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Antibiotic toxicity screening on seedling emergence: beyond traditional species

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Antibiotics are prevalent environmental pollutants with documented plant uptake and effects. Germination and seedling emergence are critical stages of plant development, making toxicity tests valuable for assessing the terrestrial risk. This study aims to address the need to understand species and antibiotic-dependent effects of 10 antibiotics across 23 plant species, including non-cultivated and geographically diverse species, through a Tier I phytotoxicity screening in which all antibiotics were tested at a single nominal concentration of 1 mg L^{-1}, an upper-bound environmentally relevant scenario. Results revealed that antibiotic toxicity is highly species and antibiotic-dependent, highlighting the need to evaluate effects on under-studied species. Some global trends were observed; fluoroquinolones (ciprofloxacin, enrofloxacin, ofloxacin) stimulated germination and root growth in legumes and grasses, while black knapweed (*Centaurea nigra*) consistently exhibits germination reductions (30–53%) and root growth inhibition under all antibiotic exposures. Florfenicol exposure decreased root length and biomass in Chinese cabbage (*Brassica rapa subs. Pekinensis*) by nearly 100%, contrasting with stimulation observed in rice (*Oryza sativa*). The importance of assessing sublethal effects, as root and biomass changes, in addition to germination, for a more comprehensive phytotoxicity assessment is demonstrated. Standardized test conditions may overlook species requiring specific germination conditions. Sorption of antibiotics to filter paper affected exposure concentrations, emphasizing the importance of chemical quantification before and after tests. This study highlights the need for adaptive phytotoxicity protocols, careful experimental design to obtain statistically significant results, and inclusion of non-cultivated species as bioindicators to better assess antibiotic risks to terrestrial plants.

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Environmental significance

Antibiotics are increasingly recognized as emerging environmental pollutants due to their widespread occurrence and persistence in agricultural systems. This study provides a comprehensive cross-country Tier I screening, evaluating the phytotoxic effects of ten antibiotics across 23 cultivated and non-cultivated plant species from China, the UK, and Mexico. The results revealed that antibiotic toxicity is both species- and antibiotic-dependent, showing variable effects on germination, root elongation, and biomass. This study identifies, for the first time, sensitive non-cultivated species as potential bioindicators for environmental monitoring, like black knapweed. By highlighting species-specific responses and limitations of current standard testing protocols, these findings contribute to improving environmental risk assessment frameworks and promoting more representative and ecologically relevant methods for evaluating impacts of antibiotics on terrestrial ecosystems.

Introduction

Antibiotics are extensively used worldwide in human and veterinary medicine[1] and are routinely applied in agricultural systems for disease prevention and growth promotion.[2,3] As these pharmaceuticals are not fully absorbed by humans or animals, a substantial fraction of the consumed dose, and their intermediates, are excreted in faeces or urine,[4] leading to their introduction into soils *via* wastewater irrigation and the land application of wastewater-derived biosolids, animal manures, and slurries.[5] Antibiotic concentrations in agricultural soils have been reported to range from the nanogram to microgram per gram range, with higher levels typically associated with soils receiving livestock-derived amendments.[6,7] Sulfonamides and tetracyclines are among the most frequently detected classes at elevated concentrations,[6,7] although marked regional variability has been observed depending on soil properties and amendment practices. For example, soils amended with chicken

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manure in China contained higher fluoroquinolone concentrations than sulfonamides or tetracyclines, with ciprofloxacin levels reaching up to 18.42 μg kg^{-1},[8] whereas soils irrigated with raw wastewater in Cameroon presented substantially lower ciprofloxacin concentrations (0.44–2.33 ng g^{-1}), along with sulfamethoxazole, trimethoprim, enrofloxacin, and oxytetracycline at similar or lower levels.[9] Information remains comparatively scarce for other regions, including Latin America. In the Tula Valley (Mexico), where raw wastewater has been used for agricultural irrigation for over a century,[10] antibiotic concentrations in irrigation water have been reported between 337.9 ng L^{-1} (oxytetracycline) and 9059.8 ng L^{-1} (erythromycin),[11] while corresponding soil measurements are limited, with triclosan reported at 7.7 ng g^{-1} (ref. 12).

Once present in soils, antibiotics can be taken up by plants to varying extents depending on the exposure scenario.[5,13,14] Studies have reported antimicrobial residues in irrigation water, soils, and crops, although accumulation in plant tissues varies widely. For example, antimicrobials were detected in 85% of water and soil samples but at lower frequencies and concentrations in crops irrigated with reclaimed water in Israel,[15] whereas sulfamethoxazole concentrations exceeding 30 mg kg^{-1} were reported in tomato (*Solanum lycopersicum* L.) following irrigation with treated wastewater in Spanish glasshouses.[16] Similarly, a meta-analysis documented plant accumulation of tetracyclines and sulfonamides above 50 mg kg^{-1} in studies from China, Germany, and Spain following exposure to pig slurry and manure.[17]

