INTERNATIONAL JOURNAL OF APPLIED AND EXPERIMENTAL BIOLOGY
(ISSN PRINT: 2790-6523; ISSN ONLINE: 2790-6531)
2024, Vol. 3, NO. 1, 69-74.
doi: 10.56612/ijaaeb.v3i1.72
https://eminentscientists.com/

Understanding the risks of antibiotics released from livestock on agricultural productivity: Evidence from mung bean [*Vigna radiata* (L.) Wilczek] pot experiment

Kashif Yasin[1], Muhammad Waseem[1*], Muhammad Zakria[2], Maryam Daud[1]

[1]Department of Biology & Environmental Sciences, Allama Iqbal Open University, Islamabad, Pakistan
[2] Department of Statistics, Allama Iqbal Open University, Islamabad, Pakistan

Abstract
Antibiotics of veterinary origin are generally released to agricultural fields via grazing animals or manure. Antibiotics can also be delivered to agricultural lands by reclaimed wastewater irrigation as they have been found in both raw and processed sewage effluents. Possible effects of antibiotics on crop plants, particularly of mung bean (*Vigna radiata* cv. NM-11) were investigated in the present study. A pot experiment comprising four different treatments (0, 1 µg L^{-1}, 5 µg L^{-1}, and 10 µg L^{-1}) of amoxicillin was carried out under natural atmospheric conditions. Five applications each of 200 mL of different concentrations of amoxicillin and one concentration of distilled water (control) were administered at two leaf stage onwards to the respective groups of mung bean plants. The effects of amoxicillin were assessed on the post-germination traits (agronomic parameters) of mung bean during ontogenesis and at the time of full development. The antibiotic adversely affected the plant agronomic traits which were determined twice at four-week intervals. The amoxicillin, even in small concentrations, adversely affected the growth as well as yield parameters of mung bean plants. Moreover, varying concentrations of amoxicillin used in the experiment also declined chlorophyll biosynthesis especially at the later stages of plant development.

SECTION
Plant Biology (PB)

HANDLING EDITOR
Athar, H.R. (PB)

ARTICLE HISTORY
Received: 29 May, 2023
Accepted: 24 Oct, 2023
Published: 02 Jan, 2024

KEYWORDS
Growth attributes;
Growth inhibitors;
Plant pigments;
Pulse crops;
Toxic chemicals

Introduction

An antibiotic is a chemical compound produced by microorganisms that has the ability to inhibit the growth of bacteria and other microorganisms, as well as to destroy them (Waksman, 1947; Kasanah and Hamann, 2004). Antibiotics are used by animals, humans and plants, and have both positive and adverse effects. Nowadays, antibiotics have become an integral part of living organisms. There are many antibiotics that have different chemical nature and have different effects on living things (Sengupta et al., 2013). Antibiotics are made from different chemical compounds that suppress (bacteriostatic) or kill (bactericidal) microorganisms such as bacteria, fungi, and protozoa, as well as they act as animal growth regulators in veterinary and human medicine (Peng et al., 2014). Antibiotics have been utilized in the veterinary sector for many years as a successful method of animal husbandry since these chemical agents increase animal growth, while also preventing and treating bacteria (Kümmerer, 2009; Manyi-Loh et al., 2018). The positive element of adding antibiotics to animal feed was found in 1950s, according to an American Cyanamid report (Ogle, 2013). Thus, the use of veterinary antibiotics has increased many-fold these days. According to some projections for the global economy from 2017 to 2050, worldwide

***CONTACT** Muhammad Waseem, waseembotanist@yahoo.com; waseemmuaf@hotmail.com, Department of Biology & Environmental Sciences, Allama Iqbal Open University, Islamabad Pakistan

TO CITE THIS ARTICLE: Yasin, K., Waseem, M., Zakria, M., Daud, M. (2024). Understanding the risks of antibiotics released from livestock on agricultural productivity: Evidence from mung bean [*Vigna radiata* (L.) Wilczek] pot experiment. *International Journal of Applied and Experimental Biology* 3(1): 69-74.

increases in healthcare expenses might range from 0.33 trillion USD to over 1.2 trillion USD per year by 2050 due to antibiotic resistance in bacteria (World Bank Group, 2017).

