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Detection of the Toxic Effects of the Antibiotic Florfenicol by Seed Germination Test

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OVER 60,000 tons of veterinary antibiotics are used globally annually, with an anticipated increase to 67% by 2030. Florfenicol is a veterinary drug its residues enter the environment through fertilizer (manure) application and waste water in agricultural areas, potentially leading to plant toxicity. In the current study, the veterinary antibiotic florfenicol was tested in two ways: first, in Petri dishes during cultivation, and second, by adding the antibiotic to the soil using germination trays. Various antibiotic concentrations were applied (0.005%, 0.01%, 0.02%, and 0.04% v/v, ml/L). Results from the first experiment exhibited significant decrease in germination rates after 8 days it reached to 18.00, and in shoot , root length to 1.26, 0.92 cm, and fresh ,dry seedling weights to 0.47,0.28 mg , with increased concentration compared to the control group. The second experiment demonstrated significant differences in germination rates after 8 days, along with changes in shoot and root length, fresh and dry seedling weights, and leaf count, with most traits decreasing as concentrations increased except for root length, which notably decreased at the 0.04% concentration , it record 4.86 cm. Changes in root structure, including the disappearance of fibrous roots and reduction in root system length, were observed. Germination rates after 4 days showed no significant difference in either experiment. The findings suggest that florfenicol concentrations in the first experiment affected the germination and growth of yellow corn (*Zea mays* L) plants, starting from the lowest concentration of 0.005%. In the second experiment, using organic-rich soil mitigated the toxic effects of florfenicol at low concentrations, except for the highest concentration of 0.04%. Florfenicol did not cause damage to chloroplasts, as the seedlings recovered their usual appearance.

Keywords: Environment; Risk; Veterinary antibiotics; Residues; Plants.

1. Introduction

Residues from antibiotics have emerged as a persistent environmental concern due to their extensive usage in both veterinary and human medicine (Zhang et al., 2020). Florfenicol (Flo) is among the veterinary antibiotics used to treat fish, cattle, pigs, and chickens. Its usage dates back to 2007 in Brazil, and globally, it is one of the most prevalent types used in aquaculture due to its broad-spectrum efficacy against Gram-positive and Gram-negative bacteria (Shiroma et al., 2020). Structurally, florfenicol resembles chloramphenicol but replaces the nitro group in the benzene ring with a sulfomethyl group. It is a fluorinated thiamphenicol (TAP) derivative, incorporating a fluorine atom at position C-3 of the benzene ring instead of a hydroxyl group (Ciuca, 2018), as depicted in Figure 1.

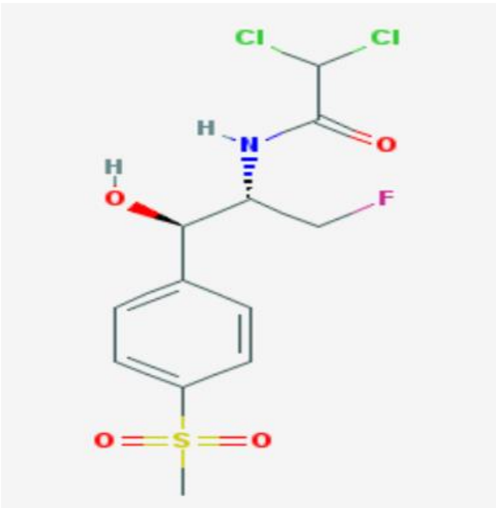

Fig. 1. Structure of florfenicol (N-dichloroacetyl derivative of (1R,2S)-2-amino-3-fluoro-1-[4-(methanesulfonyl)phenyl]propan-1-ol).

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The mechanism of action of florfenicol depends on its ability to hinder the synthesis of ribosomal protein S by binding to the bacterial 50S subunit (Pentecost et al., 2013; Shiroma et al., 2020). Additionally, florfenicol induces cell damage, halts the cell cycle, and inhibits cell division and proliferation, leading to programmed cell death (apoptosis) (Han et al., 2020; Wang et al., 2021).