Following plant uptake, antibiotics have been shown to exert phytotoxic effects, including alterations in germination, growth, and photosynthesis. These effects can arise through multiple pathways, such as disruption of electron transport, reductions in vascular bundle size and stomatal conductance, and disturbances to photosynthetic pigments and chloroplast structure.[18] Three out of the four antibiotic biomolecular targets, intended for animals, are shared by plants, therefore antibiotics may easily interfere with plant biomolecular pathways.[13] Phytotoxic responses have been reported to be antibiotic class-dependent. For instance, amoxicillin did not affect germination of lettuce (*Lactuca sativa*), alfalfa (*Medicago sativa*), or carrot (*Daucus carota*), whereas other antibiotics, such as sulfamethoxazole, induced effects under comparable conditions.[19] Despite growing evidence of antibiotic occurrence in soils, plant uptake, and adverse effects, toxicity studies have predominantly focused on a limited number of fast-growing cultivated species, including spinach (*Spinacia oleracea*), lettuce (*L. sativa*), radish (*Raphanus sativus*), and thale cress (*Arabidopsis thaliana*),[17,20,21] as well as major agricultural crops such as wheat (*Triticum aestivum*),[21–23] rapeseed (*Brassica campestris*), maize (*Zea mays*)[24] and rice (*Oryza sativa* L.).[21,25] In contrast, far less is known about antibiotic effects on non-cultivated species and on agriculturally relevant species from understudied regions.[13,18,26] Addressing this gap is critical, as antibiotic exposure in agricultural systems extends beyond cultivated crops, and effects on non-cultivated species may have cascading consequences for ecosystem functioning.[5,13] Furthermore, current risk assessments typically consider a limited range of antibiotic classes[17] and rarely evaluate comparable endpoints across multiple compounds, constraining our understanding of differential phytotoxic responses. Given that antibiotic use has increased globally since their introduction in the 1940 decade and is projected to continue rising in many regions,[2,27,28] the generation of globally representative toxicity data across species and antibiotic classes remains essential for robust environmental risk assessment.

This study presents, for the first time, an international Tier I hazard-screening of antibiotic phytotoxicity during seed germination, designed to address key gaps in terrestrial plant risk assessment across species and regions. The screening focuses on non-cultivated plant species from the UK, selected based on Annex 3 of OECD Guideline 208 for non-crop terrestrial plant testing,[29] alongside agriculturally and culturally relevant species from Mexico and China. Experiments were conducted in three laboratories using standardized protocols, enabling cross-country comparability within a collaborative international framework. In line with a Tier I screening objective, all antibiotics were tested individually at a single nominal concentration of 1 mg L^{-1},[30,31] selected as an upper-bound environmentally relevant exposure scenario. Antibiotic concentrations in pore water of wastewater-irrigated or manure-amended soils have been reported in the high μg L^{-1} to mg L^{-1} range,[32–34] supporting the relevance of this concentration for conservative screening. Tier I assays intentionally use a single, elevated concentration to identify potential species–antibiotic sensitivities and to prioritize compounds and taxa for further investigation, rather than to derive dose–response relationships, consistent with established screening frameworks, such as EPA Protocol 600/3-88/029A. Hydroponic filter-paper assay was used to expose germinating seeds directly to the dissolved, bioavailable fraction of each antibiotic, providing a functional analogue of pore-water exposure while minimizing confounding effects associated with soil sorption.[30,35–37] In addition to biological endpoints, antibiotic concentrations were quantified before and after exposure using liquid chromatography-mass spectrometry, an approach not typically included in seedling toxicity assays, but critical for evaluating exposure dynamics and informing interpretation of screening results. Overall, this study provides a comparative, cross-country assessment of early-stage plant responses to prioritize antibiotics and species that warrant further investigation. In addition, it provides an assessment of existing toxicity guidelines, by applying them in a cross-country, multi-laboratory screening framework. Accordingly, the outcomes of this Tier I screening are intended to identify sensitive species–antibiotic combinations and to inform the design of subsequent Tier II studies, where full dose–response experiments will be required to characterize concentration–effect relationships and derive effect thresholds.

Experimental

Chemicals

For the Mexico experiments, the chemicals used were HPLC grade Methanol and Acetonitrile (≥99.9%, Sigma-Aldrich), ammonium acetate and formic acid (≥97.8 and ≥98% respectively, both from Sigma-Aldrich), and sterile water was from ultrapure water (18.6 MΩ cm, Elix 5 tandem with Synergy® UV High Flow from Millipore®), then filtered through 0.22 μm

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Table 1 Taxonomic classification and supplier for the tested species in China, UK, and Mexico