Antibiotics have played a critical role in the treatment of infectious diseases that were previously thought to be fatal to people and animals. However, their widespread usage as a feed additive in animal production has created worries about the antibiotic-resistant bacteria developing in the environment and antibiotics appearing in food and water supplies (Kumar et al., 2005; Wallinga et al., 2022). The application of animal manure and bio-solids containing excreted antibiotics to agricultural soil as a fertilizer is the most common route for antibiotic discharge in the terrestrial environment (Kemper, 2008; He et al., 2020). Antibiotics found in animal feces might reach the agricultural soil through grazing also. Furthermore, the use of some antibiotics as pesticides in crop production (e.g., oxytetracyclin and sulfonamides) may contribute to the existence of residues in soil (McManus et al., 2002; Conde-Cid et al., 2020*)*. Antibiotics can also be delivered to agricultural land by reclaimed wastewater irrigation as they have been found in both raw and processed sewage effluents (Yang et al., 2005; Kulik et al., 2023). In context of fertilization, the uptake of veterinary and human antibiotics by plants, as well as their consequences, is of great interest in agriculture. Several recent investigations have shown that plants can absorb medicinal substances from the growing media through their roots (Kumar, et al. 2005; Gworek et al., 2021) and they cause alteration in growth and physiological processes of plants. For example, the presence of ciprofloxacin (Cipro) in maize irrigation water caused morpho-physiological changes in plants, resulting in lower seed yield (Gomes et al., 2017; 2019). Another research on Sulfadiazine (SDZ) effect on maize and willow found that at low concentrations (10 mg kg^{-1}), the mean number of leaves per plant was around 81 and 9, respectively, and the stem lengths were around 38 and 23 cm for willow and maize. On the other hand, 200 mg kg^{-1} of SDZ resulted in a significant reduction in the number of leaves (28 for willow and 4 for maize) and the stem length (17 cm for willow and 5 cm for maize), respectively (Michelin et al., 2012).

Although mung bean is a widely used potential pulse crop, it is believed that the crop is sensitive to a variety of abiotic and biotic stresses (Nair et al., 2019). Thus, it is naïve to expect that this crop is also sensitive to toxic chemicals such as antibiotics. Keeping in view the above-mentioned facts, the present study was conducted to investigate the effects of varying levels of amoxicillin on agronomic and yield parameters of mung bean plants in a pot experiment.

Materials and Methods

To study the effect of antibiotic (Amoxicillin) on mung bean [*Vigna radiata* (L.) Wilczek], a pot experiment was carried out in the Botanic Garden of the Allama Iqbal Open University, Islamabad during June 2021 to September 2021. The seed of the mung bean variety NM-11 was obtained from the Agronomy Department, National Agricultural Research Council (NARC), Islamabad, Pakistan. The antibiotic used in this study was Amoxicillin ($C_{16}H_{19}N_3O_5S$). The effects of varying levels of the antibiotic were studied on different growth parameters, i.e., shoot and root lengths, fresh and dry shoot and root weights, above-ground and below-ground absolute growth rate (AGR), as well as total biomass. The experiment set-up plan was a completely randomized design (CRD) with two time periods and antibiotic levels under natural atmospheric conditions of Islamabad. Significant differences among the mean values were appraised using the least significance difference (LSD) test at the 5% probability level.

Preparation of amoxicillin solutions

Primarily, 1 mg of amoxicillin powder was dissolved in 1 mL of ethanol and then its volume was made up to 1 liter by adding distilled water. Furthermore, dilutions of this stock solution were made by taking 1 mL, 5 mL and 10 mL of stock solutions and making the volume of each up to 1000 mL getting final antibiotic concentrations as 0, 1 µg L^{-1}, 5 µg L^{-1} and 10 µg L^{-1}, respectively.

In June, 2021, a total of 48 seeds of mung bean were sown equidistantly in 12 pots each containing 4.0 kg of good quality soil. A total of four seeds were sown in each pot. The pots were regularly watered with good quality irrigation water. Germination of seeds was initiated after 7 days from the date of sowing. Twelve pots were divided into 4 groups, each containing three pots as a replicate. At two leaf stage, the soil in each pot was supplied with 200 mL of the appropriate concentration of antibiotic solution.

