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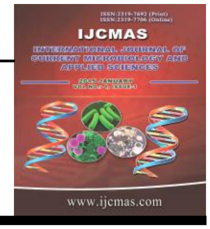

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Original Research Article

Effect of antibiotics on seed germination and root elongation of wheat

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A B S T R A C T

We applied a screening-level phytotoxicity assay to evaluate the effects of thee on germination and root elongation of 3 species of Wheat (*Triticum aestivum* L.). Each antibiotic test had 8 treatments with tetracycline concentrations of 0 (CK), 0.1, 1, 10, 100, 1000, 10000 mg/L (same for amoxicillin and levofloxacin). The range of phytotoxicity of the antibiotics was on three types of wheat plant species at the concentration of EC_{25}s 0.1 > 10,000 mg/L. None of the antibiotics caused a significant decrease in seed germination for three types of wheat plant species. Compared with Percentage of germination, shoot and total length measurements, root elongation was consistently the most sensitive end point. The use of screening assays as part of a tiered approach for evaluating environmental impacts of antibiotics can provide insight into relative species sensitivity and serve as a basis by which to screen the potential for toxic effects of novel compounds to plants.

Keywords

Antibiotics, Germination, Phytotoxicity, *Triticum aestivum*

Introduction

Wheat (*Triticum aestivum* L.) is one of the important leading cereal crop which ranks first among world food crops, measured either by cultivated area (211.06m ha) or by the production (566.8 m t) achieved (Jagshoran *et al.*, 2004). Wheat, with its root ramifying into the depths of human culture has an evolutionary history parallel with history of human civilization itself. Even today, it decides the feast or famine for millions of people. Wheat attained its premier position by virtue of its unique protein gluten, which is responsible for bread making properties of wheat flour. It is highly nutritious cereal foodstuff and its amino acid yield per acre far exceeds that of animal products (Mac. Gillivrary and Basley, 1962).

In India, wheat is the second most important cereal crop next to rice and a key crop of the green revolution and post green revolution era. India stands second among wheat producing countries with respect to area and production. During the crop year 2005-06, wheat was grown over an area of 26.8 m ha with production of 69.35 m t with an average productivity of 2,586 kg per ha. In

Karnataka, wheat is grown over an area 2.23 lakh ha with a production of 1.25 lakh tonnes and with an average productivity of 564 kg per ha which is much lower than national average (Anon, 2007) Wheat plays an important role in the cropping programme of Karnataka and has already been proved to be the best component crop under multiple cropping system of the state.

Imbibition simply means the absorption of a liquid. Before attempting to germinate a seed, it is important to know whether the seed (or fruit) will imbibe water. In the laboratory this is determined by placing the seeds on moist filter paper at room temperature and then at hourly intervals for 8-10 hours, blotting the seeds dry and weighing them (Baskin C. and Baskin J., 2001). A gradual increase in seed weight indicates that the seed is absorbing water and is therefore 'water permeable'.

Pharmaceuticals and personal care products (PCPPs) in the environment came to the attention of the scientific community in the late 1990 s (Daughton and Ternes, 1999; Halling-Sørensen *et al*., 1998), and research relating to the concentrations, fate, and effects of PPCPs has increased significantly since that time. Much of this work has focused on aquatic systems. Agricultural systems are recognized as a potentially significant source of PPCPs to aquatic environments by way of runoff and leaching after the application of biosolids from wastewater treatment plants (WWTPs), manure from livestock operations, or excretion from free-ranging livestock, but the effects on agricultural soil systems have not been well documented (Thiele-Bruhn, 2003; Pope *et al*., 2009).

In terrestrial systems, research has generally focused on effects on invertebrates, particularly those associated with decomposition (Floate *et al*., 2005; Boxall *et al*., 2006; Barrett *et al*., 2009). Although the effects of selected PPCPs has been investigated in some terrestrial plant species (Batchelder ,1982; Migliore *et al*., 1998, 2003), in general, our current understanding of the potential response of terrestrial plants to these compounds after exposure in soil is limited. Since the beginning of the "antibiotic era," the effects of antibiotics on plant growth have been tested, in general with inhibitory results. The reason, perhaps, for this type of result is that few workers tested levels below 50 p.p.m,. and that these earlier preparations were impure and in many instances plant hormones more toxic than most antibiotics were present (Bein *et al*, 1947). This should be kept in mind when evaluating early work with antibiotics in field of plant growth. More attention has been paid to tetracycline contamination in view of its rapid increasing concentration in the environment. Therefore, it is important to set up rapid, simple, and accurate methods for monitoring tetracycline ecotoxicity.

The effect of various antibiotics such as amoxicillin, levofloxacin and tetracycline on the seed germination of different seeds of wheat (*triticum aestivum L*.) in which highest phytotoxicity of the levofloxacin and similar to tetracycline. Amoxicillin give in significant decreases root and total lengths. Root length was more sensitive than total length,have lower phytotoxicity than levofloxacin and tetracycline (Brain *et al*., 2008).