Family	Genus	Common name	Species (Latin name)	Use	Seed supplier
China					
Fabaceae	Glycine	Soybean	*Glycine max*	Food crop	Shandong Xuhong Seed Technology Co, Ltd, China
Poaceae	Triticum	Wheat	*Triticum aestivum*	Food crop	Zhoukou Academy of Agricultural Sciences, China
Fabaceae	Vigna	Mung bean	*Vigna radiata*	Food crop	Gengniu Seeds Business Dept., Xinhe Town, Shuyang, China
Asteraceae	Lactuca	Lettuce	*Lactuca sativa*	Vegetable crop	Shouhe Seed Co, Ltd, China
Poaceae	Oryza	Rice	*Oryza sativa*	Food crop	Jiangsu Academy of Agricultural Sciences, China
Brassicaceae	Brassica	Chinese cabbage	*Brassica rapa* subsp.*Pekinensis*	Vegetable crop	Guangzhou Yunong Seedling High-Tech Co, Ltd China
Asteraceae	Cichorium	Chicory	*Cichorium intybus*	Forage/medicinal	Suqian Zeshun Landscaping Co, Ltd, China
Asteraceae	Leucanthemum	Shasta daisy	*Leucanthemum maximum*	Ornamental	Shouguang Firefly Agricultural Technology Co, Ltd, China
UK					
Fabaceae	Phaseolus	French bean	*Phaseolus vulgaris*	Food crop	Suttons
Fabaceae	Pisum	Pea	*Pisum sativum*	Food crop	Suttons
Fabaceae	Trifolium	Wild white clover	*Trifolium repens*	Forage	Yellow Flag Wildflowers
Fabaceae	Trifolium	Red clover	*Trifolium pratense*	Forage	Yellow Flag Wildflowers
Poaceae	Avena	Oats	*Avena sativa*	Food/forage crop	—
Poaceae	Triticum	Wheat	*Triticum aestivum*	Food crop	—
Poaceae	Hordeum	Barley	*Hordeum vulgare*	Food/forage crop	KWS Feeris
Poaceae	Festuca	Sheep's fescue	*Festuca ovina*	Forage/turfgrass	Yellow Flag Wildflowers
Poaceae	Festuca	Red fescue	*Festuca rubra*	Forage/turfgrass	Yellow Flag Wildflowers
Poaceae	Briza	Quaking grass	*Briza media*	Ornamental/wild	Yellow Flag Wildflowers
Asteraceae	Lactuca	Lettuce	*Lactuca sativa*	Vegetable crop	Suttons
Asteraceae	Leucanthemum	Oxeye daisy	*Leucanthemum vulgare*	Ornamental/wild	Yellow Flag Wildflowers
Asteraceae	Centaurea	Black knapweed	*Centaurea nigra*	Wildflower	Yellow Flag Wildflowers
Asteraceae	Centaurea	Cornflower	*Centaurea cyanus*	Ornamental/wild	Yellow Flag Wildflowers
Mexico					
Solanaceae	Solanum	Tomato	*Solanum lycopersicum*	Food crop	Hortaflor
Asteraceae	Tagetes	Mexican marigold (cempasúchil)	*Tagetes erecta*	Ornamental/cultural	Hortaflor
Caryophyllaceae	Dianthus	Carnation	*Dianthus caryophyllus*	Ornamental	Hortaflor
Fabaceae	Medicago	Alfalfa	*Medicago sativa*	Forage crop	Hortaflor

nitrocellulose membrane. In the UK, Methanol (99.9%, Fisher) and sterile water (ultrapure water 18.6 MΩ cm, Sartorius arium® comfort, EDI UV), which was then autoclaved in (LTE Touchclave-R autoclave at 126 °C for 15 min), were used. In China, methanol (≥99.5%, Aladdin) and sterile water (ultrapure water 18.6 MΩ cm, Shanghai Hitech Instruments Co., Ltd Hitech), which was then autoclaved in ZEALWAY G-85D), were used. In the SI, the details of the antibiotics used, the supplier, and the purity are provided (Table S1). Table 1 contains the supplier and species details of the seeds used for the germination tests. The filter papers used were 100 mm Camlab grade 122P [114V in the UK, 110 mm, Labshark Qualitative filter paper no. 130209069 in China, and 150 mm, Whatman Cat no 1002-147 in Mexico.

Seedling germination

Seed set-up. Germination assays were conducted using clean, sterile Petri dishes and seeds (SI, Section S1 and Table S2) in all three countries. For each species, the initial dry mass of 30 seeds was recorded. In Mexico, these seeds were subdivided into three replicates of 10 seeds, which were weighed separately. Seeds were pre-soaked overnight in sterile water in the dark to promote uniform imbibition (4 °C in the UK; 22 °C in China and Mexico). Any seeds that had germinated during this pre-soaking period were excluded prior to exposure. A hydroponic filter-paper system was used to ensure direct exposure of germinating seeds to the dissolved, bioavailable fraction of the antibiotics under standardized conditions. The number of seeds per Petri dish was adjusted according to seed size and local standard practice to avoid overcrowding (UK: 5 large or 10 small seeds; China: 7–12 seeds; Mexico: 10 seeds per dish, in triplicate).

Antibiotics were tested individually at a single nominal concentration of 1 mg L^{-1}. In the UK and China, 5 mL of the working solution (0.1% solvent volume) was added per Petri dish, while in Mexico, 40 mL were used to accommodate larger dish formats. The same nominal concentration was applied across all antibiotics and species to enable direct comparative

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screening. Reported environmental concentrations of the studied antibiotics in irrigation water and soil pore-water, together with the concentration used in this study, are summarized in Table S3 to provide environmental context and illustrate that the selected test concentration represents a conservative upper-bound dissolved-phase exposure relevant for Tier I hazard screening. Treatment details by country are provided in Table S2, and species-specific information is given in Table 1. Control dishes were prepared identically, replacing antibiotics with the equivalent volume of methanol. An additional control without seeds was included in the Mexico experiments to assess potential antibiotic degradation and sorption onto filter paper.