Previous studies indicated that antibiotics have toxic effects on plants by disrupting their physiological activities (Opriş et al., 2013). Residues from veterinary medications enter the environment by applying natural fertilizers (manure) and waste water in agricultural areas, posing a risk of plant poisoning due to antibiotics (Ajibola et al., 2022; Richter et al., 2016; Pan et al. 2016). These residues may accumulate in plant tissues like leaves and seeds (Marques et al., 2021) or influence microbial and enzymatic activities in the soil (Liu et al., 2009). conducted a study assessing ten antibiotics, including chlortetracycline, levofloxacin, and sulfamethoxazole, at concentrations ranging from 1-10,000 micrograms/liter, They used a seed germination test and evaluated root mass elongation in clover, lettuce, and carrots. The findings revealed that root mass elongation was most affected by antibiotics compared to green mass, with carrots being the most sensitive plant. Among the antibiotics, chlortetracycline exhibited the highest toxicity to plants. (Hillis et al., 2011). Another study evaluating five antibiotics, including chloramphenicol and tetracycline, on seed germination and root mass elongation in lettuce, tomatoes, carrots, onions, and cucumbers found significant inhibition of root elongation, with the root end being the most sensitive endpoint for plant toxicity testing (Pan & Chu, 2016).

Florfenicol is commonly detected at very low concentrations in aquatic environments, ranging from micrograms per liter to nanograms per liter (Shiroma et al., 2020). The bacterial ribosome serves as a crucial target for various antimicrobials, and since mitochondria and green plastids contain bacterial-type ribosomes, they are more vulnerable to antimicrobials targeting the ribosome, resulting in toxic effects on both terrestrial and aquatic plants (Guo et al., 2024; Hu et al., 2022). Researchers observed that plants exhibited greater sensitivity to florfenicol compared to macrolides and tetracycline, emphasizing the need for further investigation to comprehensively understand the toxic effects of antibiotics on plants to conserve plant biodiversity (Carballo et al., 2022).

In an experiment involving wheat seedlings (*Triticum aestivum* L.) to elucidate florfenicol's toxic mechanism, researchers found that florfenicol inhibited wheat seedling growth, elevated antioxidant enzyme activities (peroxidase, catalase, and superoxide dismutase), malondialdehyde content, and membrane permeability with increasing florfenicol concentrations. It impeded photosynthesis and **disrupted** green plastid components. Despite global attention on the environmental impacts of antibiotics, research on florfenicol toxicity remains limited (Chen et al., 2023).

Considering the adverse effects of veterinary antibiotics on environmental elements, including plants, this study aimed to uncover florfenicol's toxic and inhibitory effects on corn plants using a germination test as a model.

2. Materials and Methods

The experiment was conducted in the research laboratory of the Environmental Research Center at the University of Mosul. It comprised two experiments: the first involved Petri dishes with five replicates for each concentration, and the second involved adding the antibiotic florfenicol to the soil using germination pots.

2.1. Preparation of florfenicol antibiotic concentrations: Five concentrations of the antibiotic florfenicol were prepared, sourced from veterinary medicine stores, and produced by the company Eurovet in Turkey, under the trade name EuroFlor antibiotic with a concentration of 10% (as indicated on the original box). The following concentrations were prepared: 0.005%, 0.01%, 0.02%, and 0.04% v/v, ml/L in distilled water from the original concentration.

2.2. Planting seeds

Experiment No. 1 (Planting in Petri Dishes)

10-15 seeds of the yellow corn variety *Drachma syngenta* were distributed into each of the five Petri dishes. The antibiotic concentrations were then added to each dish. The dishes were placed in a Memmert incubator at a temperature of 30-35°C. The following measurements were performed:

1. Germination rate after 4 and 8 days from planting the seeds, using the formula (Copeland & McDonald, 2012):

$$Percentage = \left(\frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds}\right) \times 100$$

2. Length of the green mass (cm)
3. Length of the root mass (cm)
4. Fresh seedling weight (mg)
5. Dry seedling weight (mg)
6. Number of leaves

Experiment No. 2 (germination using peat moss trays)

Yellow corn seeds were planted in ready-made organic soil of Turkish origin, characterized by the following specifications: Table 1.