Agronomic parameters

Data for the growth parameters, i.e., shoot and root lengths, and fresh and dry shoot and root weights were recorded twice after four and eight weeks of amoxicillin application. Absolute growth rate of above-ground, below-ground and total biomass were also calculated by using the following formula (Reford, 1967).

$$\text{AGR (Dry matter weight)} = \frac{W_2 - W_1}{t_2 - t_1}$$

W_2 *and* W_1 refer to the plant dry matter weight (g) at times t_1 and t_2, respectively (Reford, 1967).

SPAD values of chlorophyll

The SPAD values of chlorophyll were recorded four times with an interval of 15 days each after sowing and application of the antibiotic. Furthermore, at the end of the experiment, the following yield parameters were recorded: time (days) taken to pod formation, number of pods per plant, pod length, number of seeds per pod, 100 seed weight, and seed yield per plant.

Results

The growth of mung bean plants was recorded to be reduced due to increasing concentration of amoxicillin in the rooting medium (**Figure 1**).

Figure 1. Growth of mung bean plants when different concentrations of amoxicillin antibiotic were applied through the rooting medium

The data for shoot length (**Table 1**) show that there was a marked adverse effect of exogenously supplemented amoxicillin in different concentrations on this growth attribute. As the exogenous concentration of the antibiotic increased, the shoot length of the mung bean plants decreased exponentially. The most inhibitory effect of the antibiotic was of the highest level, i.e., 10 µg L^{-1} amoxicillin. As with the shoot length, the root length of the mung bean plants also decreased markedly with the application of the antibiotic in different concentrations (**Table 2**). A consistent decrease in root length of the mung bean plants was observed with increase in the level of externally applied antibiotic. Again, the most severe effect of the antibiotic on root length was observed due to its highest level.

Varying levels of the antibiotic applied through the root growing medium had a significant inhibitory effect on different yield and yield components of the mung bean plants (**Table 3**). For example, number of pods per plant, pod length, number of seeds per pod, 100 seed weight, seed yield per plant, below-ground biomass per plant, and above-ground biomass per plant decreased considerably with increase in exogenous level of the antibiotic.

Chlorophyll content measured as SPAD values (**Table 4**) also decreased consistently with increase in the level of amoxicillin applied though the rooting medium. The highest level of the antibiotic had the most drastic effect on SPAD values of the mung bean plants.

Table 1. Shoot lengths (cm) of amoxicillin-fed mung bean plants after four and eight weeks of application

Amoxicillin (µg L^{-1})	After Four Weeks	After Eight Weeks
0	32.75 a	59.38 a
1	27.75 a	48.88 b
5	20.50 b	41.62 c
10	17.25 b	32.00 d

Same letters on mean values indicate non-significant difference among means at $P \leq 5\%$.

Table 2. Root lengths (cm) of amoxicillin-fed mung bean plants after four and eight weeks of application

Amoxicillin (µg L^{-1})	After Four Weeks	After Eight Week
0	13.5 a	30.50 a
1	12.0 a	22.25 b
5	8.5 b	16.75 c
10	7.0 c	13.75 d

Same letters on mean values indicate non-significant difference among means at $P \leq 5\%$.

Table 3. Agronomic and yield attributes of amoxicillin-fed mung bean plants after four and eight weeks of application

Parameters	Amoxicillin concentration (µg L^{-1})			
	0	1	5	10
Number of pods per plant	10.50 a	9.50 a	3.50 b	2.00 b
Pod length (cm)	7.40 a	5.80 b	4.80 b	3.50 c
Number of seeds per pod	8.52 a	6.85 b	5.40 c	4.75 c
100 seed weight (g)	4.04 a	2.28 b	1.91 c	1.41 c
Seed yield per plant (g)	3.34 a	1.85 b	0.42 c	0.41 c
Below-ground biomass per plant (g)	0.49 a	0.43 a	0.14 b	0.10 b
Above-ground biomass per plant (g)	0.75 a	0.484 b	0.145 c	0.450 c

Same letters on mean values indicate non-significant difference among means at $P \leq 5\%$.

Table 4. SPAD values of amoxicillin-fed mung bean plants measured at an interval of two weeks up to 8-week growth.

Amoxicillin (µg L^{-1})	After Two Weeks	After Four Weeks	After Six Weeks	After Eight Weeks
0	37.0 a	40.2 a	48.9 a	74.9 a
1	31.3 a	31.5 b	40.6 b	49.3 b
5	32.5 a	30.0 b	31.5 c	31.1 c
10	31.5 a	31.4 b	28.8 c	31.7 c

Same letters on mean values indicate non-significant difference among means at $P \leq 5\%$.