Petri dishes were sealed with parafilm and incubated in the dark for 7 days at temperatures selected to maximise germination (UK: 18 °C; Mexico and China: 22 °C). Dark conditions were used to simulate below-ground germination environments and to minimise confounding light-dependent developmental responses. This approach allows germination and early seedling emergence to be assessed independently of photomorphogenic processes. Seed germination was monitored every weekday, and it was noted when they had germinated. Seeds were considered germinated when the length of plumules (shoots) and radicles (roots) reached at least 2 mm, which is a commonly used criterion in seed germination studies.[38]

Data collection. After 7 days, the final germination rate was recorded. The seedlings were gently removed from the Petri dishes and placed on a piece of paper (black in China and Mexico and white in the UK), and images were captured, alongside a ruler to be able to measure radicle length. In the UK, a Flatbed scanner (Epson Expression 11000XL, model J331A) was used to obtain images, and in China and Mexico, images were taken with a phone (China: focal length was 6.765 mm and F number was f/1.78; Mexico: 6 mm actual focal length, equivalent to 26 mm in 35 mm format at f/1.6 aperture). Image J software (Java) was used to measure the length of the radicles. The Smart root plugin (UCLouvain, open source) was implemented to assist with radicle measurement.

Seedling dry biomass was measured at the end of the seven-day germination assay, in Mexico, considering each Petri dish as an individual experimental unit, and in China and the UK, considering the total of 30 seeds. The relative inhibition index (RCI, defined in eqn (1) is commonly used to quantify the degree of inhibition on plant growth based on dry biomass measurements, and was calculated as follows:[39]

$$\mathrm{RCI}_i(\%) = \frac{Y_{\mathrm{Control}} - Y_{\mathrm{Treatment},i}}{Y_{\mathrm{Control}}} \times 100 \quad (1)$$

where $Y_{\mathrm{Treatment}}$ is the total dry biomass of the i-treatment, and Y_{Control} the biomass of the control. A positive RCI value indicates an inhibitory effect, whereas a negative value indicates stimulation.[40] For the Mexico experiments, a standard deviation was calculated. Additionally, the change in biomass (Δ biomass, defined in eqn (2) was calculated as the difference between the final dry biomass and the initial seed dry weight.

$$\Delta\mathrm{Biomass}_i = Y_{\mathrm{final},i} - Y_{\mathrm{initial},i} \quad (2)$$

Data analysis. Statistical analyses were performed for germination and root-length data from the Mexico experiments only, where replicated treatments allowed inferential testing. Due to non-normal data distributions, overall differences among treatments were assessed using the non-parametric Kruskal–Wallis test. When significant effects were detected, pairwise comparisons between treatments and controls were conducted using the Dwass–Steel–Critchlow–Fligner (DSCF) post hoc procedure. All analyses were performed using OriginPro software (OriginLab Corporation, version 2025b), with statistical significance defined at $p < 0.05$. Effect size was estimated using Cohen's f, and post hoc power analysis was conducted using the "Power and Sample Size" function in OriginPro to evaluate the number of replicates required to achieve 70%, 80%, and 90% statistical power at a significance level of 0.05.

Pharmaceutical quantification. To assess antibiotic sorption to filter paper and potential degradation during the hydroponic assay, antibiotic concentrations were quantified before and after exposure. This analysis was conducted as part of the Mexico experiments to provide mechanistic insight into antibiotic dynamics under the assay conditions and to inform interpretation of exposure across all treatments. From each Petri dish, a 1.0 mL aliquot was collected and transferred into 2 mL amber vials for HPLC-MS/MS analysis. Pharmaceutical concentrations were quantified using an Agilent Technologies 6420 HPLC-ESI-MS/MS system.

Chromatographic separation was achieved on a Zorbax SB-C18 column (250 × 4.6 mm, 5 μm) maintained at 20 °C, using an isocratic mobile phase consisting of acetonitrile (20%), methanol (40%), and aqueous 0.1% formic acid with 10 mM ammonium acetate (40%) at a flow rate of 0.4 mL min^{-1}. The injection volume was 10 μL. Electrospray ionization was operated in positive mode, with a drying gas temperature of 300 °C and flow rate of 11 L min^{-1}, a nebulizer pressure of 15 psi, and a capillary voltage of 3000 V.