Materials and Methods

Collection of antibiotics: Three antibiotics were tested: The different types of antibiotic including, Amoxicilin (98% purity), Levofloxacin (98% purity) and Tetracyclin (98% purity) were collected from agriculture university.

Seed storage : 3 species of wheat (*Triticum aestivum*) seeds from the seed collection. The seeds were stored at 4^{o}C for at least 1 week before treatment.

Surface sterilization of seed: Laboratory tests to evaluate the effects of antibiotics on seed germination of three species (wheat) were carried out using the filter paper method. After having been sterilized using 0.1% NaCl and pretreated by soaking in distilled water for six hours, different species seeds of wheat (20), which depended on the size of the seeds.

Germination test: After Surface sterilization, seeds were placed on a filter paper (9 cm diameter) kept in each Petri dish (10 cm diameter). For each antibiotic compound, the filter papers in Petri dishes were treated with 5 mL of the antibiotic solution at different concentrations and covered before placing in an incubator. Seeds were germinated in the incubator under the conditions of darkness. The seed germination was evaluated using root length of seedlings as endpoint (primary root _5 mm) after 4–5 days (Tiquia *et al*., 1996). Each antibiotic test had 8 treatments with tetracycline concentrations of 0 (CK), 0.1, 1, 10, 100, 1000, 10000 mg/L, amoxicillin concentrations of 0 (CK), 0.1, 1, 10, 100, 1000, 10000 mg/L, levofloxacin concentration of 0 (CK),0.1, 1, 10, 100, 1000, 10000 mg/L. Each treatment including controls was carried out in three replicates.

End-Point Measurements: At the germination of each experiment, the following end points were measured: % germination, root length, shoot length, and total length. Percent germination was calculated as the number of seeds that germinated per dish divided by the total number of seeds per dish × 100. Root length was measured from the tip of the primary root to the hypocotyl. Total length was measured from the tip of the primary root to the tip of the shoot. Both root length and total length were recorded to the nearest millimeter using a ruler. Shoot length was determined by subtracting root length from total length.

Statistical Analysis: All four end points were analyzed using ‘‘proc GLM’’ of SAS version 9.1.3 (SAS Institute 2003) using nominal concentrations. The end points were evaluated after 12 days for seed A, seed B and seed C using one-way analysis of variance (ANOVA) to identify significant effects with a type I error rate (a) of 0.05. To test the assumptions of an ANOVA, the data set was subjected to an analysis of residual error for each end point to ensure that errors were independent, homogeneous, and randomly distributed. When a significant effect on an end point was determined, a lowest observable effect concentration (LOEC) for that end point was computed using Dunnett’s adjustment (a = 0.05), which allows for unplanned comparisons between all means and the control. After the performance of ANOVA, the raw data were analyzed using regression models in SigmaPlot, version 9 (Systat Software 2004).The regression model with different significant was selected to calculate the effective concentrations at 50%, 25%, and 10% levels.

Result and Discussion

Effect of antibiotics on seed germination of three types of wheat plant species at the concentration of EC25s 0.1 > 10,000 mg/L.(Figure 1, 2 and 3).

None of the antibiotics caused a significant decrease in seed germination for three types of wheat plant species (Tables 1, 2 and 3).

Levofloxacin: Root length was the most sensitive end point for all three of the tested species exposed to levofloxacin. Seed B was the most sensitive species and had significantly decreased root growth at a concentration of 10 mg/L, resulting in an EC25 of 3.9 mg/L. A significant decrease in seed C root length was also observed at 10 mg/L, which corresponded to an EC25 of 363 mg/L. Levofloxacin did not cause significant decreases in seed A root length, except at 10,000 mg/L, despite the best-fit model indicating a much lower threshold of toxicity, with an EC25 of 112 mg/L as described by a hormetic response curve.

Tetracycline: Of the tetracycline compound was most phytotoxic antibiotic to wheat species A, with EC50 of 6050 mg/L; root length were decreased at 100 mg/L. For the other two types of seed, significant decreases in root length were observe at 1000 mg/L.The EC25s determined for root lengths of seed A and seed C were 33 and 110 mg/L, respectively.

The responses of the three species of wheat to levofloxacin was highly variable, although root length was again the most sensitive end point. The relative sensitivity ranking from most to least sensitive was seed B > seed C > seed A. Significant decreases in root length of seed A and seed C were observed at concentration of 1000 and 10,000 mg/L, respectively; root length of seed A was unaffected by exposure to highest concentration of 10,000 mg/L.

Amoxicillin: Amoxicillin resulted in significant decreases only to seed a root and total lengths. Root length was more sensitive than total length, with an EC25 of 9,342 mg/L compared with 9,994 mg/L for total length. Amoxicillin had no significant effect on either seed B or seed C, with corresponding EC25s >10,000 mg/L for all end points. Root growth was found to be significantly stimulated at the lowest exposure concentration of 1 mg/L for seed C.

