Journal of Materials and
Environmental Science
ISSN : 2028-2508
e-ISSN : 2737-890X
CODEN : JMESCN
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J. Mater. Environ. Sci., 2026, Volume 17, Issue 6, Page 964-978

http://www.jmaterenvironsci.com

Phytotoxic Effects of Veterinary Antibiotics on Seed Germination and Early Seedling Development of *Vigna radiata* L.

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Received *08 May 2026,*
Revised *11 June 2026,*
Accepted *16 June 2026*

Keywords:
✓ *Antibiotics;*
✓ *EC_{50};*
✓ *Phytotoxicity index;*
✓ *Root elongation;*
✓ *Seed germination;*
✓ *Vigna radiata*

Citation*: Mohan B., Hema V., Prabha D. (2026) Phytotoxic Effects of Veterinary Antibiotics on Seed Germination and Early Seedling Development of Vigna radiata L., J. Mater. Environ. Sci., 17(6), 964-978*

Abstract: Antibiotics are emerging contaminants in agro-ecosystems and may pose ecological risks to non-target organisms, including plants. A screening-level phytotoxicity assay was conducted to evaluate the phytotoxic effects of eleven commonly used veterinary antibiotics-enrofloxacin (ENR), ciprofloxacin (CIP), sulfadiazine (SDZ), sulfadimidine (SD), oxytetracycline (OTC), chlortetracycline (CTC), doxycycline (DOX), amoxicillin (AMX), ampicillin (AMP), ceftriaxone (CEF), and gentamicin (GENTA)-on seed germination and early seedling development of *Vigna radiata* L. Seeds were exposed to antibiotic concentrations ranging from 0 to 300 µg mL^{-1} for eleven days. Germination percentage decreased significantly at concentrations > 25 µg mL^{-1} compared with treatments < 10 µg mL^{-1}. High-dose exposure (> 10 mg L^{-1}) delayed germination, extending the germination time to 60–74 h. Complete inhibition of germination was observed at concentrations > 200 µg mL^{-1} for ENR, CIP, OTC, CTC, DOX, AMX, CEF, and GENTA. Root elongation and total seedling length were significantly reduced at concentrations > 1 µg mL^{-1} for all antibiotics tested, while shoot length reduction was observed from > 5 µg mL^{-1}. The phytotoxicity index ranged from 0.32 to 1.00, indicating moderate to high toxicity toward root development. EC_{25} and EC_{50} values further identified doxycycline (EC_{50} = 116.03 µg mL^{-1}; EC_{25} = 186.12 µg mL^{-1}) as the most phytotoxic antibiotic, whereas sulfadiazine (EC_{50} = 346.40 µg mL^{-1}; EC_{25} = 513.06 µg mL^{-1}) exhibited the lowest toxicity toward *V. radiata*. These findings highlight the potential ecological risks of veterinary antibiotic contamination in agricultural soils and their implications for crop establishment and productivity.

1. Introduction

Advances in animal husbandry practices to meet the increased demand for food of animal origin have led to a substantial increase in antibiotic use (Tian *et al.*, 2021). To meet rising demand, antibiotics are used extensively worldwide in animal husbandry for therapeutic, prophylactic, and growth promotion (Abreu *et al.*, 2023; Kupczynski *et al.*, 2024). It has been estimated that veterinary antibiotics account for approximately 52% of global antibiotic consumption, with the remaining proportion attributed to human consumption (Decheng *et al.*, 2018; Liu *et al.*, 2018).

The widespread application of veterinary antibiotics in livestock production and human medicine has raised significant environmental and public health concerns in recent decades. Since their half-life in animals is typically short, ranging from days to a few weeks, a substantial proportion of administered antibiotics (approximately 30–90%) are rapidly excreted through urine and feces (Sarmah *et al.*, 2006; Lofrano *et al.*, 2017), either as parent compounds or as active metabolites (Jansen *et al.*, 2020). These antibiotic residues can reach agricultural soils through the frequent direct application of contaminated animal manure as fertilizer, and by irrigation with wastewater and the application of sewage sludge (Akhter *et al.,* 2023; Patra and Dubey, 2024). Antibiotic residues thus introduced into agricultural soils may subsequently sorb onto soil matrices, leading to persistence, ranging from several days to several months. This likely explains the frequent detection of veterinary antibiotic residues in farmland topsoil and sediments worldwide, with reported concentrations spanning from µg kg^{-1} to mg kg^{-1} (Ho *et al.,* 2012; Hu *et al.,* 2012; Albero *et al.,* 2018; Patyra *et al.,* 2020; Akhter *et al.,* 2023).

The presence of antibiotic residues in soils can significantly influence species richness, community composition, and grazing behaviour of non-target organisms, including microbial communities and earthworms (Zhao *et al.,* 2022; Omosigho *et al.,* 2022). This may accelerate the development and spread of antimicrobial resistance genes among beneficial environmental microorganisms. Moreover, antibiotic residues in soil can be taken up by plants through their root systems (Diass *et al.,* 2021; Yu *et al.,* 2022; Akhter *et al.,* 2023) and subsequently accumulate in stem and leaf tissues, as plants have limited capacity to excrete these compounds (Tasho *et al.,* 2020; Marques *et al.,* 2021; Zhang *et al.,* 2017). This accumulation facilitates their entry into the food chain via consumption of contaminated plant products, posing risks to ecosystem health and human well-being.

Several studies have documented the occurrence of antibiotic residues in edible crops, including tetracyclines, quinolones, and sulfonamides (Feng *et al.,* 2018), as well as enrofloxacin and ciprofloxacin (Marques *et al.,* 2021). These residues can disrupt key physiological and biochemical processes in plants, such as chlorophyll synthesis and glycine betaine accumulation, ultimately inhibiting plant growth and development, including root elongation and biomass production (Salem *et al.,* 2015; Zhang *et al.,* 2017; Tasho *et al.,* 2020). Exposure to elevated concentrations (> 40 µg mL^{-1}) of antibiotics like oxytetracycline and enrofloxacin has been shown to reduce root length, fresh weight, root number, and root surface area, while enhancing antioxidant enzyme activities (Li *et al.,* 2023; Riaz *et al.,* 2017).

