. 10. The changes in the Catalase unit activity (CAT) in Vigna radiata.
Data presented in mean ± SD (n = 3)

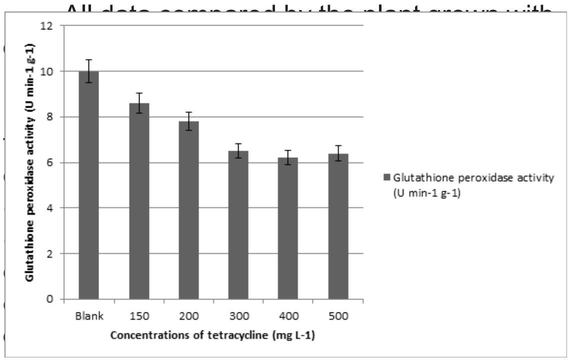

Figure. 11 The changes in the Glutathione peroxidase activity (GPX) in Vigna radiata.
Data presented in mean ± SD (n = 3).

OTC can interact with CAT with one binding site by van der Waals' interactions and hydrogen bonds, and then CAT activity was inhibited. In AMX treated plants, highest catalase was found in blank (48.18-unit min-1 g-1) and 17.9 %, 33.3 %, 39.8 %, 56.2 % and 59.1 % reduction were observed in A1, A2, A3, A4 and A5 due to toxicity of amoxicillin (Fig.10). The reason for this

Vol. 15 (3) 299-314, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI:10.5530/ctbp.2021.3.1

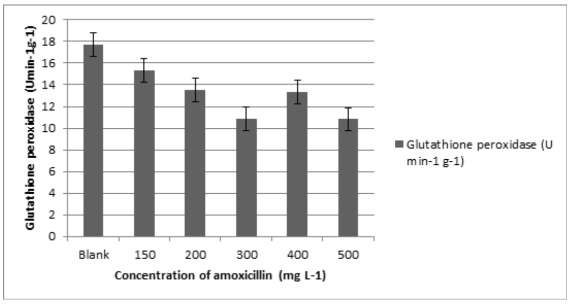

Figure. 12 The changes in the Glutathione peroxidase activity (GPX) in Vigna radiata. Data presented in mean ± SD of (n = 3).

change may toxicity due to antibiotics on antioxidative enzymes. Statistically significant difference was observed in CAT activity (p=0.128) in tetracycline treated plants and in case of AMX plant samples not significant difference was found (p=0.189).

In tetracycline treated plants, glutathione peroxidase activity was highest in blank sample of plants (10.02 U min $^{-1}$ g^{-1}) and with increasing concentrations of tetracycline GPX activity was decreased (Fig.11) and 14%- 36% reduction (T1 –T5) was found. Similar trend was found in amoxicillin treated plants where GPX activity

Table 1. The content (mean ± SD, n=3) of TC in different samples of Vigna radiata (in shoot and root) after completion of experiment (4W). % Remediation, Translocation factor (TF) and Bioconcentration factor (BCF).

S. no.	Samples (Concentrations of TC, mg L^{-1})	Initial TC in media (mg/ml)	TC in shoot (mg)	TC in root (mg)	After experiment TC in H.M. (mg)	TF of tetracycline	BCF of tetracycline	% remediation
1	B (Blank)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	C (Control)	5			4.520			9.6%
3	T1 (150)	1.5	0.315	0.41	0.64	0.6	7.2	48.3 %
4	T2 (200)	2	0.615	0.59	0.80	0.95	7	60.13 %
5	T3 (300)	3	0.294	0.60	0.88	0.24	3.6	29.6 %
6	T4 (400)	4	0.96	1.4	0.81	0.67	6.06	59 %
7	T5 (500)	5	0.744	1.095	1.953	0.57	4.06	36.7 %

Table 2. The content (mean ± SD, n=3) of AMX in different samples of *Vigna radiata* (in shoot and root) after completion of experiment (4W). % remediation, Translocation factor (TF) and bioconcentration factor (BCF)