The EC25 of the most sensitive end point measured (germination, root length, shoot length, or total length) for a given species is presented.

Ranking of the geometric mean of EC25s for the most sensitive end point for the three types of seeds to a given antibiotic indicated an order of most to least phytotoxic of levofloxacin > tetracycline > amoxicillin. As a whole, the range of phytotoxicity of the antibiotics was large, with EC50s ranging from 113 to >10,000 mg/L, EC25s ranging from 3.9 to > 10,000 mg/L and EC10s ranging from 0.7 to >10,000 mg/L.

Interclass and Intra-class Differences

Comparisons of interclass and intraclass responses to antibiotics were conducted using EC25 values for the most sensitive end point (Fig. 10). Based on regression analysis, only levofloxacin produced an EC25 < 10 mg/L; the additional compound tetracycline had EC25s < 100 mg/L. Interestingly,tetracycline was the only compound that showed a consistent phytotoxic response, with EC25s within a single order of magnitude for all three wheat seeds. Of the 3 tested compounds, amoxicillin did not induce measureable phytotoxic effects on any plant seeds based on EC25s. Amoxicillin was the only antibiotic not to produce a significant lowest observable adverse effect level in any species up to the maximum concentration tested of 10,000 mg/L. Ranking of the geometric mean of EC25s for the most sensitive end point for the three types of seeds to a given antibiotic indicated an order of most to least phytotoxic of levofloxacin > tetracycline > amoxicillin. As a whole, the range of phytotoxicity of the antibiotics was large, with EC50s ranging from 113 to > 10,000 mg/L, EC25s ranging from 3.9 to > 10,000 mg/L and EC10s ranging from 0.7 to >10,000 mg/L.

With few exceptions, intraclass differences in potency were relatively small. Among the tetracyclines, within a plant seeds, potency was moderate, and phytotoxic responses to levofloxacin and tetracycline were typically within an order of magnitude of each other. Similar trends have been found in aquatic plants exposed to tetracyclines (Brain *et al*., 2008). The relative similarity in threshold values for a given class of pharmaceutical is to be expected because these compounds, despite structural differences, behave similarly and elicited similar responses in the three types of wheat tested.

Germination and Root Elongation as Relevant End Points: The results of this study suggest that germination is not a useful end point for plant testing. No significant effects on germination were observed for any of the 3 antibiotics up to the highest treatment concentration of 10,000 mg/L. Fundamentally, this confirms that plant germination is a highly conserved process, with many of the nutrients, carbohydrates, and proteins stored and available for seedling emergence even if cellular processes to convert these compounds to more bioavailable forms are negatively affected (Basset *et al*., 2002, 2005).

The increased sensitivity of root growth compared with germination to phytotoxic compounds is a common observation in plant studies. With few exceptions, root elongation was the most sensitive end point of the three length measurements for each of the plant species. Compared with germination, root elongation detected antibiotic-induced phytotoxic responses regularly, with all compounds eliciting EC25 values < 10,000 mg/L in wheat seed A, except Levofloxacin. This result supports the continued use of root elongation as the primary end point in conducting shortduration culture experiments (Kapustka, 1997).

Risk Assessment: The determination of toxicity of antibiotics to plants provides a good example of why the application of a tiered risk assessment is beneficial. For example, in this study, levofloxacin was determined to be one of the more toxic compounds at environmentally relevant concentrations, with significant effects observed as low as 1,000 mg/L and EC25s ranging from 3.9 to 112 mg/L (Kumar *et al*., 2005a). Another important consideration in assessing the risk of antibiotics to the environment is the specific use of the antibiotic, such as whether it is primarily used in human or veterinary treatment as well as whether exposures result from low-level prophylactic uses or a large pulse after a disease outbreak. Of importance in an agricultural context is how biosolids or manures containing pharmaceuticals are stored and treated before they are applied to a terrestrial system (Haller *et al*., 2002). For plants, anaerobic digestion of animal manure in holding tanks before application onto agricultural fields might decrease the risk associated with sulfonamide toxicity from land application of manure (Göbel *et al*., 2005).

In conclusion, we assessed the toxicity of 3 antibiotics to 3 types wheat plant seeds and found a range of phytotoxicity (EC25 values) ranging from 3.9 to > 10,000 mg/L. These values would exceed the EC25 of amoxicillin evaluated on wheat seed A, and a few of the compounds, such as tetracycline and levofloxacin, for seed B and seed C. This could pose a problem for seedling establishment if biosolid or manure applications to agricultural fields occur shortly before planting, particularly if the amendment is not thoroughly tilled into the soil or applied using more current soil-injection techniques. This could lead to aggregates of antibiotic-associated soil creating local hot spots that the seed or plant root may contact during the critical early stages of plant development. The application of screening assays can provide valuable insight into species sensitivity and serve as a basis by which to screen the potential toxic effects of novel compounds. These low-cost tests should continue to be used on known toxicants but more importantly on emerging pollutants.