Table 1. Characteristics of the growing medium (i.e., peat moss).

Property	Values
Total nitrogen	**0.2- 0.45%**
Waterholding capacity	**46-174%**
Organic matter	**75%**
pH	**5-7**

At a rate of 3 replicates for each concentration (i.e., 3×5), 10-15 seeds were planted for each concentration. The antibiotic concentrations (Flo) were added to the replicates, except for the control group, which was watered with distilled water. The seeds were then transferred to the incubator, and their growth was monitored until the seedlings reached two weeks old. Fig.2.

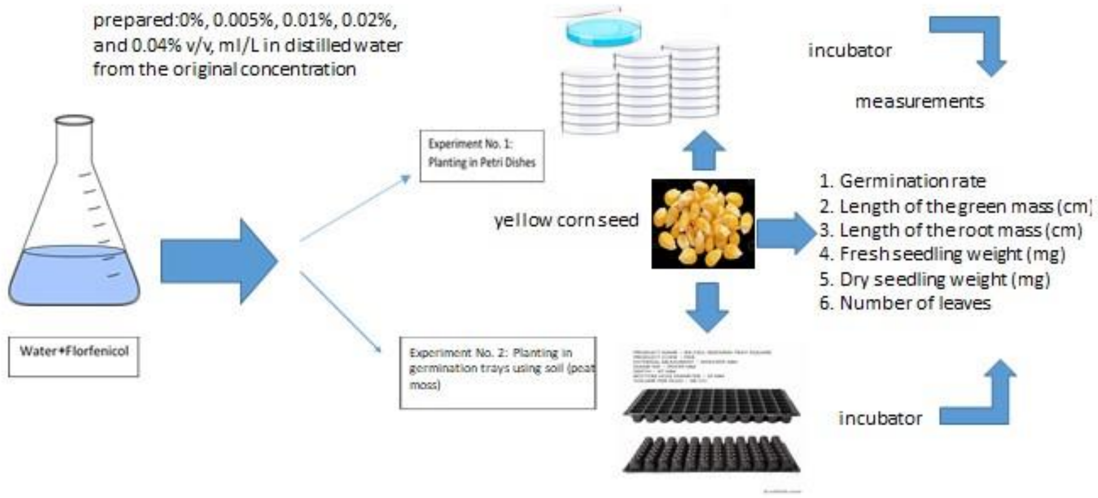

Fig. 2. The flowchart of the current experiment.

2.3 Statistical Analyses

The data were analyzed using the factorial experiments system with a complete random design (C.R.D). According to Duncan's multiple range test, different treatments were significantly distinguished by alphabetical letters (Al-Zubaidy, 2021; Duncan, 1955).

3. Results

3.1 Experiment 1

Table 2. shows the effect of different concentrations of the antibiotic florfenicol on the germination of yellow corn seeds in Experiment No. 1, planted in Petri dishes. In Experiment 1, the study results indicated the inhibitory effect of the antibiotic (Flo) on the germination of yellow corn seeds. After 8 days of antibiotic treatment, the germination rate decreased notably, with the lowest rate observed at the high concentration of 0.04% ml/L it reached to18.0000 Similarly, shoot length, root length, fresh seedling weight, and dry seedling weight all decreased post-antibiotic treatment, with the 0.04% ml/L concentration showing the most significant impact. However, the germination rate after 4 days did not show a significant difference.

Table 2. Effect of different concentrations of the antibiotic florfenicol on the germination of yellow corn seeds planted in Petri dishes (Experiment 1).

Applied dose of antibiotic (flo; v/v in ml/L)	Germination rate after 4 days	Germination rate after 8 days	Shoot length (cm)	Root length (cm)	Fresh seedling weight (mg)	Dry seedling weight (mg)
0%- Control	36.00 a	68.00 a	5.00 a	5.50 a	1.30 a	0. 97 a
0.005%	20.00 a	24.00 b	4.20 b	5.10 ab	1.24 a	0.89 b
0.01%	18.00 a	20.00 b	3.90 b	4.80 b	0.97 b	0.85 b
0.02%	28.00 a	32.00 b	3.00 c	3.60 c	0.94 b	0.74 c
0.04%	16.00 a	18.00 b |	1.26 d	0.92 d	0.47 c	0.28 d

Different letters indicate significant differences at a probability of 0.05, according to Duncan's multiple range test.