S.no.	Samples (Con. Of AMX, mg L^{-1})	Initial AMX in media (mg/ml)	AMX in shoot (mg)	AMX in root (mg)	After experiment AMX in H.M. (mg)	TF of amoxicillin	BCF of amoxicillin	% remediation
1	B (Blank)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	C (Control)	5			4.257			14.86
3	A1 (150)	1.5	1.17	0.213	None	1.4	8.7	92.2 %
4	A2 (200)	2	0.321	0.48	0.892	0.22	5.1	40 %
5	A3 (300)	3	0.604	0.63	1.295	0.82	0.48	41.1 %
6	A4 (400)	4	0.564	0.579	1.649	0.56	3.25	28 %
7	A5 (500)	5	2.109	0.537	2.220	1.13	5.76	52.2 %

Vol. 15 (3) 299-314, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI:10.5530/ctbp.2021.3.1

was also highest in blank (17.72 U min $^{-1}$ g^{-1}) sample of plants and 13.6%- 38.9 % reduction trend was found (A1-A5) (Fig.12). As compared to A2 and A3, GPX activity was increased at A4 (400 mg l^{-1}). It is reported that, Glutathione peroxidase is present in various parts of plant cells and selenocysteine is their active site Thus, GPX activity in stress condition is activated to maintain the appropriate H_2O_2 level in stress plant. Moreover, Glutathione is the substrate of glutathione peroxidase reactions and play a very important role in the removal of ROS and thus glutathione was increased in A4. Statistical differences were observed in Glutathione peroxidase activity (p=0.0129) in tetracycline and amoxicillin treated plants (p=0.0154).

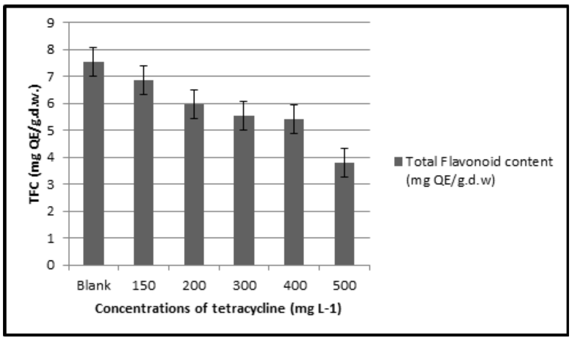

Figure. 13 The changes in the flavonoid (TFC) in Vigna radiata. Data presented in mean ± SD of (n = 3).

Reason for H_2O_2 removal from plants may be due to presence of peroxidases in plant cells. Glutathione peroxidases are heme containing proteins and essential enzymes which are involved in phase I metabolism of organic compounds in plants and showed high activity in stress condition. In phase I organic compounds are combined with peroxidase and converted into its less toxic forms. Singh et al. (2018) [23] has also found a similar kind of observation for removal of amoxicillin (1 mg L^{-1}) by *Spiro delapolyrhiza* grown in Hoagland media.

TPC and TFC in vigna radiata in response of TC and AMX

Total phenolic (TPC) and flavonoid content (TFC) was also estimated as a biomarker of toxicity. TPC was higher in tetracycline treated plant as compared to amoxicillin. TFC standard was prepared with quercetin, Regression equation y = 0.0004x+ 0.2199 and R^2 = 0.9979 was observed. For AMX Regression equation y = 0.0008x + 0.24 and R^2 = 0.9234 was calculated.

In tetracycline treated plants, TFC was found to be highest in blank sample of plant (7.533mg GAE/g D.W.) while with increasing concentrations of tetracycline TFC was seen to decrease (Fig.13). Similar trend was observed in amoxicillin treated plants, where TFC was high-

Table 3. Total TC content in plant (n=3) (on dry wet basis), remaining TC content in Hoagland media (mg by total volume of HM in culture tube), difference of TC (Total TC in plant – degraded TC amount).

Samples (Concentrations of TC, mg L^{-1})	Initial content of TC on media (mg)	Total TC accumulated in plant (mg)	After experiment TC content in Hoagland media (mg)	Degraded tetracycline content (mg)
T1(150 mg L^{-1})	1.5	0.725	0.64	0.135
T2 (200 mg L^{-1})	2	1.20	0.80	-
T3 (300 mg L^{-1})	3	0.894	0.88	1.25
T4 (400 mg L^{-1})	4	2.36	0.81	0.83
T5 (500 mg L^{-1})	5	1.839	1.95	1.211

Vol. 15 (3) 299-314, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI:10.5530/ctbp.2021.3.1

Table 4. Total average of AMX content in plant (n=3) (on dry wet basis), remaining AMX content in Hoagland media (mg by total volume of HM in culture tube), difference of AMX (Total AMX in plant -degraded AMX amount).

