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EFFECT OF ANTIBIOTICS ON MORPHOMETRIC AND BIOCHEMICAL PARAMETERS OF GREEN GRAM (*Vigna radiata*)

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ABSTRACT

The present study was to investigate the impact of antibiotics such as Ampicillin and Doxycycline on morphometric and biochemical parameters of green gram (*Vigna radiata*). Antibiotics at different concentrations were mixed with soil and water environment. Morphometric parameters such as shoot length, root length, leaf length, leaf width and stomatal index were measured. Biochemical parameters such as protein, carbohydrate and electrical conductivity were analysed. Ampicillin showed an increase in total protein content and total carbohydrate in liquid medium. Shoot length and root length were severely affected by antibiotic treatment. This study indicate that only ampicillin in soil was shown to have a significant effect on *Vigna radiata*. This may be due tothe long exposure durations experienced by plants grown in soil medium. Antibiotics were found to cause less toxicity in minimum concentrations, though it causes toxicity when the exposure period is long. The leaves and stem of the plants were generally found to be most sensitive to the antibiotic exposure. The phytotoxic effects of antibiotics were found to be less severe for lower concentrations.

KEYWORDS: Antibiotics, Bioassay, Morphometric and Biochemical Parameters.

Bio-medical wastes are generated during diagnosis and treatment. Unlike Western countries, India does not have stringent rules to regulate the uncontrolled disposal of Pharmaceuticals and personal care products (PPCPs), especially antibiotics. Antibiotics are used for the treatment of disease and for promoting growth by farmers(Kim et al; 2013),veterinarians (Mishra et al; 2011) and also in aquaculture (Swapna et al; 2012). It has beenshown that the antibiotic residues in the soilwill affect the flora(Chen et al; 2011) andfauna (Elmolla and Chaudhuri;2010).Continuous use of Antibiotics will develops resistance in bacteria (Purohit et al; 2017).Although, toxicity data of some antibiotics on certain animals are available at present, there is a paucity of information on antibiotic phytotoxicity. At present only a few reports are available on the phytotoxic effect of PPCP, especially antibiotics on vascular plants.This study presents the phytotoxicity of two antibiotics (Ampicillin and Doxycycline) on Green gram (*Vigna radiata*).Toxicity studies on *Vigna radiata*with reference to heavy metals were available (Abbasiet al; 2012), (Singh et al; 2017).Bioassayswere carried for the determination of the relative strength of a particular substance by comparing its effects on a test organism .The toxicity tests are used for the determination of beneficial and harmful concentration of environmental factors or toxicity of a substance to a test species (Aery; 2010).

MATERIALS AND METHODS

The study was conducted at the School of Environmental studies, Cochin University of Science and Technology. In order to find out Phytotoxicityof Antibiotics liquid bioassay and soil bioassay were performed. Morphometric parameters such as shoot length, root length, leaf length, leaf width and stomatal index were measured. Biochemical parameters such as protein, carbohydrate and Electrical conductivity were studied in antibiotic added soil and liquid medium.

EXPERIMENT USING ANTIBIOTICS INDIVIDUALLY IN LIQUID MEDIUM (LIQUID BIOASSAY)

Antibiotic stock solutions were diluted to obtain a 0 to 7.51 g/L and 0 to 0.00995 g/L concentration series for ampicillin and doxycycline respectively. Then seeds are introduced into petri plate and were kept for 4 days in room temperature.Biochemical parameters were measured by using spectrophotometer.Lowry's Method was used to detect protein content in the plant samples. Total carbohydrate content was estimated by the Anthrone reagent method of (Hedge and Hofreiter 1962).

EXPERIMENT USING ANTIBIOTICS INDIVIDUALLY IN SOIL MEDIUM (SOIL BIOASSAY)

It was carried out using disposal containers (250ml) with uncontaminated soil. The antibiotic stock solutions were diluted to obtain a 0 to 1.501 g/Kg and 0 to 0.0199 g/Kg.Plant 3 seeds in three corners of the container.The moisture content of the soil was maintained everyday by adding equal quantities of distilled water. On the 7th day, the plants were taken out from the container, and analysis were carried out.Morphometric characters such as root length, shoot length, leaf length and leaf

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width were measured.Electrical conductivity were measured by using conductivity meter, stomata was observed by using compound microscope.

Statistical analysis were performed using R software of version 3.1.2 (R core team; 2017).