Oxytetracycline, trimethoprim, azithromycin, and ciprofloxacin were identified and quantified using the multiple reaction monitoring (MRM) mode (Table S4). Method validation details are provided in the SI (Table S5). The concentration of the antibiotic solution of each Petri dish was determined, including the controls without seeds. Changes in antibiotic concentration ($\Delta C\%$, eqn (3) and seed removal rates eqn (4) were calculated based on measured concentrations before and after exposure.

$$\Delta C\% = \frac{C_{\mathrm{treatment,inital}} - C_{\mathrm{treatment,final}}}{C_{\mathrm{treatment,initial}}} \times 100 \quad (3)$$

$$\text{Seed removal}(\%) = \overline{\Delta C_{\mathrm{seeds}}} - \overline{\Delta C_{\mathrm{no\ seeds}}} \quad (4)$$

In eqn (3), $C_{\mathrm{treatment,initial}}$ and $C_{\mathrm{treatment,final}}$, were the initial and the final concentration in mg L^{-1}, respectively, determined by HPLC-MS/MS analysis. Seed removal was defined as the difference between the mean concentration change ($n = 3$) observed in assays with seeds and corresponding controls without seeds eqn (4). This metric reflects the net effect associated with the presence of seeds and does not distinguish among individual

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processes, such as plant uptake, sorption to filter paper, or other transformation pathways occurring during the assay. The associated error eqn (5) for the seed removal was estimated by propagating the standard deviations of concentration changes measured in assays with seeds and in controls without seeds (SD_{seeds} and $SD_{no\ seeds}$), using three replicates per treatment ($n = 3$).

$$SD = \sqrt{\frac{SD_{Seeds}^2}{n} + \frac{SD_{no\ seeds}^2}{n}} \quad (5)$$

To describe antibiotic partitioning between water and filter paper in control Petri dishes without seeds, a linear partitioning model was applied eqn (6).

$$q = k_d C_{eq} \quad (6)$$

where q is the amount adsorbed per unit mass of filter paper, C_{eq} is the equilibrium concentration of the antibiotic in water, and K_d is the apparent partitioning coefficient. A mass balance approach was then used to derive eqn (7).[41,42]

$$\phi = \frac{\Delta C}{1 - \Delta C} = K_{d,app} \frac{m}{V} \quad (7)$$

where ΔC is the pharmaceutical fraction removed from water (*i.e.*, concentration change), m, is the mass of the filter paper, V is the volume of the solution in the Petri dish, and $K_{d,app}$ is the apparent partition coefficient between the filter paper and water. This mass-balance formulation describes the extent to which antibiotics partition onto the filter paper relative to the aqueous phase under the experimental conditions, thereby supporting the interpretation of concentration losses observed in control dishes without seeds.

Results and discussion

Germination

Following exposure to antibiotics at 1 mg L^{-1}, germination responses were evaluated for 10 antibiotics across 7 species in China, 10 antibiotics across 14 species in the UK, and 4 antibiotics across 4 species in Mexico (Table S6). Reported environmental concentrations of the target antibiotics in irrigation water and soil pore-water, together with the concentration applied in this study, are summarized in Table S3 to provide environmental context for this screening-level assessment. Replicate-level variability could only be quantified for the Mexico dataset ($n = 3$), where within-treatment standard deviations ranged from 5 to 20% (Fig. 1; Mexico study). Due to experimental constraints, replicate variability could not be assessed for the UK and China datasets.

Given the Tier I hazard-screening nature of this study, a ± 20% difference in germination relative to the control was used as a descriptive indicator of biologically relevant change, rather than as a criterion for statistical significance, to facilitate comparative interpretation across datasets.[43] This threshold was informed by the observed variability in the Mexico experiments and by reported coefficients of variation for seed

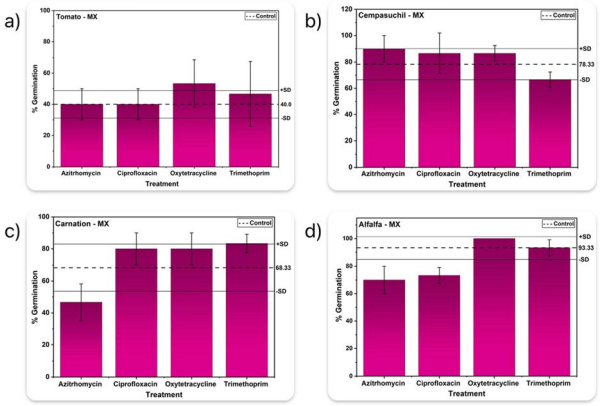

Fig. 1 Mexico germination results: (a) tomato (*S. Lycopersicon*), (b) cempasuchil (*T. Erecta*), (c) carnation (*D. Caryophyllus*), (d) alfalfa (*M. Sativa*), with standard deviation (SD, $n = 3$), the dotted line is the average % germination and their ± SD (fine lines) in the control.

germination in the literature, typically below 20%. For example, values in the range of 6.7 to 11.4% have been reported for *M. sativa* and *L. sativa*, respectively,[19] while an average of 17.66% was reported across 20 Fabaceae species.[44] Formal statistical inference was conducted exclusively for the Mexico dataset using Kruskal–Wallis tests followed by Dwass–Steel–Critchlow–Fligner (DSCF) post hoc comparisons, and these results are presented separately in Table 2

For germination, Kruskal–Wallis tests indicated a significant overall treatment effect for alfalfa (*Medicago sativa*; $p = 0.013$; Table 2). However, DSCF post hoc comparisons did not identify statistically significant differences between individual antibiotic treatments and the control. This discrepancy reflects the limited resolution of pairwise comparisons under low replication ($n = 3$), a known constraint of non-parametric post hoc testing in screening-level studies. Accordingly, while global distributional differences were detected, individual treatment effects could not be resolved statistically, and germination responses were interpreted descriptively within the Tier I screening framework.