3.2 Experiment 2

Table 3 displays the impact of various concentrations of the antibiotic florfenicol on the germination and growth of yellow corn seeds in Experiment 2, which was conducted by planting in germination trays using soil. The results revealed that the inhibitory impact of the Flo extended beyond seed germination in Petri dishes to encompass germination and growth of yellow corn seeds in peat moss planting medium. Table 3 illustrates a decrease in germination rate after 8 days, with the 0.04% ml/L high concentration showing the greatest reduction in Root length (cm) compared to other concentrations. Additionally, the germination rate after 4 days was not significant, aligning with the Petri dish germination experiment results, indicating growth instability and seed variability in germination ability. As shown in the results of the Table (3), a significant decrease occurred at a 0.05 probability level in the Shoot length (cm), fresh and dry seedling weight and Number of leaves.

Table 3. Effect of different concentrations of the antibiotic florfenicol on the germination and growth of yellow corn in the planting medium (peat moss), Experiment 2.

Applied dose of antibiotic flo (v/v in ml/L)	Germination rate after 4 days	Germination rate after 8 days	Shoot length (cm)	Root length (cm)	Fresh seedling weight (mg)	Dry seedling weight (mg)	Number of leaves
Control 0%	36.67 a	86.67 a	23.44a	14.33a	1.78 a	0.89 a	4.5000 a
0.005%	70.00 a |	56.67 ab	17.33 bc	14.41a	1.50 ab	0.75 ab	3.2167 b
0.01%	36.67 a	43.33 b	14.64 c	14.39 a	1.36 b	0.68 b	3.83 ab
0.02%	43.33 a	73.33 ab	21.83 ab	15.66 a	1.53 ab	0.77ab	4.07ab
0.04%	50.00 a	46.67 b	21.58ab	4.86 b	1.57ab	0.79 ab	4.10 ab

Different letters indicate significant differences at a probability of 0.05, according to Duncan's multiple

Fig. 3. demonstrates a significant decrease in root length, particularly noticeable with the high 0.04% concentrations affecting main root and fibrous roots lengths, with fibrous roots disappearing compared to the control, as depicted in Figure 3.

Fig. 3. The effect of the antibiotic florfenicol on root length at a concentration of %0.04 and fibrous roots lengths, with fibrous roots disappearing compared to the control.