Seed germination and root elongation tests are sensitive and significant bioassays used to assess the phytotoxic effects of veterinary antibiotics on plants (Wang *et al.,* 2019). Numerous studies have demonstrated the impact of veterinary antibiotics on plant growth parameters through pot experiment using different class of veterinary antibiotics (e.g., tetracycline, sulfonamides, quinolones, macrolides, aminoglycosides, beta-lactams, and lincosamides) and with variety of plant species such as wheat (Li *et al.,* 2023), corn (Michelini *et al.,* 2012; Gomes *et al.,* 2019), rice (Liu *et al.,* 2009; Motwani and Mehta, 2018; Cheong *et al.,* 2020), lettuce (Pan and Chu, 2016; Tasho *et al.,* 2020), cabbage (Pan and Chu, 2016; Cheong *et al.,* 2020), spinach (Tao *et al.,* 2022), pakchoi (Duan *et al.,* 2018), carrot (Pan and Chu, 2016; Tasho *et al.,* 2020), cucumber (Pan and Chu, 2016), tomato (Bellino *et al.,* 2018), pepper (Tasho *et al.,* 2020), and Basil (De Mastro *et al.,* 2023).

Although several studies have investigated the phytotoxic effects of antibiotics on various plant species, their impacts on the common pulse crop *Vigna radiata* is very limited, except for a few studies by Sharma and Malan (2017), who reported phytotoxic responses in *V. radiata* exposed to paracetamol and ibuprofen, which focused on oxidative stress-mediated toxicity due to growth inhibition. Similarly, exposure to oxytetracycline and levofloxacin has been shown to disrupt nitrogen metabolism and induce oxidative stress, resulting in reduced plant growth at higher concentrations (Fiaz *et al.,* 2023). Since *Vigna radiata* is a widely cultivated pulse crop in India serving as an affordable and significant source of dietary protein, an understanding of its response to antibiotic contamination in South Indian soils is crucial for assessing potential risks to crop productivity and food security, owing to the difference in phytotoxic effects for plant species, antibiotics, soils and different concentrations (Hills *et al.,* 2011; Wang *et al.,* 2019; Sun *et al.,* 2021).

To address this research gap, the present study investigated the phytotoxic effects of eleven frequently used veterinary antibiotics in our area, Coimbatore, Tamil Nadu, India, to evaluate their impact on seed germination and early seedling development of *Vigna radiata*. The identified antibiotics belonged to five major classes: tetracyclines (oxytetracycline, chlortetracycline, and doxycycline), quinolones (enrofloxacin and ciprofloxacin), sulfonamides (sulfadiazine and sulfadimidine), aminoglycosides (gentamicin), and β-lactams (amoxicillin, ampicillin, and ceftriaxone).

2. Methodology

2.1 Standards

The antibiotics enrofloxacin (ENR), ciprofloxacin (CIP), oxytetracycline (OTC), chlortetracycline (CTC), doxycycline (DOX), gentamicin (GENTA), amoxicillin (AMX), ampicillin (AMP), ceftriaxone (CEF), sulfadiazine (SDZ), and sulfadimidine (SD), representing five or six major classes of antibiotics, were used for analysis. They were procured from Sigma Aldrich with a purity > 99%.

2.2 Seed collection

Certified seeds of *Vigna radiata* L. were obtained from Tamil Nadu Agricultural University (TNAU), Coimbatore, India, with a certified germination rate greater than 98%.

2.3 Stock and working standard solutions

Stock solutions were prepared by dissolving 30 mg of each antibiotic in 30 mL of methanol to obtain a final concentration of 1000 µg mL^{-1}. Working standard solutions with concentrations of 1, 5, 10, 25, 50, 100, 200, and 300 µg mL^{-1} were prepared by appropriate dilution of the stock solutions using deionized water. All standard solutions were freshly prepared 1 h prior to the experiment to ensure stability.

2.4 Experimental design and germination assay

The filter paper method was followed to evaluate the effect of the veterinary antibiotics on seed germination and root elongation in *Vigna radiata* L. (International Seed Testing Association, ISTA, 1985). *V. radiata* seeds were surface sterilised using 0.1% mercuric chloride and then rinsed, followed by soaking in deionised water for 4 hours. Twelve healthy seeds of *V. radiata* L. were placed on a filter paper (Whatman 22 µm, 9 cm diameter) in a Petri dish (10 cm diameter). The filter papers were treated with 5 mL of each antibiotic solution at concentrations 0, 1, 5, 10, 25, 50, 100, 200, and 300 µg mL^{-1} and covered before placing in a dark growth cabinet. Seeds were germinated under the conditions of 25 ºC, with 60% of humidity. Deionized water was used for the control experiment. Water loss from the Petri dishes was monitored daily, and 3 mL of the respective antibiotic solutions was added when necessary to maintain the target concentration. The experiment was terminated after 11 days. All experiments were performed in triplicate.

These concentrations were selected based on previous reported antibiotic residues in Tamil Nadu soils, with aminoglycosides (0.40 µg kg^{-1}), β-lactams (1.40 µg kg^{-1}), fluoroquinolones (16.80 µg kg^{-1}), tetracyclines (19.90 µg kg^{-1}), and sulfonamides (26.80 µg kg^{-1}) detected in paddy fields (Kuppusamy *et al.,* 2023), tetracyclines (8.30–61 µg kg^{-1}), sulfonamides (0.20–48.40 µg kg^{-1}), and quinolones (1.80 µg kg^{-1}) frequently reported in Indian farmland soils (Kuppusamy *et al.,* 2024), and fluoroquinolones occurring at 20–26 µg g^{-1} in dumpsite soils of Chennai, Tamil Nadu (Arun *et al.,* 2022).

2.5 Growth parameter measurements

At the end of the experiment (day 11), growth parameters, including germination percentage, root length, shoot length, and total seedling length, were measured using a standard millimeter-scale ruler. Germination was considered successful when the radicle visibly emerged from the seed coat.

Percent seed germination was calculated as follows,

$$\%\,Germination = \frac{Number\ of\ seeds\ germinated\ at\ 11^{th}\ day}{Total\ number\ of\ seeds} \times 100 \qquad \text{...... } \textbf{Eqn.1}$$

Phytotoxicity index (PI) was estimated based on germination and root elongation of *Vigna radiata* seeds using the following equation:

$$PI = 1 - \frac{R_{LT}}{R_{LC}} \qquad \text{...... } \textbf{Eqn.2}$$

where, RLT is the root length in the antibiotic-treated seeds, and RLC is the root length in the control treatment. The values of the PI range between (0) and (1), in which a higher value means a negative (i.e., toxic) effect and a lower value means a positive (i.e., stimulatory) effect (Mekki *et al.,* 2007; Rusan *et al.,* 2017).