Samples (Con. Of AMX, mg L^{-1})	Total TC in plant (mg)	After experiment AMX content in Hoagland media (mg)	Degraded tetracycline content (mg)
A1(150 mg L^{-1})	1.383	Not detected	0.117
A2 (200 mg L^{-1})	0.801	0.89	0.309
A3(300mg L^{-1})	1.23	1.29	0.48
A4 (400mg L^{-1})	1.143	1.64	1.217
A5 (500 mg L^{-1})	2.646	2.22	0.134

est in blank sample (25.9 mg GAE/g D.W.) and high dose of amoxicillin (400-500 mg L-1) cause the decrease in TFC content (all data compared by the blank sample of plant) (Fig.14).

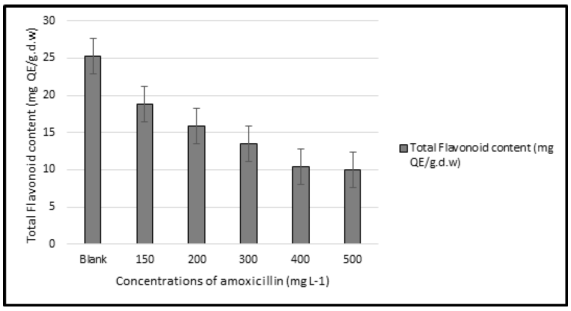

Figure. 14 The changes in the flavonoid (TFC) in Vigna radiata. Data presented in mean ± SD (n = 3).

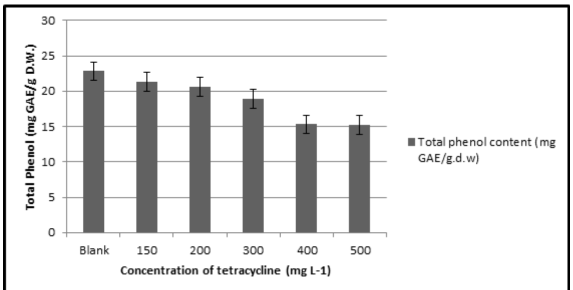

Figure. 15 The total phenolic content (TPC) in Vigna radiata exposed to different concentration of tetracycline. Data presented in mean ± SD (n = 3).

In tetracycline, TPC standard prepared with gallic acid and Regression equation y= 0.0004 x + 0.2122 and R^2 = 0.9789 was calculated. In case of amoxicillin, Regression equation y = 0.0004 x + 0.3974 and R^2 = 0.948 was found. In tetracycline plants highest TPC (22.83 mg GAE/g D.W.) was found in blank which was decreased with respect to increasing concentrations of tetracycline and 6.70 % - 33.4 % (T1 – T5) reduction range was found (Fig. 15). Similarly, in amoxicillin setup highest TPC was also found in blank (7.66 mg GAE/g D.W.) and due to amoxicillin toxicity TPC was decreased by 5.6 %, 6.8 %, 7.3 %, 12.9 % and 20 % in A1, A2, A3, A4 and A5 (all reduction percentage was calculated by blank sample of plants) (Fig. 16). It is reported that, Phenols generally prevent oxidative damage by scavenging active oxygen species and by grating the radical chain reaction during lipid peroxidation.

Remediation potential of Vigna radiata for tetracycline and amoxicillin in hydroponic system

Standard graph of tetracycline and amoxicillin was prepared by applying various concentrations of these antibiotics in silica gel plate and then chromatograms were viewed under UV region. In UV region between 150- 500 nm, amoxicillin showed highest peak at λ = 195 nm

Vol. 15 (3) 299-314, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI:10.5530/ctbp.2021.3.1

and tetracycline showed at 374 nm. In standard graph, X-Axis is the concentrations of antibiotics and Y-Axis denoted area under the peak (Figure 16). A linear relationship between the concentration of tetracycline and peak area was derived and correlation coefficient R^2 = 98.05 was achieved for amoxicillin and in case of tetracycline correlation coefficient R^2 = 99.98 was achieved. Retention factor 0.33 was calculated in tetracycline and in case of amoxicillin 0.23 retention factor was found.