Discussion

It is a fact that the extensive use of antibiotics is not only harmful for animals, but also for plants. Antibiotics are usually not retained in the bodies of livestock and some of the residues of these antibiotics are released in the environment through their excretory system (He et al., 2020). In most of the countries, the feces of livestock are used as manure for crop production. Although antibiotics in plants had been intensively studied in the context of possible detrimental effects on human health (Kumar et al., 2005; Grote et al., 2007; Kang et. al., 2013; Pan et al., 2014), however, their effects on plants had not been documented with the same attention. The present experiment was conducted to study the effect of three different concentrations (1 µg L^{-1}, 5 µg L^{-1} and 10 µg L^{-1}) including control of amoxicillin on mung bean variety NM-11 within the natural climatic conditions of Allama Iqbal Open University, Islamabad. The study demonstrates that even comparatively small concentrations of the antibiotic (amoxicillin) as typically found in the soil of agricultural landscapes, adversely affected the growth of mung bean plants. Furthermore, our study revealed that antibiotics in concentrations similar to those detected in grassland soils (Li et al., 2022) had significant effects on mung bean growth. This assumption, however, does not take into account (i) that antibiotics may also accumulate in the soil (Hamscher et. al., 2002; Minden et al., 2017), which can increase total soil concentrations over time and therefore they change the response patterns in plant communities, (ii) that antibiotics are often found in mixtures in agricultural soils with likely interactive effects between antibiotics, and (iii) that antibiotics may also interact with microorganisms in the soil, potentially affecting the response of plants. The study illustrates that crop plant species in terms of plant biomass can respond to concentrations of antibiotics as typically found in agricultural soils, which may negatively affect yield in farmland fertilized with antibiotic treated manure (Huygens et al., 2021). Different agronomic plant traits of later ontogenetic stages were also adversely affected by the three different concentrations of amoxicillin administered. The mung bean variety NM-11 showed a clear response to the antibiotic treatments, especially in terms of growth and biomass related traits.

Post-germinative development of the mung bean variety NM-11 was significantly affected by the addition of antibiotic to the rooting medium, but the effects again differed among different antibiotic concentrations. All the agronomic parameters were measured twice after 4 and 8 weeks of the antibiotic treatment, while the chlorophyll content was measured four times in the course of plant development. The different amoxicillin concentrations used in the experiment also significantly affected the chlorophyll content of the mung plants, especially during the later stages of plant development, which means that the amoxicillin concentration hampered the chlorophyll biosynthesis with time. The same pattern was found for other functional traits determined at the time of harvest; almost all trait values were lower than the controls. The different concentrations of amoxicillin were also responsible for the marked reduction of yield per plant. So, it is concluded that the amoxicillin concentration even in µg L^{-1} can adversely affect the plant growth and yield. These results corroborate with the findings of other studies (Migliore et al., 2010; Hillis et al., 2011; Michelin et al., 2012; Gomes et al., 2017, 2019) in which reduction in plant growth and yield was observed in different plant species by the application of different types of antibiotics such as oxytetracyclin, sulfadiazine, sulfadimethoxine and ciprofloxacin. All these studies including ours clearly depict that the antibiotics released from animals to the environment can adversely affect growth and metabolism of plants and are injurious for human health.

Acknowledgment

The authors gratefully acknowledge the technical support of the staff of the Botanical Garden of the Allama Iqbal Open University, Islamabad.

Declaration of Author(s), Editor(s) and Publisher

Conflict of interest
The authors declare no conflict of interest.

Source of funding
Declared none.

Contribution of authors
Conceptualized and designed the study, MW. Conduction of the experiment and recording of data, KY. Data analysis and preparation of tables, MZ. Wrote up the first draft of the manuscript, MD.

Ethical approval
This study does not involve human/animal subjects, and thus no ethical approval was required.

Handling of bio-hazardous materials

The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of the experiment, all materials were properly discarded to minimize any types of bio-contamination(s).

Availability of primary data and materials

As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher/journal management. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent

All authors contributed in designing and writing the entire article. All contributors have critically read this manuscript and agreed for publishing in IJAaEB.