Using the ±20% descriptive threshold, antibiotic exposure was associated with increased germination relative to controls for several species–antibiotic combinations. Across datasets, germination stimulation was more frequently observed in legumes and grasses exposed to fluoroquinolones (ciprofloxacin, enrofloxacin, and ofloxacin) (Table 2 and Fig. 2). These responses were identified descriptively within the Tier I screening framework and highlight species–compound combinations that merit further investigation. Previous studies have reported similar stimulatory responses in crops exposed to low concentrations of fluoroquinolones, including maize, rice, and alfalfa, where enhanced germination and root elongation have been discussed in the context of hormetic effects.[45–48] Such responses have been linked to stress-related biochemical pathways, including reactive oxygen species (ROS) signalling and the activation of antioxidant defence mechanisms.[35,49,50] However, it is important to note that the mechanistic relationship between fluoroquinolone modes of action and

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Table 2 Kruskal–Wallis and DSCF post-hoc test results for germination percentage and root length in Mexico experiments

		Kruskal Wallis			DSCF Test							
					AZI		CPX		OTC		TRM	
		g. l.	χ^2	*p*-Value	*W*	*p*-Value	*W*	*p*-Value	*W*	*p*-Value	*W*	*p*-Value
Tomate (S. *lycopersicum*)	% Germination	4	2.14	0.710	0.000	1.000	0.000	1.000	1.898	0.665	0.380	0.999
	Root length (mm)	4	3.52	0.475	−0.617	0.993	2.278	0.491	2.362	0.453	0.514	0.996
Cempasuchil (*T. erecta*)	% Germination	4	7.05	0.133	1.89	0.669	1.311	0.887	1.554	0.807	−2.070	0.586
	Root length (mm)	4	13.9	0.007	−0.199	1.000	−3.486	0.099	1.792	0.712	1.705	0.748
Carnation (*D. caryophyllus*)	% Germination	4	8.78	0.067	1.671	0.762	2.06	0.591	−2.611	0.347	1.671	0.762
	Root length (mm)	4	5.53	0.237	0.287	0.637	0.49	0.345	−1.959	1.000	−2.617	0.997
Alfalfa (*M. sativa*)	% Germination	4	12.7	0.013	1.964	0.635	−0.200	1.000	−3.184	0.161	−3.198	0.158
	Root length (mm)	4	18.4	0.001	1.294	0.891	3.635	0.076	−5.132	0.003	−4.100	0.031

germination-associated biochemical pathways remains poorly understood, and direct causal links have not been established.

Additional species–antibiotic combinations exhibiting > 20% increases in germination included carnation (*D. caryophyllus*) under trimethoprim exposure, which did not reach statistical significance in post-hoc testing but is interpreted here as a descriptive trend, and in French bean (*Phaseolus vulgaris*), where 33.4% and 53.4% more germination was observed under ciprofloxacin and enrofloxacin exposure, respectively, compared to the control (for which only 3.3% germination was observed). Similarly, pea (*P. sativum*), germination increased under ciprofloxacin, enrofloxacin, florfenicol, and sulfonamide exposure, ranging between 23 and 50%. Oats (*A. sativa*), showed up to 40% increase under florfenicol exposure, and red clover (*T. pratense*) exhibited 20% increases under azithromycin, sulfonamide, and trimethoprim exposure, suggesting hormetic effects, consistent with previous findings in crops, such as maize and alfalfa.[45,46,48] These phenomena may involve biochemical pathways related to ROS signalling and antioxidant responses, warranting further investigation to assess ecological impacts and antibiotic–plant interactions.