The seedling vigor index (SVI) was calculated according to **Eq. 3.** Higher SVI indicates more vigorous growth (Munir *et al.,* 2015).

$$\text{SVI-I} = \text{SL} \times \text{FGP} \qquad \text{...... } \textbf{Eqn.3}$$

where, SL = total length of seedling (cm), and FGP = Final germination percentage.

2.6 Statistical analysis

Data are presented as mean ± standard deviation (SD) of three replicates. Statistical analysis was performed using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to evaluate the effects of different antibiotic concentrations on germination and seedling growth parameters. When significant differences were observed, Duncan's multiple range test was applied to compare the treatment means. Statistical significance was considered at $p < 0.05$.

The No-Observed-Effective Concentration (NOEC) and the Lowest-Observed Effective Concentration (LOEC) were determined based on comparisons with the control treatment. The EC_{25} and EC_{50} values were calculated using linear regression analysis between antibiotic concentration and percentage of germination inhibition.

3. Results and Discussion

3.1 Seed germination

The impact of initial concentrations of the veterinary antibiotics on seed germination is illustrated in **Figure 1**. The results showed inhibition of seed germination compared with the control. Control seeds exhibited 100% germination, whereas germination percentage decreased progressively in seeds exposed to antibiotic treatments with increasing antibiotic concentrations. A reduction in germination exceeding 50% at a concentration of 200 µg mL^{-1} was observed for all antibiotics tested. At low concentrations (1 and 5 µg mL^{-1}), germination percentages remained comparable to the control, with no statistically significant differences ($p > 0.05$), suggesting that trace-level antibiotic exposure does not adversely affect early seed germination.

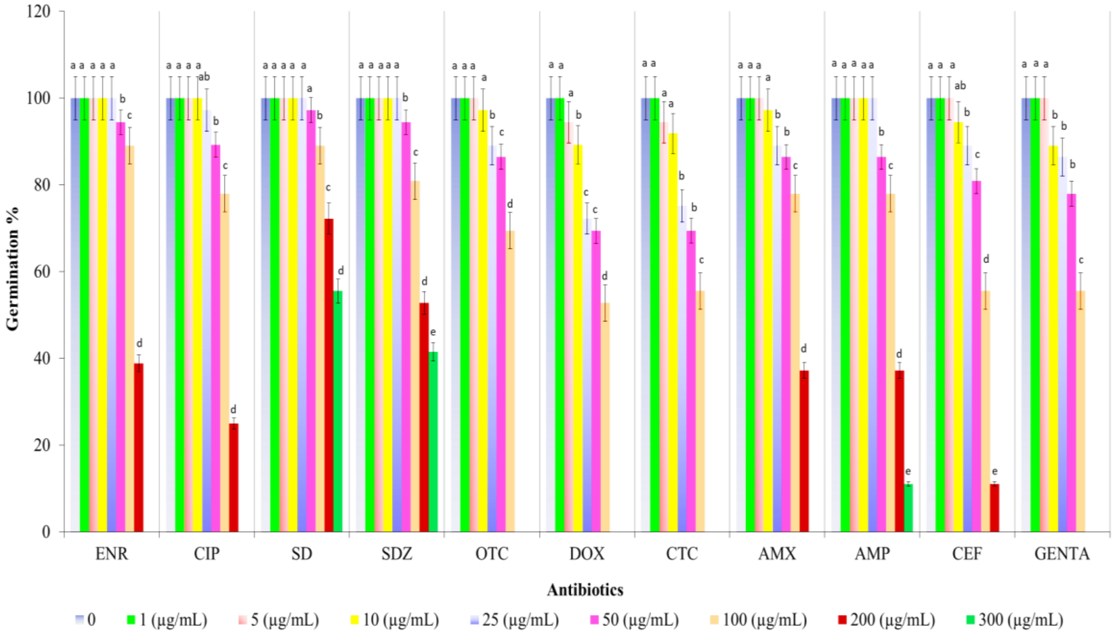

Figure 1. Effect of veterinary antibiotics on germination (%) of *Vigna radiata* after 11 days of exposure.

The bars and error bars displays the mean and standard deviation of triplicates. The same letters in the same row for each antibiotic indicates that are not significantly different between treatments according to the Duncan test at $p < 0.05$.

However, with an increase in concentrations from 10 µg mL^{-1}, a progressive decline in germination was evident with statistically significant reductions observed at 25–50 µg mL^{-1} ($p < 0.01$) for most antibiotics. The magnitude of inhibition increased with concentration, confirming a dose–response relationship. Our results align with the findings of Hammok *et al.* (2024), where florfenicol decreased the percent germination of *Drachma Syngenta* by 55% with a rise in initial concentrations from 0.005% to 0.04%. Similar inhibitory effects of antibiotics on seed germination and seedling growth have been reported in previous studies. Cheong *et al.* (2020) observed inhibition of seed germination and seedling growth at higher concentrations of chlortetracycline (> 25 mg L^{-1}) in *Brassica campestris*. Likewise, Minden *et al.* (2017) reported inhibitory effects of penicillin, sulfadiazine, and tetracycline in *T. aestivum* (wheat) and *Apera spica-venti* (wind grass), while Eichberg *et al.* (2024) documented similar effects for sulfadimidine treated with *Centaurea jacea* (brown knapweed), *Galium mollugo* (hedge bedstraw), and *Sagittaria latifolia* (duck-potato) plants. In the present study, seed germination was observed within 24 h in the control and low-concentration treatments (0–10 µg mL^{-1}) for all eleven antibiotics. In contrast, exposure to higher concentrations (> 10 µg mL^{-1}) resulted in a significant delay in germination, with seed emergence occurring after 60–74 h (approximately 2.5–3 days), irrespective of antibiotic type. Complete inhibition of germination was observed at 200 and 300 µg mL^{-1} for OTC, DOX, CTC, and GENTA, whereas ENR, CIP, CEF, and AMX exhibited complete inhibition of germination only at 300 µg mL^{-1}. Conversely, seed emergence was observed for SD (41.49%), SDZ (55.53%), and for AMP (11.10%) even at a higher antibiotic concentration of 300 µg mL^{-1} (**Figure 2**). Seed emergence in rice and wheat, even with increasing concentrations of 0-1000 µg mL^{-1} and 5 to 80 µg mL^{-1} for oxytetracycline and enrofloxacin, respectively, has been reported (Motwani and Mehta, 2018; Li *et al.*, 2023).