Disclaimer/Editors' and publisher's declaration

All claims/results/prototypes included in this manuscript are exclusively those of the authors and do not inevitably express those of their affiliated organizations/enterprises, or those of the publisher/journal management, and the editors/reviewers. Any product mentioned in this manuscript, or claim rendered by its manufacturer, is not certified by the publisher/Journal management. The journal management disowns responsibility for any injury to organisms including humans, animals and plants or property resulting from any ideas/opinions, protocols/methods, guidelines or products included in the publication.

References

Conde-Cid, M., Núñez-Delgado, A., Fernández-Sanjurjo, M.J., Álvarez-Rodríguez, E., Fernández-Calviño, D., Arias-Estévez, M. (2020). Tetracycline and sulfonamide antibiotics in soils: Presence, fate and environmental risks. *Processes* 8:1479. https://doi.org/10.3390/pr8111479

Gworek, B., Kijeńska, M., Wrzosek, J. *et al.* (2021). Pharmaceuticals in the soil and plant environment: a review. *Water Air and Soil Pollution* 232:145. https://doi.org/10.1007/s11270-020-04954-8

He, Y., Yuan, Q., Mathieu, J. *et al.* (2020). Antibiotic resistance genes from livestock waste: occurrence, dissemination, and treatment. *npj Clean Water* 3:4. https://doi.org/10.1038/s41545-020-0051-0

Huygens, J., Daeseleire, E., Mahillon, J., Van Elst, D., Decrop, J., Meirlaen, J., Dewulf, J., Heyndrickx, M., Rasschaert, G. (2021). Presence of antibiotic residues and antibiotic resistant bacteria in cattle manure intended for fertilization of agricultural fields: a one health perspective. *Antibiotics* 10*:*410. https://doi.org/10.3390/antibiotics10040410

Kasanah, N., Hamann, M.T. (2004). Development of antibiotics and the future of marine microorganisms to stem the tide of antibiotic resistance. *Current Opinion in Investigational Drugs* 5(8):827-37.

Kulik, K., Lenart-Boroń, A., Wyrzykowska, K. (2023). Impact of antibiotic pollution on the bacterial population within surface water with special focus on mountain rivers. *Water* 15:975. https://doi.org/10.3390/w15050975

Li, J., Phulpoto, I.A., Guo, L., Zeng, J., Yu, Z. (2022). Grassland ecology system: A critical reservoir and dissemination medium of antibiotic resistance in Xilingol pasture, Inner Mongolia. *Science of The Total Environment* 807:150985.

Manyi-Loh, C., Mamphweli, S., Meyer, E., Okoh, A. (2018). Antibiotic use in agriculture and its consequential resistance in environmental sources: Potential public health implications. *Molecules* 23(4):795. doi: 10.3390/molecules23040795.

Minden, V., Deloy, A., Volkert, A.M., Leonhardt, S.D., Pufal, G. (2017). Antibiotics impact plant traits, even at small concentrations. *AoB Plants* 9(2):plx010. doi: 10.1093/aobpla/plx010.

Nair, R.M., Pandey, A.K., War, A.R., Hanumantharao, B., Shwe, T., Alam, A., Pratap, A., Malik, S.R., Karimi, R., Mbeyagala, E.K., Douglas, C.A., Rane, J., Schafleitner, R. (2019). Biotic and abiotic constraints in mungbean production-progress in genetic improvement. *Frontiers in Plant Science* 10:1340. https://doi.org/10.3389/fpls.2019.01340

Peng, M., Salaheen, S., Biswas, D. (2014). Animal health: Global antibiotic issues. *Encyclopedia of Agriculture and Food Systems* 2014:346–357. doi: 10.1016/B978-0-444-52512-3.00187-X.

Sengupta, S., Chattopadhyay, M.K., Grossart, H.P. (2013). The multifaceted roles of antibiotics and antibiotic resistance in nature. *Frontiers in Microbiology* 4:47. doi: 10.3389/fmicb.2013.00047.

Wallinga, D., Smit, L.A.M., Davis, M.F. *et al.* (2022). A review of the effectiveness of current US policies on antimicrobial use in meat and poultry production. *Current Environmental Health Reports* 9:339–354. https://doi.org/10.1007/s40572-022-00351-x