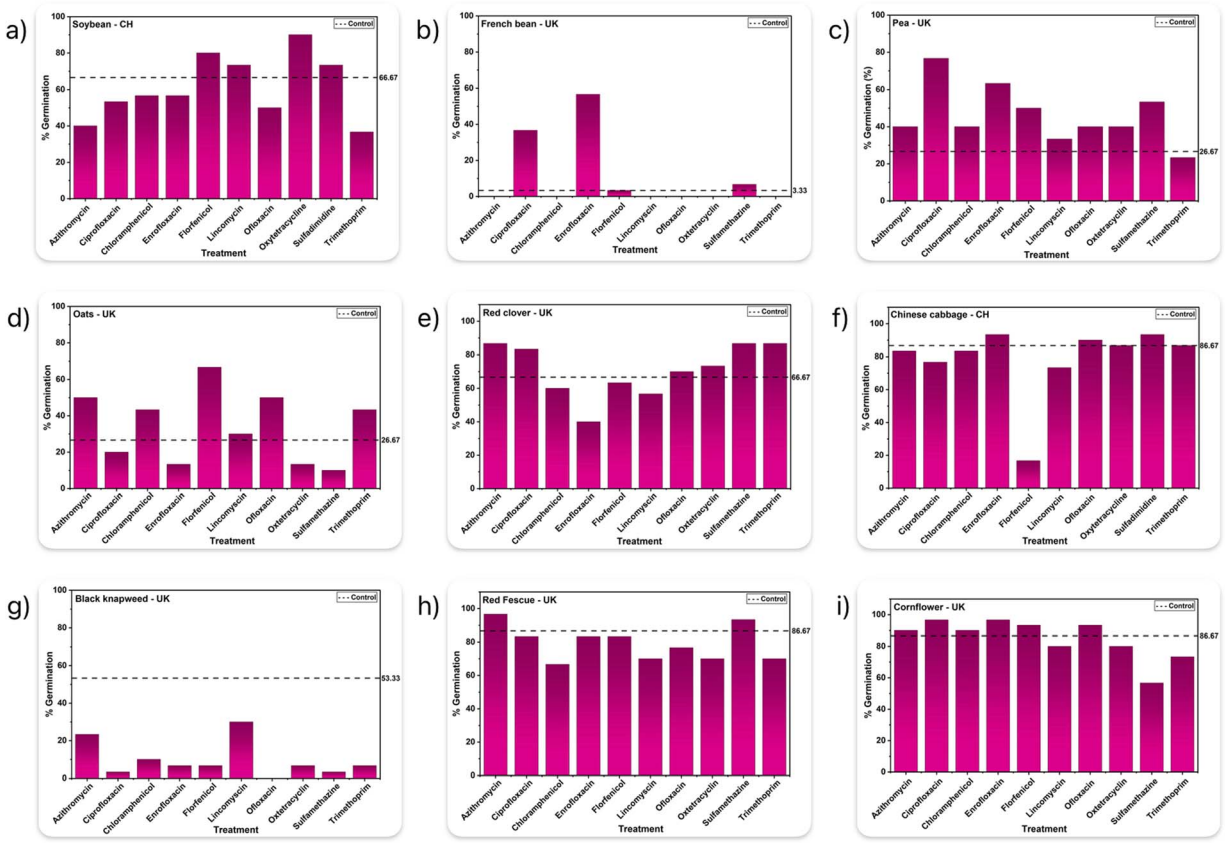

Fig. 2 Effects on germination treated with 10 antibiotics: (a) soybean (*G. Max*, China), (b) French bean (*P. Vulgaris*, UK), (c) Pea (*P. Sativum*, UK), (d) Oats (*A. Sativa*, UK), (e) Red clover (*T. Pratense*, UK), (f) Chinese cabbage (*B. Rapa subsp. Pekinensis*, China), (g) Black knapweed (*C. Nigra*, UK), (h) Red Fescue (*F. Rubra*, UK), and (i) cornflower (*C. Cyanus*, UK), the dotted line represents the germination results in the control.

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For soybean (*G. max*; Fig. 2a and Table S6), a possible increase in germination exceeding 20% was observed under oxytetracycline exposure, compared to the control (90.0 *vs.* 66.7%). In contrast, for black knapweed (*C. nigra*), near-complete inhibition of germination was detected following oxytetracycline exposure (6.7 *vs.* 53.3% in the control; Fig. 2g), and for alfalfa (*M. sativa*), no statistically significant effect on germination ($p = 0.161$) was observed between the control and oxytetracycline treatment (Fig. 1d). Previous studies report no significant effects of oxytetracycline on wheat (*Triticum aestivum*) germination in soil at concentrations of 1, 10, and 50 mg kg^{-1},[51] whereas significant inhibition has been observed under hydroponic conditions for the same species at higher concentrations (50 and 150 mg L^{-1}).[52] These discrepancies between studies and with the current results likely reflect differences in experimental setup (soil *versus* hydroponic exposure), species-specific sensitivity, and inherent challenges associated with accurately assessing germination responses.

Careful interpretation of germination outcomes is therefore required to avoid both Type I (false positive) and Type II (false negative) errors.[43] Statistical power analysis, informed by the variance observed in the Mexico dataset, was conducted to estimate the number of replicates required to minimise these errors. Accordingly, this study is considered a screening-level investigation intended to identify species–antibiotic combinations that warrant further targeted experimentation. It should also be acknowledged that antibiotics were evaluated individually. Under environmental conditions, antibiotics frequently co-occur as complex mixtures, and interactions may be additive, antagonistic, or synergistic. While the single-compound approach adopted here enables controlled comparison of compound-specific effects during early germination, it may underestimate mixture-related responses. The evaluation of antibiotic mixtures therefore represents an important priority for subsequent Tier II studies under environmentally exposure scenarios.

The most pronounced germination reduction observed across all datasets was for black knapweed (*C. nigra* and Fig. 2g, Table S6), where exposure to all antibiotics resulted in reduced germination relative to the control (53.3%). Reductions ranged from 30% under lincomycin exposure to complete inhibition under ofloxacin. These findings suggest that non-cultivated species, such as *C. nigra*, may represent sensitive bioindicators for antibiotic-related phytotoxicity. Notably, literature reports indicate that even closely related species can respond differently; for example, brown knapweed (*Centaurea jacea*) has exhibited a slight but statistically significant increase in germination under tetracycline exposure at 1 mg L^{-1}.[44] This highlights substantial interspecific variability in antibiotic responses, even within the same plant genus and antibiotic class.