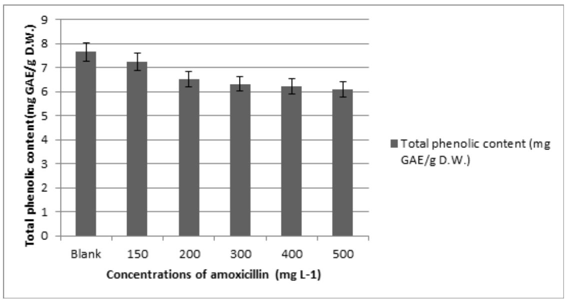

Figure. 16 The total phenolic content (TPC) in Vigna radiata exposed to different concentration of amoxicillin. Data presented in mean ± SD (n = 3).

The overall remediation rate of tetracycline and amoxicillin by *Vigna radiata* was found to be decrease as concentration of tetracycline and amoxicillin increased in hydroponic media. While calculating the phytoremediation potential of *Vigna radiata,* highest percentage remediation was found in amoxicillin treated plant as compared to tetracycline. In case of amoxicillin, highest (92.2 %) remediation was recorded in A1 (150 ppm) whereas in tetracycline highest (60.13 %) remediation was found in T2 (200 mg L^{-1}). Remediation of amoxicillin was higher as compared to tetracycline. Only in T4 sample, plant showed high remediation as compared to amoxicillin (A4). Remediation analysis was done in three sets for tetracycline and amoxicillin (Fig.17). After completion of 3 weeks in Plant tissue culture lab (PTCL), the decrease in amoxicillin in set 2 and 3 was 1.1 % and 1.2 % higher than that observed in 2 and 3 set of tetracycline at the 150 mg kg^{-1} and 300 mg kg-1 concentrations of antibiotics. Highest remediation rate was observed in set A1 while minimum was observed in A4 set and its indicates a decrease in remediation rate as the concentration of amoxicillin has exceeded. Variations in % remediation were observed in the all three replicates for amoxicillin and tetracycline concentration (from 150- 500 mg/ml) and this can be due to the variation in number of germinated seeds of *Vigna radiata.* Seeds of *Vigna radiata* was not grown properly due to the toxic effect of tetracycline. The highest (60. 13 %) remediation rate was found in T2 (200 mg L^{-1}) and lowest was observed in T3 for tetracycline. The initial

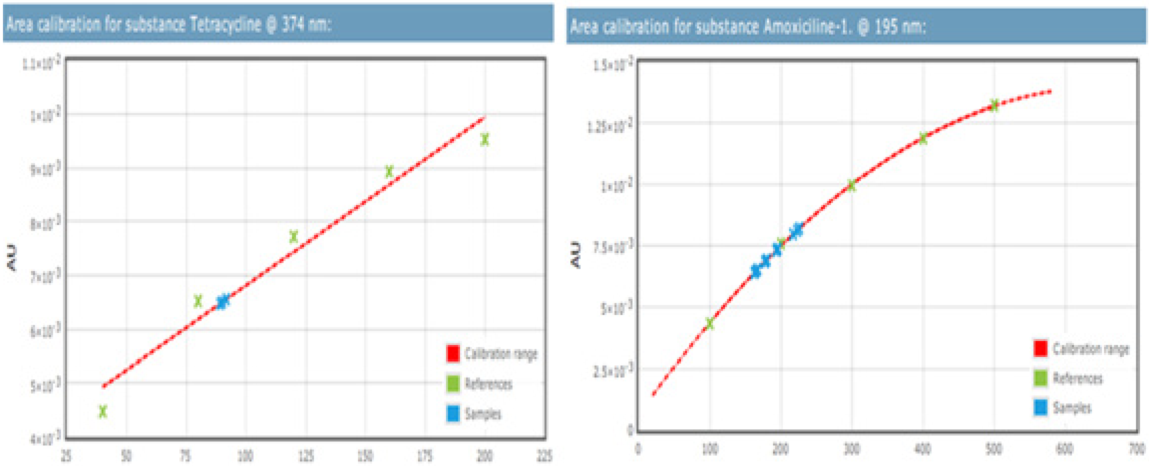

Figure 17 [A] Standard of Tetracycline (X-Axis is the concentration of amoxicillin and Y-Axis denoted area under the peak) [B] Standard of Amoxicillin (Polynomial graph