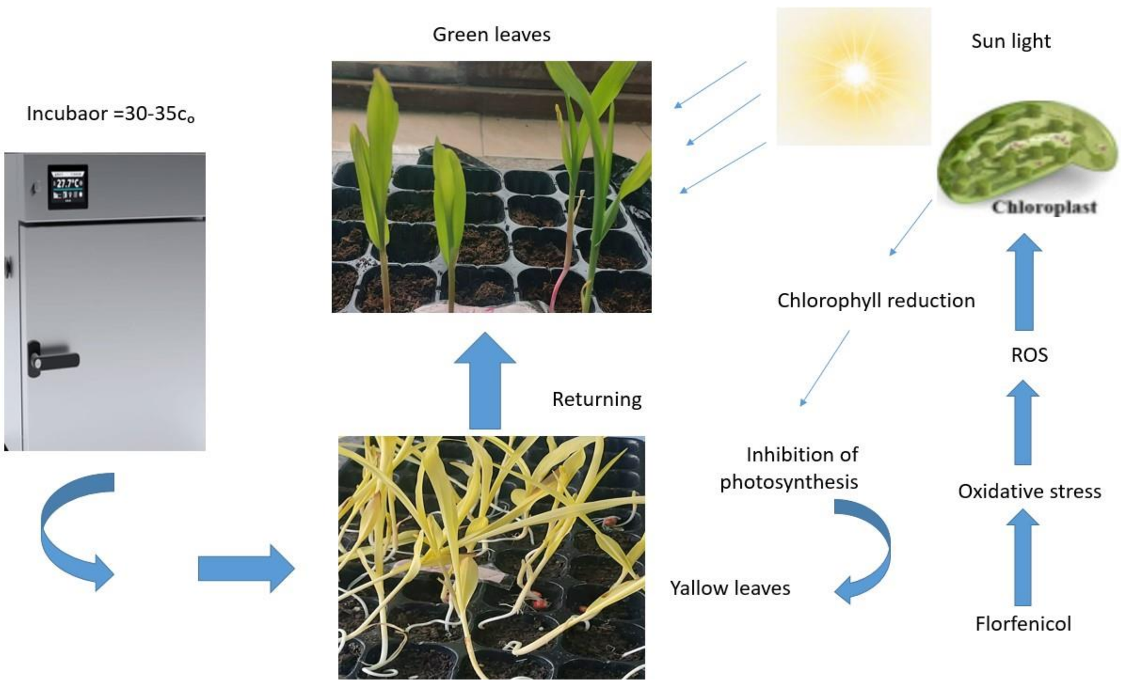

Fig. 4. The yellow corn seedlings planted in the soil. (A) Yellow corn seedlings are pale (yellow) after exposure to different concentrations of Flo. (B) The return of yellow corn seedlings to their natural green color after being placed in laboratory conditions.

4.Discussion

4.1 Experiment 1

The decrease in yellow corn seed germination post-exposure to Flo may result from its impact on the plant's physiological activities, leading to toxic effects. The antibiotic's mechanism involves inhibiting ribosomal protein S synthesis by binding to bacterial part 50 (Shiroma et al., 2020). Mitochondria and green plastids contain ribosomes of bacterial origin, rendering them more susceptible to antimicrobials targeting ribosomes (Hu et al., 2022). The germination rate after 4 days did not show a significant difference, possibly due to growth instability. Additionally, Flo induces programmed cell death, halts cell division, and impedes proliferation (Han et al., 2020; Wang et al., 2021).

Also, the decrease in Shoot length after treatment with the antibiotic (Flo) may be due to the following reasons in addition to the reasons mentioned above.

It affects the growth and physiological mechanisms the excessive accumulation of ROS induced by the high concentration of Flo exposure could further lead to the damage on the cell membrane, intracellular esterase activity and lipid peroxidation (Zhang et al., 2020; Nofal et al. 2024). The decrease in root length is due to the cessation of division at the root apical (Hillis et al. 2011) The elongation inhibition of corn plant root mass post-florfenicol treatment may stem from its impact on cell division, proliferation, and programmed cell death, similar to mechanisms observed in animals (Han et al., 2020; Wang et al., 2021). This similarity in stopping division and programmed death between animals and plants, as true nucleus organisms, could explain the observed effects.

Our experiment's findings align with Hillis et al. (2011) conclusion that root mass elongation is significantly impacted by antibiotics compared to green mass across clover, lettuce, and carrot plants (Hillis et al., 2011). Additionally, Bellino et al. (2018) noted that applying antibiotics like chloramphenicol, spinomycin, and spiramycin on tomato seeds (*Solanum lycopersicum* L.) did not affect seed germination but resulted in weak root growth, particularly at concentrations of 100 mg/L for chloramphenicol and 10 mg/L for citomycin. Flofenicol causes oxidative stress and free radicals (ROS) therefore It causes damage to the photosynthesis process and inhibits proteins associated with the photosynthesis process Florfenicol leads to metabolic imbalance and changes metabolic pathways, thus inhibiting growth and leading to stunting wheat plant (*Triticum aestivum* L.) (Chen et al., 2023).