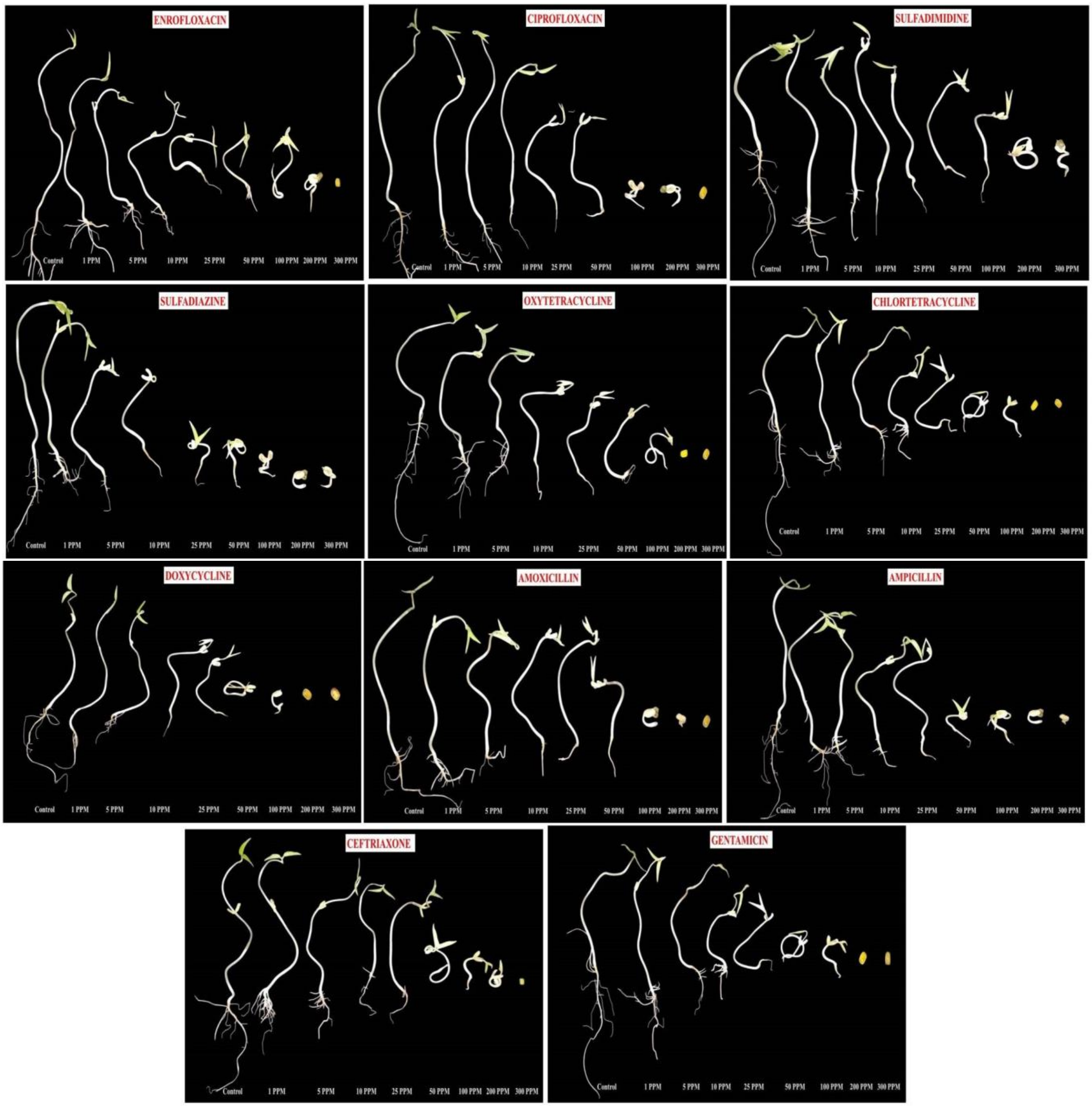

Fig. 2. Effect of veterinary antibiotics on shoot and root growth of *Vigna radiata* after eleven days of exposure

Seed germination even at higher concentrations of antibiotics may be due to weak permeability of the seed coat to antibiotics, thereby restricting the contact between the antibiotics and the embryo, probably through the production of mucilage, as explained by Teixeira *et al.* (2020) for *P. lanceolata*. The variations in germination responses of the seeds most likely relate to differences in the physicochemical properties of antibiotics, such as log K_ow, molecular weight, and mode of action, as has been explained by Pan and Chu (2015) and Bellino *et al.* (2017). This is consistent with our findings, wherein germination percentage and root elongation differed significantly among the antibiotics tested (**Figures 1 and 2**). Among the five classes of antibiotics, tetracycline showed the highest potential to reduce seed germination of *Vigna radiata*, followed by beta-lactams, aminoglycosides, quinolones, and sulfonamides.

Antibiotic stress may inhibit seed germination through different mechanisms. Disruption of H_2O_2-mediated signaling has been reported in *Brassica campestris* (Cheong *et al.,* 2020), where chlortetracycline concentrations exceeding 25 µg mL^{-1} significantly inhibited seed germination and seedling growth. In contrast, inhibition associated with disruption of oxidative signaling mechanisms has been observed in *Lactuca sativa* (Choe *et al.,* 2024). Furthermore, Gomes *et al.* (2017) reported that ciprofloxacin impairs electron transport processes and alters reactive oxygen species regulation in *Lemna minor*, even at relatively low concentrations (0.75 µg mL^{-1}).