Vol. 15 (3) 299-314, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI:10.5530/ctbp.2021.3.1

increase in remediation rate was observed for concentrations from 150 mg/mL to 300 mg L^{-1} in tetracycline setup and then it was decreased in 400 mg L^{-1} and 500 mg L^{-1}. As discussed above tetracycline is more toxic as compared to amoxicillin, so the phytotoxic effects brought by tetracycline caused the reduction in remediation rate. In control set of tetracycline only 9.6 % remediation and in case of amoxicillin 14.86 % remediation was seen.

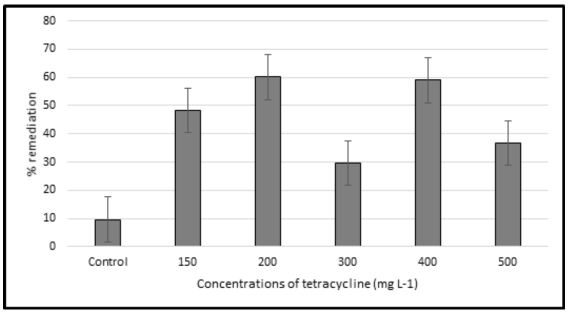

Figure.18. %Remediation of tetracycline, with respect of initial content, after 21d exposure. Each data presents the mean of three replicates (mean ± SD, n = 3).

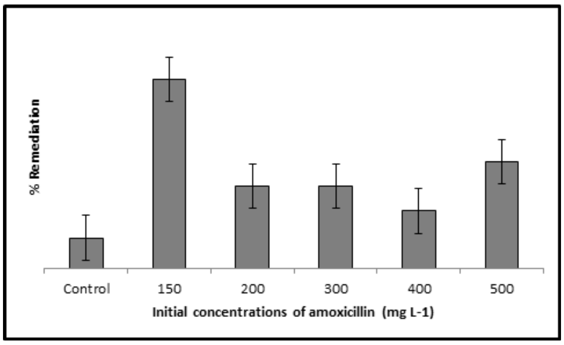

Figure. 19. %Remediation of amoxicillin, with respect of initial content, after 21d exposure. Each data presents the mean of three replicates (mean ± SD, n = 3).

The accumulated antibiotics (TC and AMX) were found maximum in root tissues followed by shoot and in the media. High accumulation of antibiotics in root part might be due to the root tissues are directly exposed to the antibiotics. *Vigna radiata* absorb antibiotics by various mechanisms such as gas exchange, aqueous channel uptake and lipid channel uptake and then degrade them in tissues via mixed function oxidases. Our results indicated that phytoremediation contributed primarily (removal > 85%) in antibiotic removal from the media and it suggested that phytoremediation is the main mechanism of antibiotic removal. While, hydrolysis and photodegradation have played a minor role in antibiotic removal. It is documented that the uptake and translocation of toxicants within plants is also performed by the process of diffusion within the plants. After uptake, these compounds can be degraded in plant tissues by the metabolic processes like monooxygenases mediated enzymatic degradation in plant cells [32]. To our best knowledge present study is first study to investigate remediation potential of leguminous plant (*Vigna radiata*) for tetracycline and amoxicillin removal.

Translocation factor (TF) and Bioconcentration factor (BCF)

TF and BCF was calculated by method given by Michelini et al. (2012) [33] in which TF shows translocation of antibiotics from root to shoot while BCF showed total accumulation in plants, calculated as follows

Translocation factor=C shoot∴C root----------- (2)

Bioconcentration factor=C plant ∴C soil------------- (3)

Where C represents concentration (mg L^{-1}) of antibiotics. The use of bioaccumulation and translocation factors has proven to be effective method for identifying the capability of the plants for antibiotics uptake. Translocation of these antibiotics (Tetracycline and Amoxicillin) increases as their concentration increases in media similarly BCF values are also shows increase. In plants grown with tetracycline translocation factor showed decrease due to toxicity caused by tetracycline.