This decrease in shoot fresh weight may be attributed to the effect of florfenicol on physiological processes within the cell, such as photosynthesis, respiration, and enzyme activities (Chen et al., 2023; Ganzour et al. 2020). Additionally, the antibiotic's impact on root growth and its ability to absorb water and nutrients could lead to shoot weight loss. Furthermore, the reduction in dry weight in plants may result from decreased accumulation of materials like proteins and carbohydrates due to the antibiotic's influence on photosynthesis and plastid breakdown after exposure (Opriş et al., 2013; Zhang, Y. et al., 2020)

4.2 Experiment 2

The germination rate also decreased in the second experiment. The decrease in the seed germination rate may be due to the reasons mentioned previously in Experiment No. (1), such as the effect on the physiological and enzymatic processes in the plant and the cessation of protein synthesis, as the antibiotic flo targets ribosomes in eukaryotic organelles such as mitochondria and plastids, which affects plant growth (Shiroma et al., 2020 ;Chen et al., 2023). The decrease in the shoot and root system may be due to the reasons mentioned above in the first experiment, in addition to the effect of flo on the division areas in the apical meristem. This decrease in shoot fresh weight may be attributed to the effect of florfenicol on physiological processes within the cell, such as photosynthesis, respiration, and enzyme activities (Chen et al., 2023;Ganzour,s.et al,2020). Additionally, the antibiotic's impact on root growth and its ability to absorb water and nutrients could lead to shoot weight loss. Furthermore, the reduction in dry weight in plants may result from decreased accumulation of materials like proteins and carbohydrates due to the antibiotic's influence on photosynthesis and plastid breakdown after exposure (Opriş et al., 2013; Zhang, Y. et al., 2020).

Moreover, significant changes in leaf numbers post-exposure to various florfenicol concentrations were noted, possibly due to effects on division areas within flower buds, thus impacting leaf numbers, as confirmed by Han et al. (2020) and Wang et al. (2021) regarding florfenicol's effect on cell division. In Figure 3, the absence of fibrous roots from the main root of the corn plant is noticeable. The low concentrations (0.05%, 0.015%, and 0.02%) did not significantly impact root length . This lack of effect could be attributed to the organic matter present in the soil, which may reduce the antibiotic's toxicity, particularly at lower concentrations (Amer 2017; Abd El-kader 2018; Abd El-Hafees and Bashandy 2019). Wyszkowska et al. (2022) discussed the role of grass and corn compost in soil fertilization and their use in biologically treating soil contaminated with the antibiotic tetracycline.

Experiment 2 revealed yellowing and pallor in yellow corn seedlings post-florfenicol exposure, depicted in Figure 4. Flo did not cause damage to green plastids, as evidenced by seedlings returning to produce chlorophyll after exposure to laboratory conditions across all concentrations, including the high concentration. This may be due to the method of application, as (Flo) was added to the soil and was not in direct contact with the leaves. Researchers have noticed that Flo reduces chlorophyll content and inhibits photosynthesis in algae ***I. galbana***, especially at high concentrations due to oxidative stress and the formation of free radicals caused by Flo (Zhang et al. 2020).

Conclusions

In the current study, it revealed the toxicity of the antibiotic florfenicol on corn plants by testing seed germination as a model. My study shows the negative effect of florfenicol on the germination and growth of corn plants. The results of experiment (1) indicated that the antibiotic florfenicol inhibited seed germination and that the effect began at low concentrations. The results of the second experiment indicated that florfenicol affected the germination of yellow corn plants, especially the root system, where the root length decreased and the fibrous roots disappeared, and the effect was clear in the high concentrations. While the use of clean organic soil helped reduce the toxic effect of flo, as the root length was not affected when low concentrations were applied to the corn plant. Therefore, we recommend avoiding using flo in high concentrations and conducting more research on the use of organic soil to reduce the effect of residues (veterinarydrug) and others on the plant.

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References

Ajibola, A., Olatunji, D., & Bayode, O. (2022). Occurrence of veterinary antibiotics in poultry manure from two farms in Ibadan, Nigeria: Ecotoxicological implications in manure-amended soil. Environmental analysis, health and toxicology, *37*(4), e2022038 . https://doi.org/10.5620/eaht.2022038

Abd EL-Kader, N. (2018). Effect of Nitrogen Applications, Micronutrients and Cyanobacteria on Wheat Yield and the Availability of some Nutrients. Egyptian Journal of Soil Science, 58(1), 105-111. doi: 10.21608/ejss.2018.5616

Abd El-Hafeez, A., & Bashandy, S. (2019). Irrigation Schedule with Different Nitrogen Sources and Levels for Maize Productivity and Some Water Relations. Egyptian Journal of Soil Science, 59(1), 25-40. doi: 10.21608/ejss.2019.7293.1239

Amer, M. (2017). Effect of Biochar, Compost Tea and Magnetic Iron Ore Application on some Soil Properties and Productivity of Some Field Crops under Saline Soils Conditions at North Nile Delta. Egyptian Journal of Soil Science, 56(1), 169-186. doi: 10.21608/ejss.