Table.1 The effects of 3 antibiotics on germination and growth of seed A exposed for 12 days

Compound	End point	LOEC (mg/L)	EC50 (±SE) (mg/L)	EC25 (±SE) (mg/L)	EC10 (±SE) (mg/L)
Tetracycline	Germination	NSD	>10,000	>10,000	>10,000
	Total length	100	677 (1.0)	25 (0.4)	2.7 (0.1)
	Root length	100	212 (0.6)	14 (0.3)	19 (0.1)
	Shoot length	1000	2499 (2.8)	49 (1.0)	3.8 (0.3)
Amoxicillin	Germination	NSD	>10,000	>10,000	>10,000
	Total length	10,000	>10,000	9994 (4.5)	8382 (1681)
	Root length	10,000	>10,000	9342 (22)	5494 (58)
	Shoot length	NSD	>10,000	>10,000	>10,000
Levofloxacin	Germination	NSD	>10,000	>10,000	>10,000
	Total length	1000	6050 (32)	814 (6.6)	351 (4.8)
	Root length	1000	218 (3.6)	112 (2.0)	83 (2.0)
	Shoot length	1000	8866 (5.5)	989 (6.4)	188 (17)

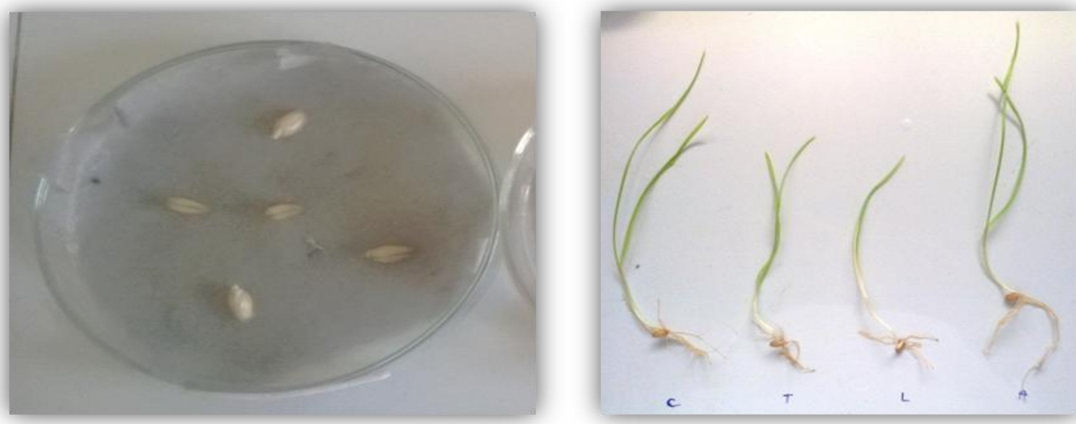
Table.2 The effects of 3 antibiotics on germination and growth of seed B exposed for 12 days

Compound	End point	LOEC (mg/L)	EC50 (±SE) (mg/L)	EC25 (±SE) (mg/L)	EC10 (±SE) (mg/L)
Tetracycline	Germination	NSD	>10,000	>10,000	>10,000
	Total length	NSD	>10,000	>10,000	2654 (4.8)
	Root length	1000	>10,000	>10,000	1433 (1.9)
	Shoot length	NSD	>10,000	>10,000	>10,000
Amoxicillin	Germination	NSD	>10,000	>10,000	>10,000
	Total length	NSD	>10,000	>10,000	>10,000
	Root length	10,000	>10,000	>10,000	>10,000
	Shoot length	NSD	>10,000	>10,000	>10,000
Levofloxacin	Germination	NSD	>10,000	>10,000	>10,000
	Total length	100	>10,000	2864 (28)	23.1(2.8)
	Root length	100	113 (17)	3.9 (5.6)	0.7 (1.8)
	Shoot length	1000	>10,000	>10,000	938 (11.3)

Table.3 The effects of 3 antibiotics on germination and growth of seed C exposed for 12 days

Compound	End point	LOEC (mg/L)	EC50 (±SE) (mg/L)	EC25 (±SE) (mg/L)	EC10 (±SE) (mg/L)
Tetracycline	Germination	NSD	>10,000	>10,000	>10,000
	Total length	NSD	>10,000	>10,000	880 (1.4)
	Root length	NSD	>10,000	>10,000	71 (1.2)
	Shoot length	NSD	>10,000	>10,000	2603 (0.9)
Amoxicillin	Germination	NSD	>10,000	>10,000	>10,000
	Total length	NSD	>10,000	>10,000	>10,000
	Root length	NSD	>10,000	>10,000	>10,000
	Shoot length	NSD	>10,000	>10,000	>10,000
Levofloxacin	Germination	NSD	>10,000	>10,000	>10,000
	Total length	NSD	>10,000	>10,000	87 (4.1)
	Root length	NSD	>10,000	363 (5.5)	9.6 (1.1)
	Shoot length	NSD	>10,000	>10,000	2280 (58)