RESULTS AND DISCUSION

Figure 1: Liquid Bioassay

Figure 2: Soil Bioassay

LIQUID BIOASSAY

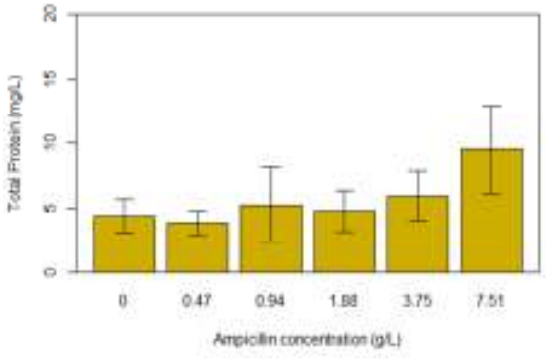

Figure 3: Effect of different concentrations of Ampicillin on protein content

An increase in protein content towards the top concentration was observed. Total protein content was high in the concentration of 7.51 g/L of Ampicillin.

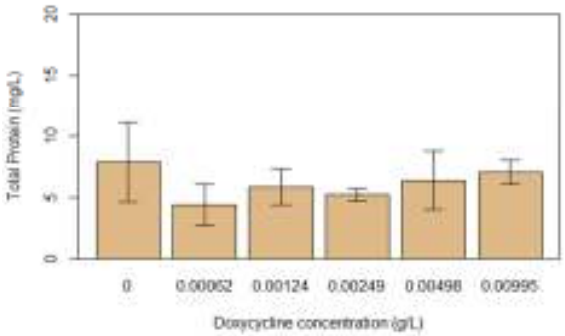

Figure 4 : Effect of different concentrations of Doxycycline on protein content

An increase in protein content towards the top concentration was observed. But total protein content was higher in control than any other concentrations.

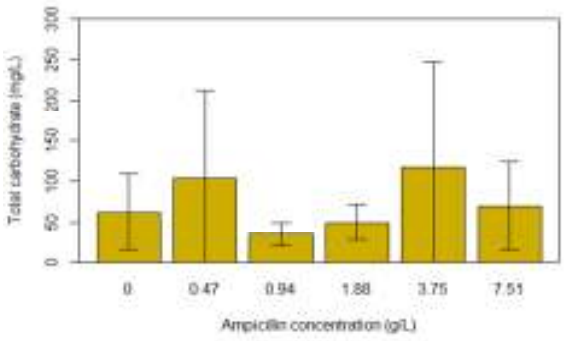

Figure 5: Effect of different concentrations of Ampicillin on Carbohydrate content

An increase in carbohydrate content was observed in0.47 and 3.75 g/L of Ampicillin concentration than control.

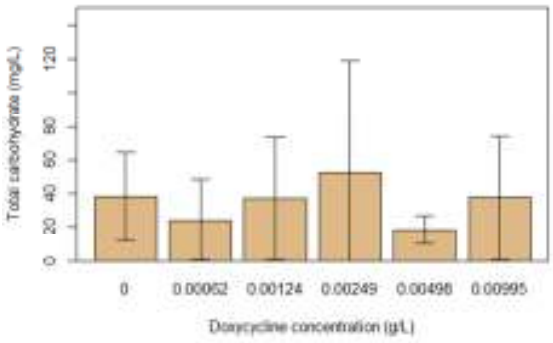

Figure 6: Effect of Doxycycline on carbohydrate content

An increase in carbohydrate content was observed in 0.00249 and 0.00995 g/Lof doxycycline concentrations than control.

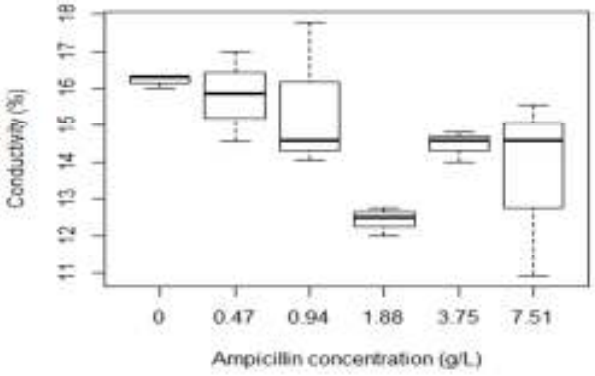

Figure 7: Effect of Ampicillin on Electrical Conductivity

No significant effect was observed in the electrical conductivity, but some stimulatory effectshave shown when compare with that of control. Ampicillin and Doxycycline does not cause membrane leakage in plant cells.