Plants with TF value less than 1 accumulated tetracycline and amoxicillin more in roots and with TF values > 1 accumulation was more in shoots. With tetracycline, translocation factor

Vol. 15 (3) 299-314, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI:10.5530/ctbp.2021.3.1

did not exceed a value of 1 (TF < 1). With amoxicillin, A1 and A5 set showed high translocation capability and TF value 1.4 (A1), 1.13 (A5) was calculated. The total accumulation of amoxicillin was calculated by bioaccumulation factors (BCF). The value of BCF > 1 was found where concentrations of tetracycline in Hoagland media was high. On increasing the concentrations of tetracycline in Hoagland media the value of BCF was also increased. Similarly, in amoxicillin setup BCF > 1 was found from 150 mg L^{-1} to 500 mg L^{-1} while in A3 bioaccumulation factor dose not exceeded the value of 1. Furthermore, BCF found to be highest in T1 (7.2) and T1 > T2 > T4 >T5 >T3 order was found in tetracycline setup similarly in amoxicillin setup BCF was highest in A1 (8.7) and A1 > A5 > A2 > T4 > T3 order was observed (Table 1) (Table 2). Higher TF and BCF values shows a greater ability of Vigna *radiata* to translocate and accumulation of these antibiotics.

After the 3W of experiment some TC and AMX amount not detected in plant as well as in H.M. It could be degraded in photolysis and hydrolysis (Table 3) (Table 4). Antioxidant producing capacity of leguminous plants could also play a very important role in antibiotics degradation in plant metabolism (phase II). According to green liver model, TC and AMX can combine with antioxidant enzyme/ metabolites and degraded in plant and converted into their less toxic form which is comparatively less toxic to plant than parent compound [34].

Conclusion.

Antibiotics occurrence in environment is now increasing the concern of researchers and policymakers because of the harmful impact on ecosystem, vegetation and human health. It is proven that, food-crops can be used for Phytoremediation and used to remove the contamination from the fields. Moreover, food crops are potential candidate for the removal of pollutants from soil and water due to their long availability and fast growth. These plants are not used for food purpose because they are grown only for 3 weeks i.e. before flowering stage or fruit formation of plants, can be disposed by safely. Brassicaceae family plants are well known for phytoremediation while Fabaceae plants are still need to be explored. Now it is confirmed that, this is the first study where *Vigna radiata* follow the sustainable approach to reduced antibiotics contamination. The study undertakes the role of leguminous crop for antibiotics removal and concentration of both the antibiotics was decreased after 3W of experiment. Highest 92% remediation was calculated in amoxicillin and in case of tetracycline about 62 % remediation was observed. In control set of tetracycline only 9.6 % and in case of amoxicillin 14.86 % remediation was found. Degraded amount of antibiotics (in both cases) estimated that antioxidant enzymes or cytochrome P-450 enzyme can degrade antibiotics in phase I and phase II in plant. In toxicity analysis preliminary results suggested that significant impact of antibiotic on length and biomass (FW and DW) of root and shoot of *Vigna radiata*. Selected samples of TC and AMX treated plant showed significant impact on total Chl, carotenoids, TPC, TFC and antioxidant enzymes (CAT and GPX). Therefore, it suggested that dose dependent impact of TC and AMX on plant physiology. In conclusion, present study confirmed that the leguminous plants (*Vigna radiata*) are successfully absorbed tetracycline and amoxicillin from hydroponic media in order to estimate human health risk due the consumption of vegetables contaminated with antibiotics. Our results are encouraging and further research will be conducted by similar work at real contaminated location in near future.

Acknowledgement

The authors are grateful to Jaypee Institute of Information Technology for providing the necessary laboratory facilities and encouragement. Animal work was not done in this study.