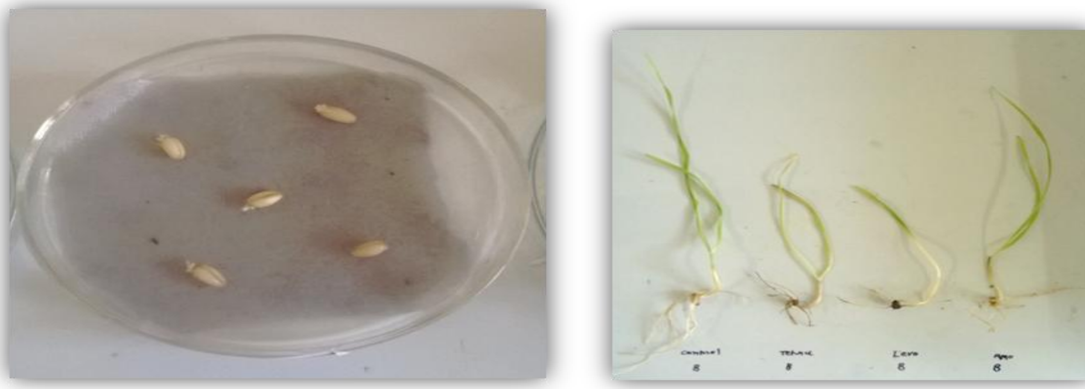
Figure.1 Effect of antibiotics on wheat (*Triticum aestivum*) seeds A



First day of germination (0.1mg/L)

After 12th day (10,000 mg/L)

Figure.2 Effect of antibiotics on wheat (*Triticum aestivum*) seed B



First day (0.1 mg/L) After 12th day (10,000 mg/L)

Figure.3 Effect of antibiotics on wheat (*Triticum aestivum*) seed C

Figure.4 The EC25s and geometric mean of the EC25 (±95% confidence intervals) of seed species A exposed to 3 different antibiotic compounds for 12 days

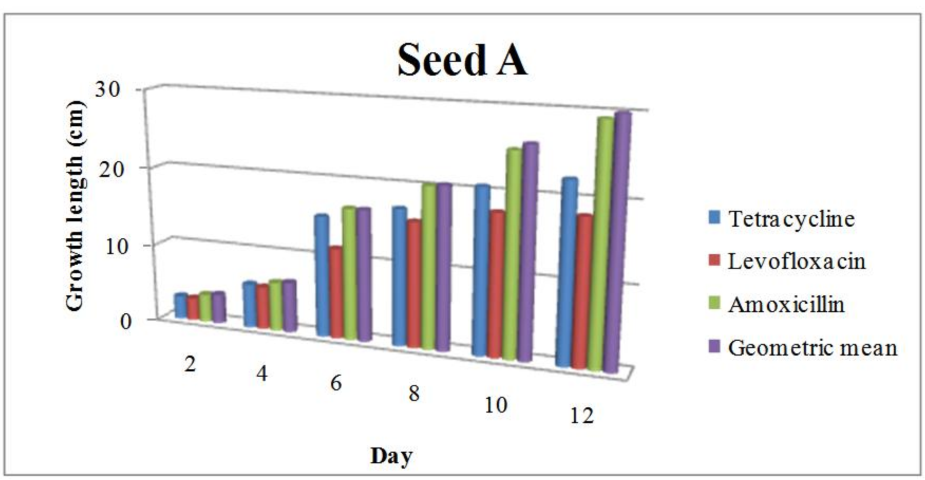


Figure.5 The EC25s and geometric mean of the EC25 (±95% confidence intervals) of seed species B exposed to 3 different antibiotic compounds for 12 days.