3.2 Root elongation

Root elongation is a significant endpoint used in plant toxicity testing. The impact of the veterinary antibiotics on root elongation of *Vigna radiata* is shown in **Figure 3**. Higher root length was observed in the control, whereas the lowest root length was recorded at 300 µg mL^{-1} treatment for all eleven antibiotics (**Figure 2**). Statistical analysis ($p < 0.05$) revealed that root length significantly decreased with increasing initial concentrations of antibiotics from 1–300 µg mL^{-1}, indicating that even low concentrations of veterinary antibiotics exert root inhibition in *Vigna radiata*. Such a significant decrease in root length was reported by Eluk *et al.* (2016) in wheat exposed to 1 µg mL^{-1} of oxytetracycline, 10 µg mL^{-1} of enrofloxacin, 15 µg mL^{-1} of kanamycin, and 0.4 µg mL^{-1} of penicillin. Similarly, inhibition of root elongation with increasing antibiotic concentrations has been reported in corn (Michelini *et al.,* 2012; Gomes *et al.,* 2019), carrot (Hills *et al.,* 2011), rice (Liu *et al.,* 2009; Motwani and Mehta, 2018; Cheong *et al.,* 2020), tomato (Bellino *et al.,* 2018), pepper (Tasho *et al.,* 2020), and spinach (Sun *et al.,* 2021; Tao *et al.,* 2022).

Approximately 50% reduction in root length was observed at 5 µg mL^{-1} for all eleven antibiotics. The percentage reduction was 47.12% for ENR, 43.56% for CIP, 50% for SD, 57.11% for SDZ, 45.37% for OTC, 68.90% for DOX, 67.56% for CTC, 62.63% for AMX, 59.34% for AMP, 58.76% for CEF, and 66.92% for GENTA at 5 µg mL^{-1}. Moreover, 100% root inhibition was observed for ENR, CIP, AMX, and CEF at 300 µg mL^{-1}, whereas complete inhibition was observed for OTC, DOX, CTC, and GENTA from 200 µg mL^{-1}. The vulnerability of roots to antibiotics may be due to their high rate of accumulation in root tissues compared to other plant organs, as reported by Michelini *et al.* (2012), Ghava *et al.* (2015), Ma *et al.* (2017), and Li *et al.* (2023), which interfere with the cell division and elongation processes in the root apical meristem, thereby restricting root growth as reported by Hillis *et al.* (2011) and Eichberg *et al.* (2024).

Radicle-type roots were observed from the 100 µg mL^{-1} treatment for all antibiotics (Figures 2 and 4). However, limited root growth in the form of radicles was still observed for SD (0.50 cm), SDZ (0.76 cm), and AMP (0.40 cm) even at the higher concentration of 300 µg mL^{-1}. The residual root development in SD, SDZ, and AMP treatments may be related to the emergence capacity of *Vigna radiata* seeds under stress conditions. The reduction in root length at higher antibiotic concentrations may be attributed to the inhibition of cell division at the root apical meristem, as reported by Hillis *et al.* (2011) and Eichberg *et al.* (2024). Furthermore, root hairs were absent from 10 µg mL^{-1} onwards (**Figures 2 and 4**), indicating stress-induced morphological changes. The absence of root hairs and the presence of radicle-type roots suggest higher stress levels in treated seedlings. However, a few root hairs were observed at 1 and 5 µg mL^{-1} for all eleven antibiotics (**Figure 2**), indicating that lower concentrations exert only mild effects on root development, possibly reflecting a hormetic response. Previous studies suggest that antibiotics can inhibit root growth by interfering with cell differentiation and key metabolic pathways, particularly folate synthesis and callus formation (Yepes and Aldwinckle, 1994). Dodds and Roberts (1981) reported that exposure to gentamicin and sulfonamides inhibited root development in *Helianthus tuberosus,* attributing it as due to disruption of folate metabolism and reduced cellular proliferation, as further supported by Basset *et al.* (2004, 2005).

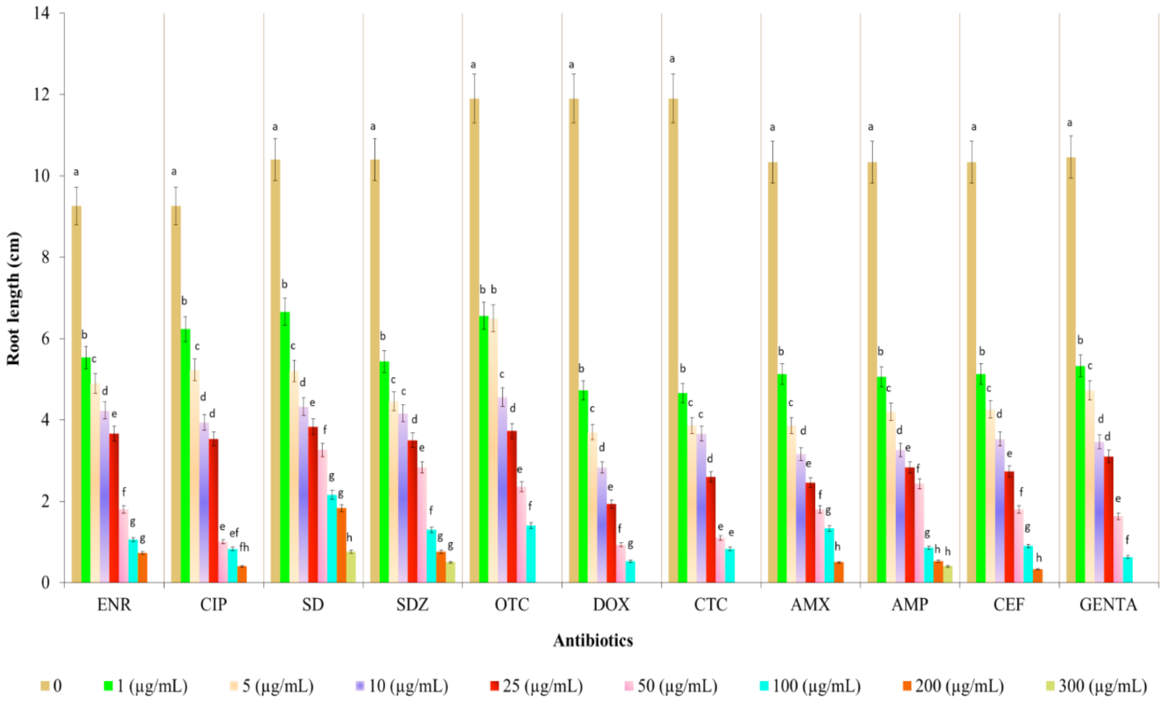

Figure 3. Effect of veterinary antibiotics on root elongation in *Vigna radiata* after 11 days of exposure
The bars and error bars displays the mean and standard deviation of triplicates. The same letters in the same row for each antibiotic indicates that are not significantly different between treatments according to the Duncan test at $p < 0.05$.