Al-Zubaidy, M. H. I. (2021). Acute neurotoxicity of acetaminophen in chicks. *Veterinarski arhiv*, *91*(4), 379-387. https://doi.org/10.24099/vet.arhiv.0950

Bellino, A., Lofrano, G., Carotenuto, M., Libralato, G., & Baldantoni, D. (2018). Antibiotic effects on seed germination and root development of tomato (Solanum lycopersicum L.). Ecotoxicology and Environmental Safety, *148*, 135-141. https://doi.org/10.1016/j.ecoenv.2017.10.006

Carballo, M., Rodríguez, A., & de la Torre, A. (2022). Phytotoxic Effects of Antibiotics on Terrestrial Crop Plants and Wild Plants: A Systematic Review. Archives of environmental contamination and toxicology, 82(1), 48-61. https://doi.org/10.1007/s00244-021-00893-5

Chen,H., Jiaojun J., Shuhao H., Luoqin S., Ping Z., Zhiheng L., Zhiguo F., Huijun L., Metabolomics and proteomics reveal the toxicological mechanisms of florfenicol stress on wheat (Triticum aestivum L.) seedlings, Journal of Hazardous Materials, Volume 443, Part B, 2023, 130264, ISSN 0304-3894, https://doi.org/10.1016/j.jhazmat.2022.130264.

Ciuca, V. (2018). Evaluarea riscului pentru mediu a produsului Florfenicol FP 10%. Veterinary Drug/Medicamentul Veterinar, *12* .(1)

Copeland, L. O., & McDonald, M. F. (2012). Principles of seed science and technology. Springer Science & Business Media .

Duncan, D. B. (1955). Multiple Range and Multiple F Tests. Biometrics, *11*(1), 1. https://doi.org/10.2307/3001478

Guo, X., Chen, H., Tong, Y., Wu, X., Tang, C., Qin, X., Guo, J., Li, P., Wang, Z., Liu, W., & Mo ,J. (2024). A review on the antibiotic florfenicol: Occurrence, environmental fate, effects, and health risks. Environmental Research, *244*, 117934. https://doi.org/10.1016/j.envres.2023.117934

Ganzour, S., Ghabour, T., Hemeid, N., & Khatab, K. (2020). Impact of Biofertilizers on Maize (Zea mays L.) Growth and Yield under Calcareous Soil Conditions.. Egyptian Journal of Soil Science, 60(4), 469-483. doi: 10.21608/ejss.2020.45922.1392

Han, C., Wei, Y., Cui, Y., Geng, Y., Bao, Y., & Shi, W. (2020).Florfenicol induces oxidative stress and hepatocyte apoptosis in broilers via Nrf2 pathway. Ecotoxicology and Environmental Safety, *191*, 110239. https://doi.org/10.1016/j.ecoenv.2020.110239

Hillis, D. G., Fletcher, J., Solomon, K. R., & Sibley, P. K .(2011) .Effects of Ten Antibiotics on Seed Germination and Root Elongation in Three Plant Species. Archives of Environmental Contamination and Toxicology, *60*(2), 220-232. https://doi.org/10.1007/s00244-010-9624-0

Hu, D., Zhang, B., Suo, Y., Li, Z., Wan ,Z., Zhao, W., Chen, L., Yin, Z., Ning, H., Ge, Y., & Li, W. (2022). Molecular Mechanisms Underlying the Inhibition of Proliferation and Differentiation by Florfenicol in P19 Stem Cells: Transcriptome Analysis. Frontiers in pharmacology, *13*, 779664-779664 . https://doi.org/10.3389/fphar.2022.779664