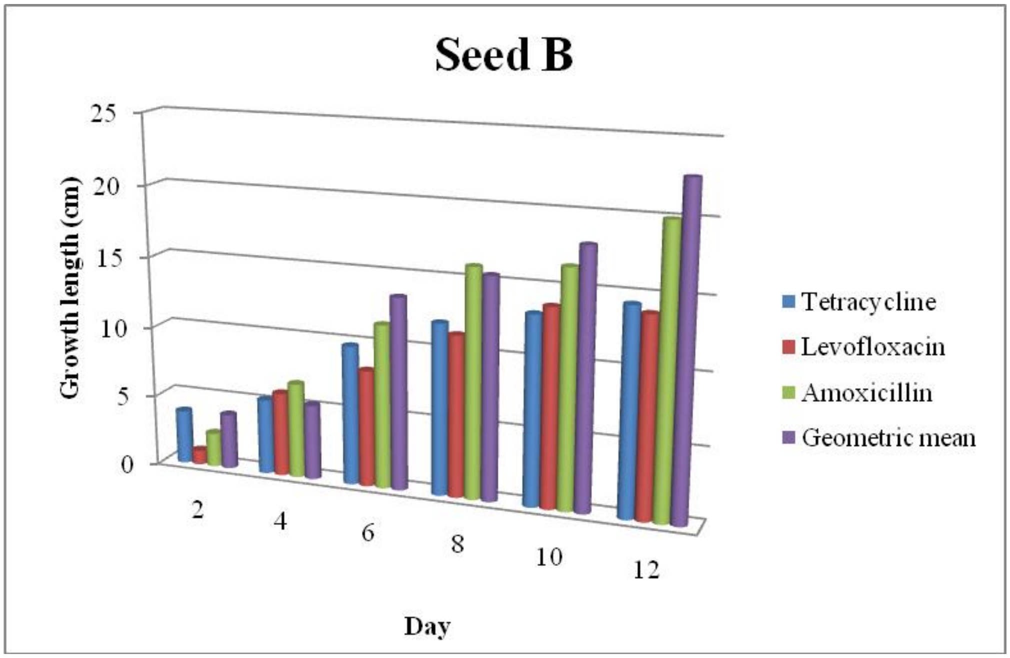


Figure.6 The EC25s and geometric mean of the EC25 (±95% confidence intervals) of seed species C exposed to 3 different antibiotic compounds for 12 days