3.3 Shoot and total seedling length

The shoot length and total seedling length of *Vigna radiata* are illustrated in **Figures 4 and 5**. The results indicated that the highest shoot length was observed at the 1 μg mL^{-1} treatment; whereas the lowest shoot length was recorded at 300 μg mL^{-1}. However, the decrease in shoot length was statistically significant only from 5–300 μg mL^{-1} ($p < 0.01$). For total seedling length, a statistically significant difference was observed from 1 μg mL^{-1} ($p < 0.01$) for all antibiotics. This difference may be attributed to the lower concentrations of antibiotics, which can exert neutral or even beneficial effects on shoot development in plants, as reported by Pan and Chu (2015). This hypothesis is consistent with the present findings, where higher shoot length was observed at 1 μg mL^{-1} compared to the control. The stimulation at lower antibiotic concentrations may be due to hormetic effects, where low doses trigger plant defense mechanisms and enhance cell division at the shoot apex, resulting in increased shoot elongation. Similar responses have been reported in *Malus* (exposed to cefotaxime, carbenicillin, and kanamycin), wheat (oxytetracycline and enrofloxacin), and rice (tetracycline, amoxicillin, and trimethoprim) plants by Yepes *et al.* (1994), Li *et al.* (2023), and Motwani and Mehta (2018). Hormetic responses at lower concentrations have also been observed in *Vigna radiata* with paracetamol and ibuprofen (Sharma and Malan, 2017) and in *Pisum sativum* exposed to fluoranthene (Kummerová *et al.,* 2012), where shoot elongation was stimulated despite reductions in total plant length. The hypocotyl structure, representing the early stage of shoot development, was observed in plants treated with > 50 μg mL^{-1} (**Figure 2**). A 50% reduction in shoot length was observed from 100 μg mL^{-1}, whereas for total seedling length, it was observed from 25 μg mL^{-1} for all antibiotics studied. Higher antibiotic concentrations may induce oxidative stress, which can impair photosynthetic activity and cause cellular damage, thereby reducing plant growth parameters. Such effects have been reported by Riaz *et al.* (2017) in *Triticum aestivum* exposed to enrofloxacin, ciprofloxacin, and levofloxacin, in

Spirodela polyrhiza exposed to amoxicillin. The significant decrease in total seedling length observed across the concentration range (1–300 µg mL^{-1}) is likely associated with the pronounced reduction in root length, which plays a critical role in overall seedling growth. Similarly, ciprofloxacin has been shown to impair electron transport processes in *Lemna minor* (Gomes *et al.,* 2017), while oxytetracycline exposure has been associated with reduced growth in alfalfa plants (Huang *et al.,* 2021). Elevated levels of reactive oxygen species (ROS) can further damage cellular components and disrupt metabolic transport systems in plants (Van Breusegem and Dat, 2006).

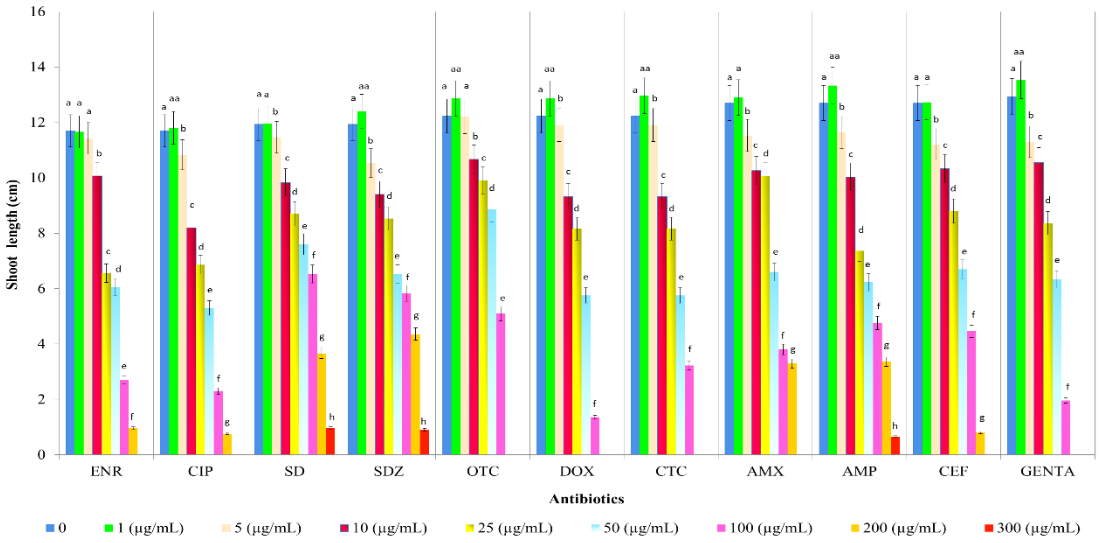

Figure 4. Effect of veterinary antibiotics on shoot elongation in *Vigna radiata* after 11 days of exposure
The bars and error bars displays the mean and standard deviation of triplicates. The same letters in the same row for each antibiotic indicates that are not significantly different between treatments according to the Duncan test at $p < 0.05$.