References

1. Klein, E.Y., Levin S.A and Laxminarayan, R, (2018). Improved policies necessary to ensure an effective future for antibiotics.

Proc Natl Acad Science. 115:8111–8112.

2. Rokni-Zadeh, H. Li. W., Vleeschouwer, De., Ghequire, M., Sinnaeve, MG., Xie, GL. D., Rozenski, J., Madder, A., Martins, JC. and Mot R, D.E. (2013). The antimicrobial compound xantholysin defines a new group of Pseudomonas cyclic lipopeptides. PLoS ONE, Vol. 21:46-62.

3. Smith, P.M., Howitt. and Panikov, N, M. R. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science. 341:569–73.

4. Ahmad N. and Saad N. (2012). Effects of antibiotics on dental implants. J Clin Med, 4:1–6.

5. Pulicharla, R., Das., K., Brar, S.K., Drogui, P., Sarma, S.J., Verma M. and Surampalli, R.Y (2015). Valero Toxicity of chlortetracycline and its metal complexes to model microorganisms in wastewater sludge. Sci. Total Environ, 532:669-675.

6. Hamscher, G., Sczesny, S., Hoper, H. and Nau, H. (2002). Determination of persistent tetracycline ¨ residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Analytical Chemistry, 74:1509–1518.

7. Sarmah, A.K., Meyer, A.K. and Boxall, A.B.A. (2006). A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics. Chemosphere, 65: 725–759.

8. Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B. and Buxton, L.B. (2002). Pharmaceuticals, hormones, and others organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance. Environment Science and Technology. 36:1202–1211.

9. Pan, X., Deng, C., Zhang, D., Wang, J., Mu, G. and Chen, Y. (2008). Toxic effects of amoxicillin on the photosystem II of *Synechocystis sp.* characterized by a variety of in vivo chlorophyll fluorescence tests. Aquatic Toxicology, 89:207–213.

10. Kűmmerer, K. and Henninger, A. (2003). Promoting resistance by the emission of antibiotics from hospitals and households into effluents. Clinic Microbio Infec. 9:1203–14.

11. Meena, V.S., Maurya, B.R., Verma, A., Aeron, A. Kumar, K., Kim, K. and Bajpai, V.K. (2015). Potassium solubilizing rhizobacteria (KSR): Isolation, identification, and K-release dynamics from waste mica. Ecological Engineering. 81:340-347.

12. Riviere, J.E., Craigmill, A.L., and Sundlof, S.F. (1991). Handbook of comparative pharmacokinetics and residues of veterinary antimicrobials. Boca Raton: **(1991)**.

13. Goyal, H. and Angur bala, Bafna. (2018). *Vigna radiata:* A Potent Phytofiltrator of Lead Grown Hydroponically in Sewage. International Journal of Agriculture. Environment and Biotechnology, 11:45 – 54.

14. Sharma, H.R. and A. Malan, A. (2017). Effect of paracetamol and ibuprofen on morphological parameters and chlorophyll content of *Vigna radiata* (Green Gram)", Environment and We: An International Journal of Science and Technology, 34: 99-108.

15. Makhijani, M., Gahlawat, S., Chauhan, K., Valsangkar, S. and Gauba, P. (2014). Phytoremediation potential of *Cicer arietinum* for tetracycline. International Journal of genetic Engineering and Biotechnology, 5:153–160.

16. Opriş, O., Copaciu, F., Soran, M.L., Ristoiu, D. and Niinemets, U. (2012). Influ-

ence of nine antibiotics on key secondary metabolites and physiological characteristics in *triticumaestivum* leaf volatiles as a promising new tool to assess toxicity. Ecotoxicology and Environmental Safety*,* 87:70–79.

17. Hillis, D.G., Fletcher, J., Solomon, K.R. and Sibley, P.K. (2011). Effects of ten antibiotics on seed germination and root elongation in three plant species. Arch. Environ Contam Toxicol, 60:220–232.

18. Arnon, DI. (1969). Copper enzymes in isolated chloroplasts. Polyphenoloxldase in *beta vulgaris.* Plant Physiology*,* 24:1-15.