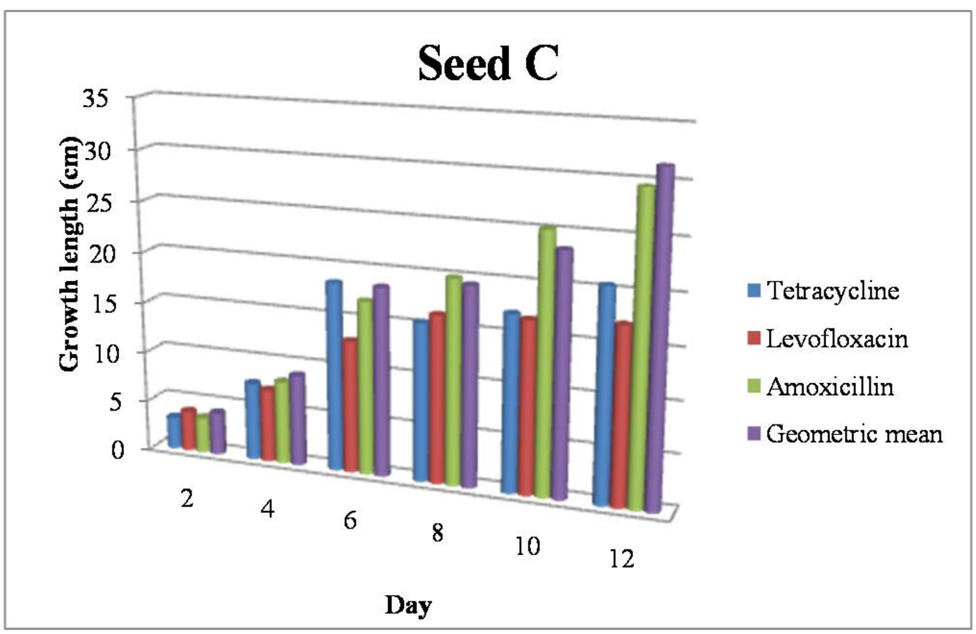


Figure.7 Effect of 3 antibiotics on wheat (*Triticum aestivum*) seed A

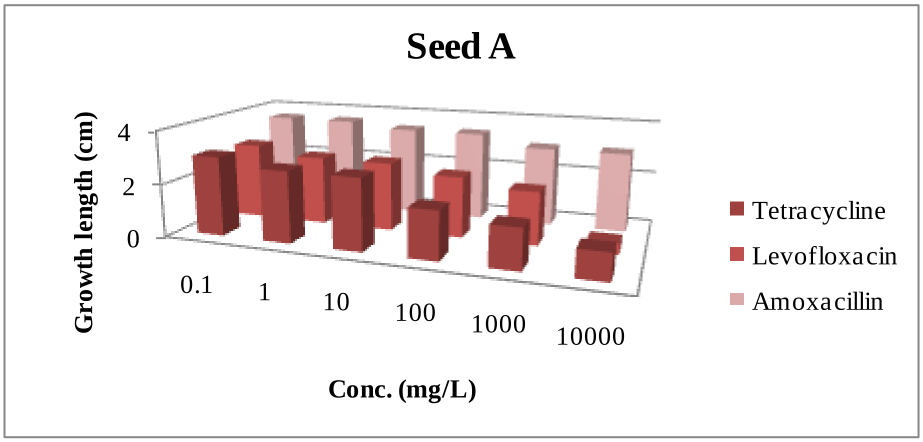


Figure.8 Effect of 3 antibiotics on wheat (*Triticum aestivum*) seed B

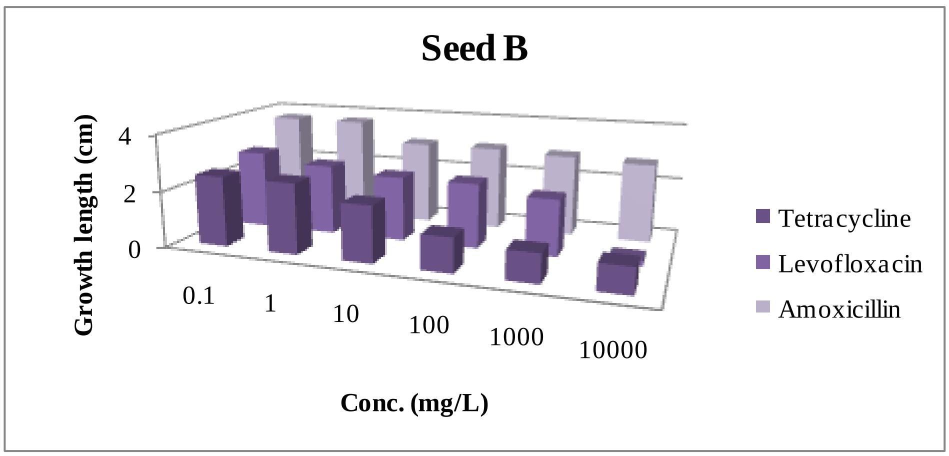


Figure.9 Effect of 3 antibiotics on wheat (*Triticum aestivum*) seed C

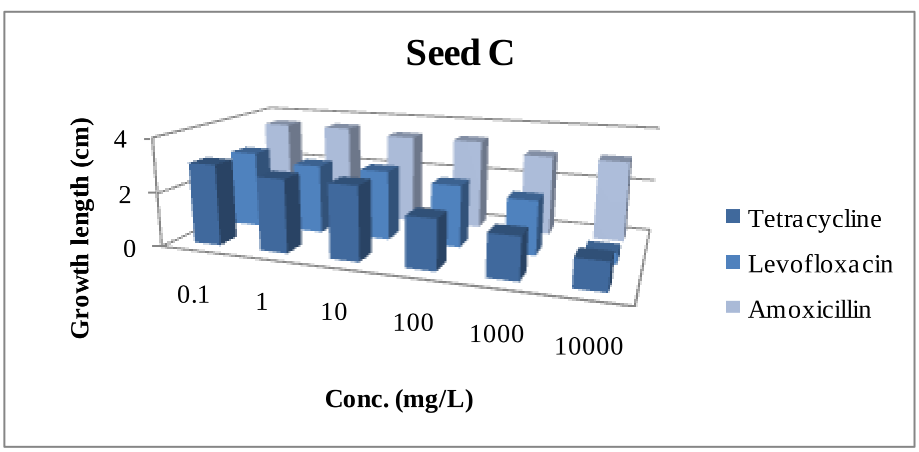
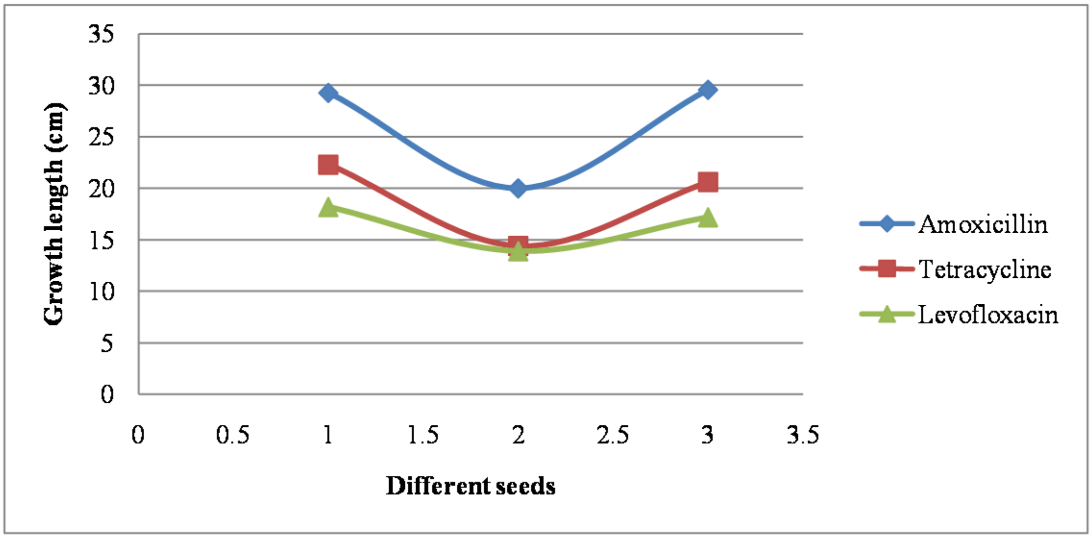


Figure.10 Effect of antibiotic on plant growth



References

Anonymous., (2007), Agriculture, Center for monitoring, Indian Economy. pp. 90-97.