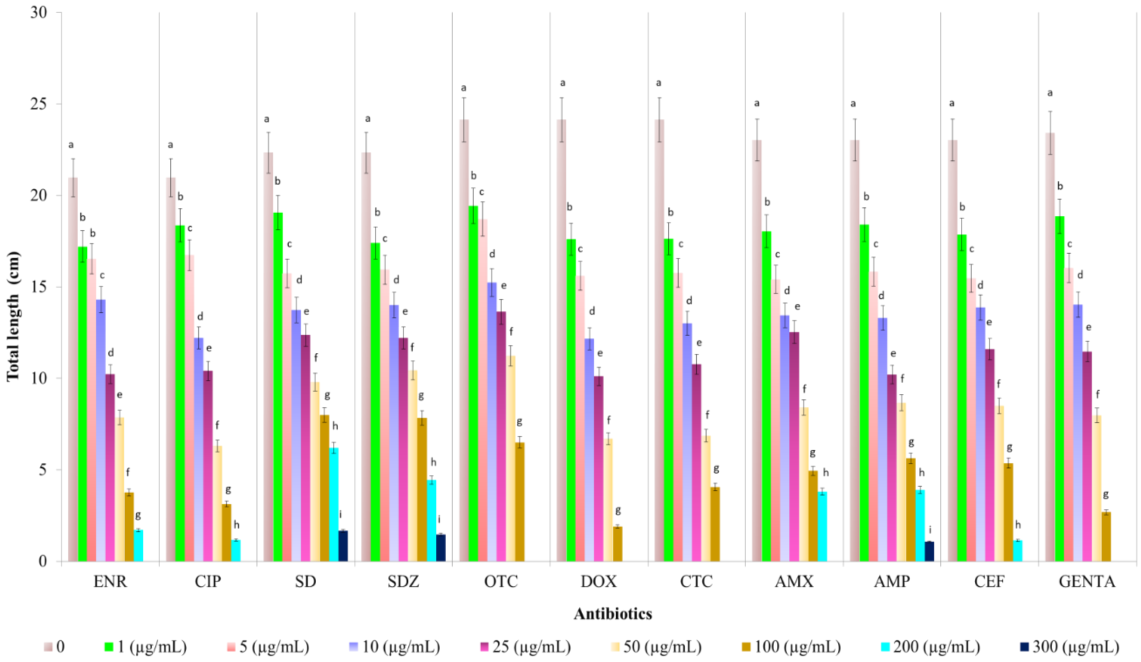

Figure 5. Effect of veterinary antibiotics on total length of seedlings of V. radiate after 11 days of exposure
The bars and error bars displays the mean and standard deviation of triplicates. The same letters in the same row for each antibiotic indicates that are not significantly different between treatments according to the Duncan test at $p < 0.05$.

3.4 Phytotoxicity assessment

Interclass and intraclass responses of veterinary antibiotics on *Vigna radiata* were evaluated using EC_{50}, EC_{25}, phytotoxicity index (PI), and seed vigor index (SVI) (**Figures 6-8**). The PI values ranged between 0.32 and 1, while SVI values ranged from 2413 to 12.54, respectively, for all antibiotics. Higher SVI values (> 2000) were observed in the control, whereas the lowest values (< 100) were recorded in 300 µg mL^{-1}. The phytotoxicity index was higher in the 300 µg mL^{-1} treatment and lowest in the 1 µg mL^{-1} treatment for all antibiotics, suggesting moderate to high toxic effects on plant root development. This observation corroborates with the findings of Munir *et al.* (2015), who reported PI values ranging from 0.02 to 0.56, indicating low to high toxicity.

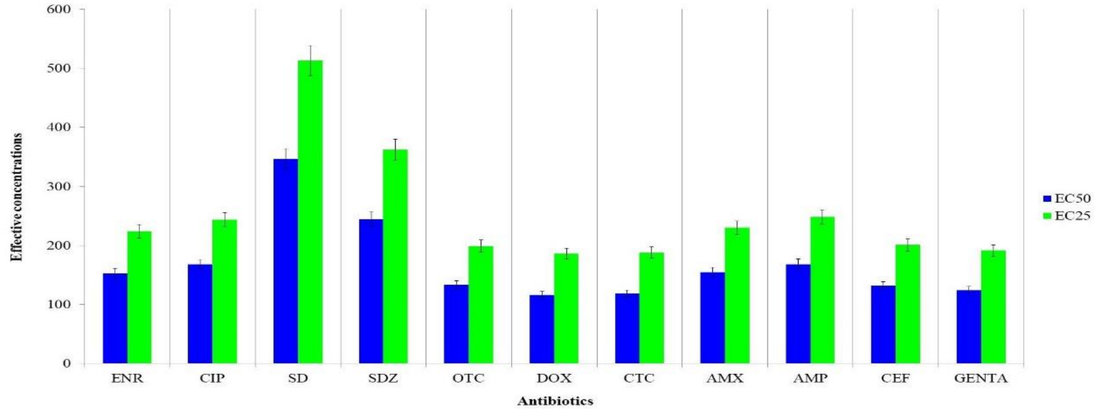

Figure 6. EC_{50} and EC_{25} values of veterinary antibiotics on *Vigna radiata*

The bars and error bars displays the mean and standard deviation of triplicates. The same letters for each antibiotic indicates that are not significantly different between treatments according to the Duncan test at $p < 0.05$.

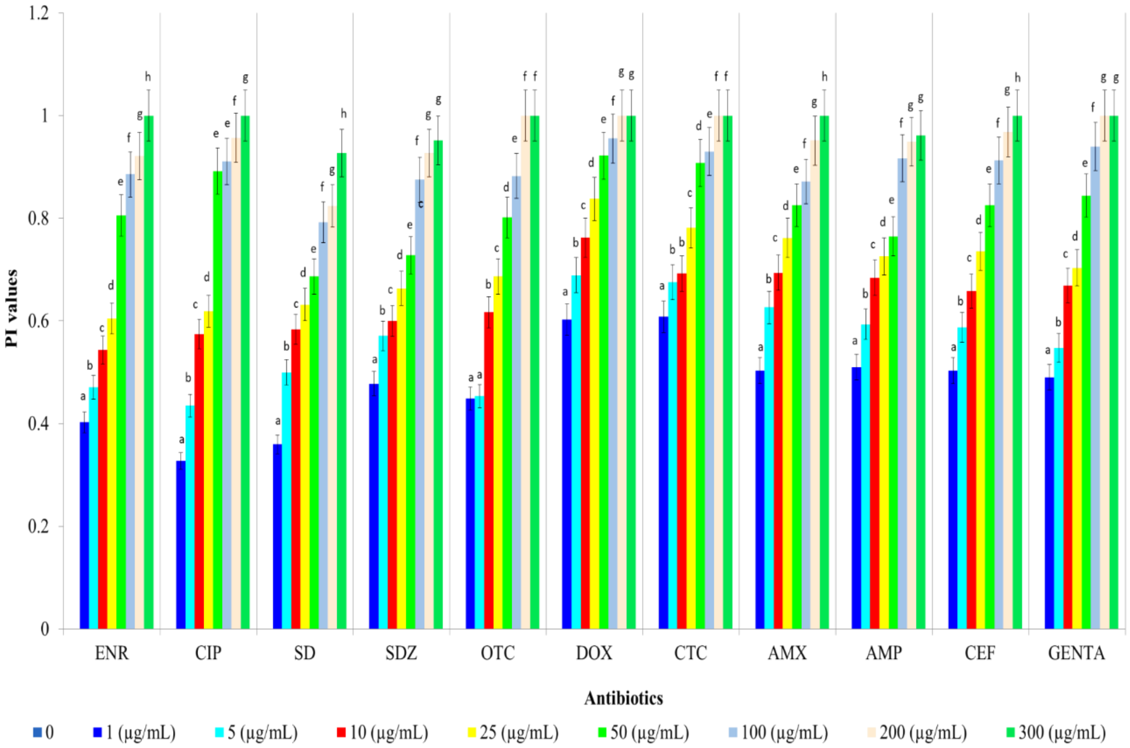

Figure 7. Phytotoxicity index of veterinary antibiotics on *Vigna radiata* after 11 days of exposure

The bars and error bars displays the mean and standard deviation of triplicates. The same letters in the same row for each antibiotic indicates that are not significantly different between treatments according to the Duncan test at $p < 0.05$.