Liu, F., Ying, G.-G., Tao, R., Zhao, J.-L., Yang, J.-F., & Zhao, L.-F. (2009). Effects of six selected antibiotics on plant growth and soil microbial and enzymatic activities. Environmental Pollution, 157(5), 1-636 .1642https://doi.org/10.1016/j.envpol.2008.12.021

Marques, R. Z., Wistuba, N., Brito, J. C. M., Bernardoni, V., Rocha, D. C., & Gomes, M. P. (2021). Crop irrigation (soybean, bean, and corn) with enrofloxacin-contaminated water leads to yield reductions and antibiotic accumulation. Ecotoxicology and Environmental Safety, *216*, 112193. https://doi.org/10.1016/j.ecoenv.2021.112193

Nofal, E., Menesy, F., M. Abd El-Hady, W., G. Shehab, E., El-Ramady, H., & Prokisch, J. (2024). Foliar Application of Nano-NPK in Combination with Nano-Micronutrients for Growth, and Flowering of Tecoma stans L. Plants. Egyptian Journal of Soil Science, 64(3), 1179-1191. doi: 10.21608/ejss.2024.288930.1768

Opriş, O., Copaciu, F., Loredana Soran, M., Ristoiu, D., Niinemets, Ü., & Copolovici, L. (2013). Influence of nine antibiotics on key secondary metabolites and physiological characteristics in Triticum aestivum: Leaf volatiles as a promising new tool to assess toxicity. Ecotoxicology and Environmental Safety, 87, 70-79. https://doi.org/10.1016/j.ecoenv.2012.09.019

Pan, M & ,.Chu, L. M. (2016). Phytotoxicity of veterinary antibiotics to seed germination and root elongation of crops. Ecotoxicology and Environmental Safety, *126*, 228-237. https://doi.org/10.1016/j.ecoenv.2015.12.027

Pentecost, R. L., Niehaus, A. J., Werle, N .A., & Lakritz, J. (2013). Pharmacokinetics of florfenicol after intravenous and intramuscular dosing in llamas. Research in Veterinary Science, *95*(2), 594-599. https://doi.org/10.1016/j.rvsc.2013.05.009

Richter, E., Berkner, S., Ebert, I., Förster, B., Graf, N., Herrchen, M., Kühnen, U., Römbke, J., & Simon, M. (2016). Results of extended plant tests using more realistic exposure scenarios for improving environmental risk assessment of veterinary pharmaceuticals. Environmental sciences Europe, 28(1), 22-22 .https://doi.org/10.1186/s12302-016-0089-2

Shiroma, L. S., Soares, M. P., Cardoso, I. L., Ishikawa, M. M., Jonsson, C. M., & Nascimento Queiroz, S. C. (2020). Evaluation of health and environmental risks for juvenile tilapia (Oreochromis niloticus) exposed to florfenicol. Heliyon, 6(12), e05716-e05716. https://doi.org/10.1016/j.heliyon.2020.e05716

Wang, X., Han, C., Cui, Y., Li, S., Jin, G., Shi, W., & Bao, Y. (2021). Florfenicol causes excessive lipid peroxidation and apoptosis induced renal injury in broilers. Ecotoxicology and Environmental Safety, 207, 111282. https://doi.org/10.1016/j.ecoenv.2020.111282

Wyszkowska, J., Borowik, A., & Kucharski, J. (2022). The Role of Grass Compost and Zea Mays in Alleviating Toxic Effects of Tetracycline on the Soil Bacteria Community. International journal of environmental research and public health, 19(12), 7357. https://doi.org/10.3390/ijerph19127357

Zhang, Y., Zhang, X., Guo, R., Zhang, Q., Cao, X., Suranjana, M., & Liu, Y. (2020). Effects of florfenicol on growth, photosynthesis and antioxidant system of the non-target organism Isochrysis galbana. *Comparative Biochemistry and Physiology Part C:* Toxicology & Pharmacology, *233*, 108764. https://doi.org/10.1016/j.cbpc.2020.108764