Barrett K, Floate K, Jensen J, Robinson J, Tolson N (2009) Assessing the effects of veterinary medicines on the terrestrial environment.

Baskin C and Baskin J.,(2001). Seeds. Ecology, Biogeography and Evolution of Dormancy and Germination. London: Academic Press. Wright, J, M,. Ann. Botany (London). 15: 493-9

Basset G, Quinlivan EP, Ziemak MJ, Dı´az de la Garza R, Fischer M, Schiffmann S et. al. (2002) Folate synthesis in plants: the first step of the pterin branch is mediated by a unique bimodular GTP cyclohydrolase I. Proc Nat Acad Sci 99:12489–12494

Basset GJC, Quinlivan EP, Gregory JFI, Hanson AD (2005) Folate synthesis and metabolism in plants and prospects for biofortification. Crop Sci 45:449–453

Batchelder AR (1982) Chlortetracycline and oxytetracycline effects on plant growth and development in soil systems. J Environ Qual 11:675–678

Bein, M.. Signer. R. and Schopfer, W. H. (1947). Eippritia, 3:291-2.

Boxall ABA, Johnson P, Smith EJ, Sinclair CJ, Stutt E et al (2006) Uptake of veterinary medicines from soils into plants. J Agric Food Chem 54:2288–2297

Brain RA, Hanson ML, Solomon KR, Brooks BW (2008a) Aquatic plants exposed to pharmaceuticals: effects and risks. In: Whitacre DM (ed) Reviews of environmental contamination and toxicology. Springer, New York, NY, pp 67–115

Daughton CG, Ternes TA (1999) Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ Health Perspect 107:907–938

Floate KD, Wardhaugh KG, Boxall ABA, Sherratt TN (2005) Fecal effects of veterinary parasiticides: non-target effects in the pasture environment. Ann Rev Entomol 50:153–179

Göbel A, Thomsen A, McArdell CS, Joss

A, Giger W (2005) Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. Environ Sci Technol 39:3981–3989

Haller MY, Müller SR, McArdell CS, Alder AC, Suter MJ-F (2002) Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography—mass spectrometry. J Chromatogr A 952:111–120

Halling-Sørensen B, Nielsen SN, Lanzky PF, Ingerslev F, Holten LHC, Jørgensen SE (1998) Occurrence, fate and effects of pharmaceutical substances in the environment—a review. Chemosphere 36:357–393

In: Crane M, Boxall ABA, Barrett K (eds) Veterinary medicines in the environment. CRC Press, New York, NY, pp 155–177.

Jagshoran, Sharma, R. K. and Tripathi, S. C., 2004, New varieties and production. The Hindu,Survey of Indian Agric., pp. 33-35.

Kapustka LA (1997) Selection of phytotoxicity tests for use in ecological risk assessments. In: Wang W, Gorsuch JW, Hughes JS (eds) Plants for environmental studies. Lewis, CRC Press, Boca Raton, FL, pp 515–548

Kumar K, Gupta SC, Baidoo SK, Chander Y, Rosen CJ (2005b) Antibiotic uptake by plants from soil fertilized with animal manure. J Environ Qual 34:2082–2083

Kumar K, Gupta SC, Chander Y, Singh AK (2005a) Antibiotic use in agriculture and its impact on the terrestrial environment. Adv Agron 87:1–54

Mac Gillivray, J. H. and Basley, J. B., 1962, amino acid production per acre by plants and animals. Acon. Bot., 16 : 25-30.

Migliore L, Civitareale C, Cozzolino S, Casoria P, Brambilla G, Gaudio L (1998) Laboratory models to evaluate phytotoxicity of sulphadimethoxine on terrestrial plants. Chemosphere 37:2957–2961

Migliore L, Cozzolino S, Fiori M (2003) Phytotoxicity to and uptake of enrofloxacin in crop plants. Chemosphere 52:1233–1244.

Pope L, Boxall A, Corsing C, Halling-Sorensen B, Tait A, Topp E (2009) Exposure assessment of veterinary medicines in terrestrial systems. In: Crane M, Boxall ABA, Barrett K (eds) Veterinary medicines in the environment. CRC Press, New York, NY, pp 129–153.

Thiele-Bruhn S (2003) Pharmaceutical antibiotic compounds in soils¯a review. J Plant Nut Soil Sci 166:145–167

Tiquia S. M.; N. F. Y. Tam and I. J. Hodgkiss (1996). Effects of composting on phytotoxicity of spent pig-manure sawdust litter. Environmental Pollution, 93: 249–56.