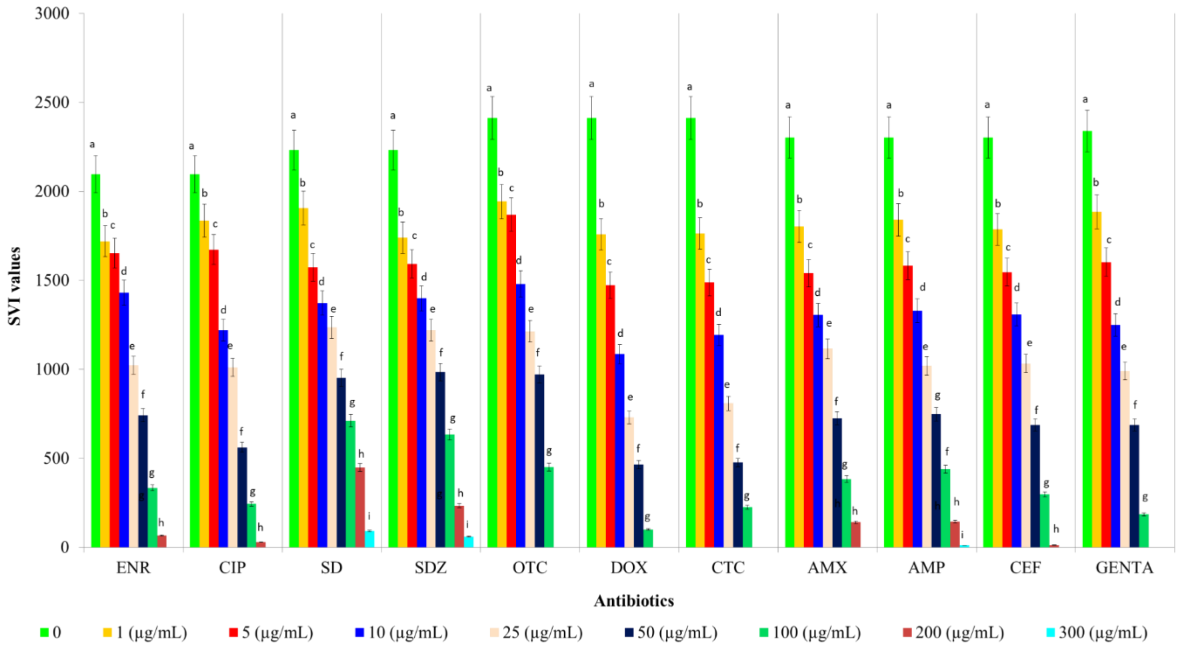

Figure 8. Seed vigor index of *Vigna radiata* exposed to veterinary antibiotics after 11 days

The bars and error bars displays the mean and standard deviation of triplicates. The same letters in the same row for each antibiotic indicates that are not significantly different between treatments according to the Duncan test at $p < 0.05$.

The EC_{50} and EC_{25} values ranged from 116.03 to 346.40 µg mL^{-1} and 186.12 to 513.06 µg mL^{-1}, respectively, revealing higher phytotoxicity of doxycycline (EC_{50} = 116.03 µg mL^{-1}; EC_{25} = 186.12 µg mL^{-1}), followed by chlortetracycline, ceftriaxone, gentamicin, oxytetracycline, ciprofloxacin, amoxicillin, enrofloxacin, ampicillin, sulfadimidine, and by sulfadiazine which exhibited lowest toxicity (EC_{50} = 346.40 µg mL^{-1}; EC_{25} = 513.06 µg mL^{-1}). The NOEC was 1 µg mL^{-1}, and the LOEC was 5 µg mL^{-1}, which confirms that even relatively low concentrations of these antibiotics can negatively impact seed germination and early seedling development.

Among the five classes of antibiotics studied, tetracyclines showed more phytotoxic effects, whereas sulfonamides exhibited a moderate phytotoxic effect. This aligns with findings of Hillis *et al.* (2011) who reported that tetracyclines were more effective than the sulfonamides. The impact of antibiotics on plant growth is multifactorial, including plant species, antibiotic concentrations, tissue types, and physiological stages (Pan and Chu, 2016).

4. Conclusion

This study evaluated the phytotoxic effects of eleven veterinary antibiotics on the germination and early seedling growth of *Vigna radiata*. The results demonstrated that exposure to these antibiotics caused significant concentration-dependent inhibition of germination and seedling development, as evidenced by increased phytotoxicity index (PI) and reduced seed vigor index (SVI). The NOEC and LOEC values of 1 and µg mL^{-1}, respectively, indicate that even relatively low concentrations of veterinary antibiotics can adversely affect plant growth. Among the eleven antibiotics, doxycycline showed the highest inhibitory effect, whereas least effect was observed with sulfadiazine.

Given that these antibiotics are commonly used in veterinary practices in the study region (Coimbatore), their release into agricultural soils, particularly through manure application or wastewater irrigation, poses a potential risk to crop productivity. This concern is especially significant in the context of the widespread cultivation of *Vigna radiata*, an economically affordable and protein-rich pulse crop. These findings underscore the need for monitoring and regulating antibiotic residues in agricultural environments. Further research focusing on physiological and molecular mechanisms is essential to better understand the long-term impacts of antibiotic exposure on crop plants.

Acknowledgement

The authors are thankful to the Tamil Nadu State Council for Higher Education (TANSCHE) for providing financial support through the Research Grant Program (Grant No.: TANSCHE–RGP Project No. RGP/2019–20/BU/HECP-0086).

Disclosure statement:

Conflict of Interest: The authors declare that there are no conflicts of interest.

Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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(2026) ; http://www.jmaterenvironsci.com