SOIL BIOASSAY

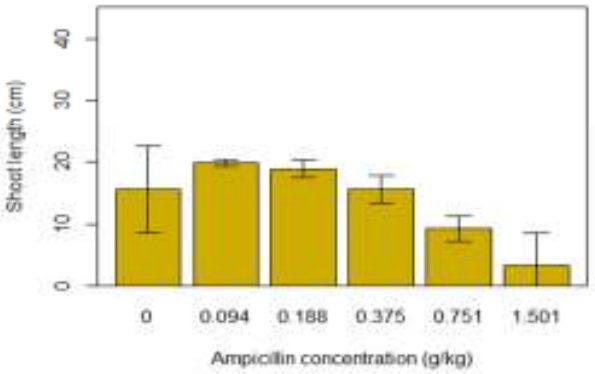

Figure 8: Effect of different concentration of Ampicillin on Shoot length

The ampicillin treatment produced significant decreasein shoot length (p<0.05) and showed a significant decrease in shoot length at 1.5 g/kg ampicillin in soil.But Doxycycline treatment does not produce any significant result in shoot length.

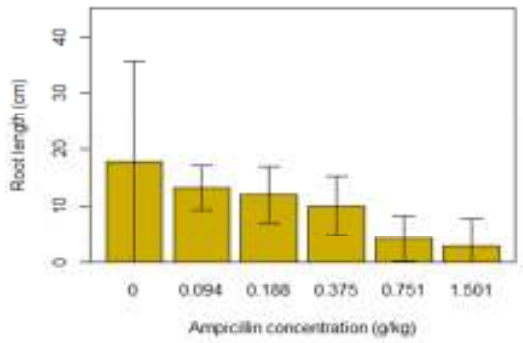

Figure 9: Effect of Ampicillin concentration on root length

A gradual decrease in root length was observed across the higher concentrations. But Doxycycline treatment in soil produced no significant change in root length.

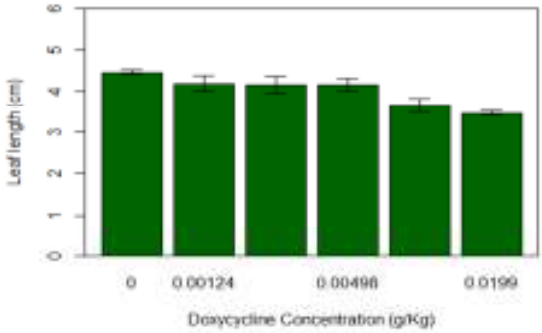

Figure 10: Effect of different concentration of Doxycycline on leaf length

Doxycycline treatment in soil produced significant change in leaf length (p< 0.05).Increasing the doxycycline concentration resulted in decreased leaf length. But Ampicillin treatment does not showed any significant result in leaf length.

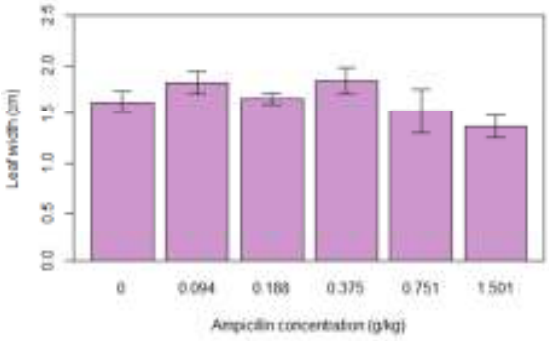

Figure 11: Effect of different concentration of Ampicillin on leaf width

A significant difference in leaf width between the ampicillin treatments was observed (p<0.05). But Doxycycline treatment does not showed significant difference in leaf width.

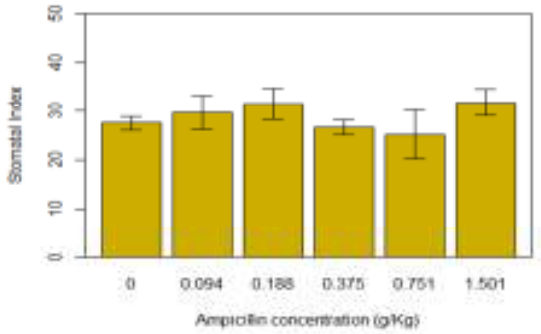

Figure 12: Effect of different concentration of Ampicillin on Stomatal Index

Ampicillin and Doxycycline doesn't produced any significant change in stomatal index.