19. Lichtenthaler, H.K. (1987). Chlorophylls and carotenolds pigments of photosynthesis. Methods Enzymology.148:350-352.

20. Caverzan, A., Casassola, A. and Brammer, S.A. (2016). Antioxidant responses of wheat plants under stress. Genetic Molecular Biology*,* 39:1–6.

21. Miao, Y., Lv, D., Wang, P., Wang, X.C. and Chen, J. (2006). An Arabidopsis glutathione peroxidase functions as both a redox transducer and a scavenger in abscisic acid and drought stress responses. Plant Cell, 18:2749–2766.

22. Chang, C.C., Yang, M.H., Wen, H.M. and Chern, J.C. (2002). Estimation of total flavonoid content in propolis by two complimentary colorimetric methods. J Food Drug Anal, 10:178–182.

23. Singh, V., Pandey, B. and Suthar, S. (2018). Phytotoxicity of amoxicillin to the duckweed *Spirodela polyrhiza*: Growth, oxidative stress, biochemical traits and antibiotic degradation. Chemosphere, 201:492–502.

24. Brain, R.A., Johnson, D.J., Richards, S.M., Hanson, M.L., Sanderson, M.W., Lam, C., Young, S.A., Mabury, P.K., Sibley, R.T. and Solomon, K.R. (2004). Microcosm evaluation of the effects of an eight pharmaceutical mixture to the aquatic macrophytes Lemna gibba and Myriophyllumsibiricum. Aquatic Toxicology, 70:23–40.

25. Gujrathi, N.P., Haney, B. and Linden, J. (2005). Phytoremediation potential of *M. aquaticum* and *P. stratiotes* to modify antibiotic growth promoters tetracycline and oxytetracycline in aqueous wastewater systems. International journal of phytoremediation, 7:99–112.

26. Kasai, K., Usami, S., Yamada, T., Endo, Y., Ochi, K. and Tozawa, Y. (2002). A RelA-SpoT homolog (Cr-RSH) identified in *Chlamydomonas reinhardtii* generates stringent factor *in vivo* and localizes to chloroplasts *in vitro.* Nucleic Acids Res., 30:4985–4992.

27. Khator. K. and Shekhawat, G.S. (2020). Cd- and Cu- induced phytotoxicity on 2–3 leaf stage of *Cyamopsis tetragonoloba* and its regulation by nitrate reductase and ROS quenching enzyme. Acta Physiol Plant, 42:120-130.

28. Baccio, DI., Pietrini, F., Bertolotto, P., Pérez, S., Barcelò, D. and Zacchini, M. (2017). Response of Lemna gibba L. to high and environmentally relevant concentrations of ibuprofen: removal, metabolism and morpho-physiological traits for biomonitoring of emerging contaminants. Science of Total Environment*,* 89:584–585.

29. Xu, S., Li, J., Zhang, X., Wei, H. and Cui, L. (2006). Effects of heat acclimation pretreatment on changes of membrane lipid peroxidation, antioxidant metabolites, and ultrastructure of chloroplasts in two cool-season turfgrass species under heat stress. Environmental Experimental Botany, 56:274–285.

Vol. 15 (3) 299-314, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI:10.5530/ctbp.2021.3.1

30. Hegedus, A., Erdei, S. and Horvath, G. (2001). Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. Plant Science, 160:1085-1093.

31. Chi, Z., Liu R. and Zhang, H. (2010). Potential enzyme toxicity of oxytetracycline to catalase. Science of Total Environment, 408:5399–5404.

32. Iatrou, E.I., Gatidou, G., Damalas, D., Thomaidis, N.S. and Stasinakis, A.S. (2017). Fate of antimicrobials in duckweed Lemna minor wastewater treatment systems. Journal Hazard Mater, 330:116–126.

33. Michelini, L., Reichel, R. and Werner, W. (2012). Sulfadiazine Uptake and Effects on *Salix fragilis* L. and Zea *mays* L. Plants. Water Air Soil Pollution, 223:5243–5257.

34. McCutcheon, S.C. and Schnoor, J.L. (2003). Overview of phyto transformation and control of wastes. Phytoremediation: transformation and control of contaminants", Wiley Inter science, 7: 3–58.