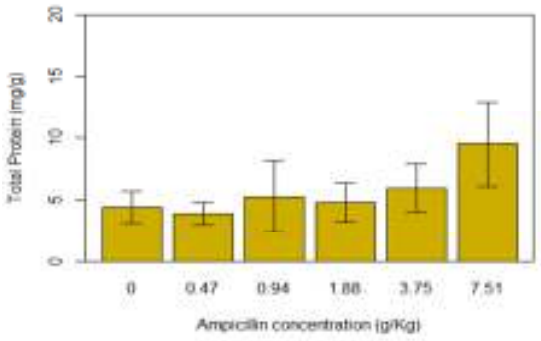

Figure 13: Effect of Ampicillin on protein content

Ampicillin showed a slight increasein total protein content at higher treatments (7.51 g/kg). And Doxycycline also showed a slight increase in total protein concentration at higher concentrations.

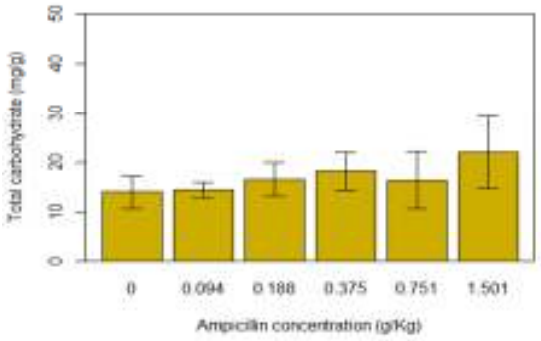

Figure 14: Effect of different concentration of Ampicillin on carbohydrate content

Ampicillin showed an increase in the total carbohydrate content of the plant

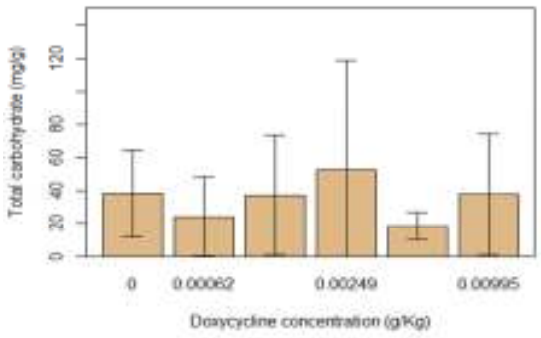

Figure 15: Effect of different concentration of Doxycycline on carbohydrate

An increase in carbohydrate content was found in 0.00249 g/Kg of Doxycycline than control.

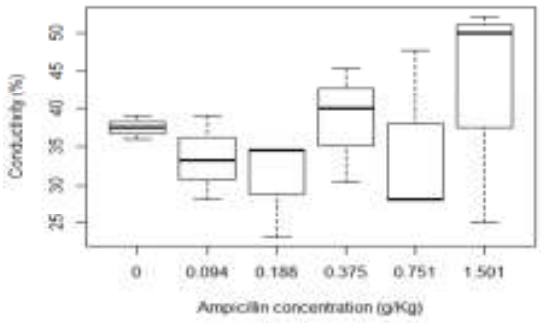

Figure 16: Effect of Ampicillin on Electrical conductivity

Ampicillin and Doxycycline does not showed any significant effect on electrical conductivity, but some stimulatory effects were observed when compared with that of control. Ampicillin and Doxycycline does not cause any membrane leakage in plant cells.

CONCLUSION

This study provides an over view of the effects of antibiotics on the morphometric and biochemical parameters of *Vigna radiata*. Antibiotics treatment in liquid medium does not showed significant effect on any of the parameters studied. Only Ampicillin in soil was shown to have a significant effect on *Vigna radiata*. This may be due to long exposure duration experienced by plants grown in the soil medium. The biotransformation of antibiotics in the plant body and the subsequent metabolic alterations can be the causes of toxicity. The leaves and

stem of the plants were generally found to be most sensitive to the antibiotic exposure. The root growth is less sensitive in detecting the toxicity of antibiotics. This findings contradicts the general observation made with other toxicants such as heavy metals and pesticides, where the root growth was found to be the most sensitive indicator of toxicity. When antibiotics reach the soil, antibiotic residues may change the microbial consortium by causing pre exposure and subsequent development of antibiotic resistance mediated through genetic transformation in microbes. The physiological changes brought about by antibiotics in the plant body is still remains to be well elucidated.Further toxicological and pharmacological investigation is required to study the mechanism of action and toxic effect by different antibiotics.

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