Another notable reduction in germination was observed for Chinese cabbage (*Brassica rapa* subsp.*pekinensis*; Fig. 2f), where a 70% decrease occurred under florfenicol exposure. Similarly, germination reductions were recorded for cornflower (*Centaurea cyanus*) under sulfamethazine exposure (−30%; Fig. 2i) and for red fescue (*Festuca rubra*) under chloramphenicol exposure (−20%; Fig. 2h). To date, no studies have evaluated the effects of florfenicol, sulfamethazine, or chloramphenicol on germination in these species. It is hypothesised that the inhibitory responses observed here may relate to interference with protein synthesis and oxidative stress pathways, mechanisms previously reported for other antibiotics in plants.[35,53] Moreover, as responses can vary widely even among closely related species,[54] these results highlight the importance of species-specific assessments when evaluating antibiotic phytotoxicity.

Azithromycin emerged as the antibiotic exhibiting the most variable effects across species, producing both inhibitory and stimulatory germination responses. In the Mexico dataset, the only observed germination reduction was for cempasúchil (*Tagetes erecta*), where azithromycin exposure resulted in a 40% decrease relative to the control. In contrast, germination stimulation exceeding 20% was observed in legumes and grasses from the UK, including *Avena sativa* and *Trifolium pratense*. In the China dataset, azithromycin exposure resulted in a 27% reduction in germination for soybean (*G. max*; Fig. 2). These contrasting responses indicate that azithromycin warrants further investigation, as its phytotoxic effects appear to be highly species-dependent.

Root elongation and biomass

Root elongation results are summarised in Table 3, which reports mean, median, and interquartile range (IQR) values for root length, alongside dry biomass differences between treatments and controls and relative change index (RCI) values. Positive RCI values indicate growth stimulation under antibiotic exposure, whereas negative values indicate growth inhibition.

High variability in root length responses was observed across species and antibiotic treatments (Table 3; Fig. 3–5), indicating heterogeneous seedling responses under exposure conditions. Such variability is consistent with species-specific differences in root sensitivity and antibiotic interactions, which have been associated in previous studies with root electrochemical properties and oxidative stress responses.[35,55] These observations highlight the need for increased replication and targeted follow-up experiments to confirm patterns identified in this Tier I screening.

For the Mexico dataset, Kruskal–Wallis tests indicated significant overall treatment effects on root elongation for cempasúchil (*Tagetes erecta*; $p = 0.007$) and alfalfa (*Medicago sativa*; $p = 0.001$), while post-hoc (DSCF) tests identified a statistically significant pairwise effect only for alfalfa under oxytetracycline exposure ($p = 0.003$; Table 2). In this case, oxytetracycline exposure resulted in a marked increase in median root length from 10.0 mm in the control to 38.0 mm (Table 3; Fig. 3d), indicating substantial stimulation of root elongation. In contrast, while oxytetracycline exposure was associated with increased germination in soybean (*Glycine max*; Fig. 2a), it resulted in pronounced inhibition of root elongation (122.35 mm compared to 160.98 mm in the control), corresponding to an RCI value of −78%. This demonstrates that

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Table 3 Root elongation and biomass indicators of seedlings exposed to pharmaceuticals[a,b]

Specie	Treatment	Mean (mm)	Median (mm)	IQR (mm)	Min – max (mm)	Δ mass (mg) ($m_{F_{Dry}} - m_i$)	RCI (%)
China							
Soybean (*G. max*)	AZI	114.10	89.17	101.53	42.77–229.22	−4698.0	47.6
	CPX	160.34	148.13	143.4	51.02–255.10	−3528.1	28.0
	CHL	163.27	181.88	71.96	32.26–239.53	−3461.9	9.1
	ERX	144.25	137.61	129.59	33.58–251.36	−3065.2	5.8
	FLOR	166.6	171.29	91.17	16.09–318.39	−3078.4	−12.1
	LIN	160.32	139.88	179.55	24.70–296.49	−2633.4	−17.8
	OFX	152.19	150.51	136.38	15.53–231.90	−4027.2	−1.5
	OTC	115.76	122.35	168.88	7.49–283.35	−263.1	−78.0
	SFD	205.00	218.93	65.95	70.89–269.60	−2480.5	−13.0
	TRM	175.83	171.73	121.42	58.75–247.35	−4518.0	35.8
	Control	147.92	160.98	61.79	22.76–296.65	−2825.0	0.0
Wheat (*T. aestivum*)	AZI	102.40	98.93	56.86	24.89–148.24	536	−10.2
	CPX	169.24	187.58	47.97	30.08–236.45	541.9	−23.7
	CHL	108.09	127.57	94.70	9.02–181.28	545.7	−12.4
	ERX	163.91	183.73	50.75	24.80–245.92	273.9	−1.6
	FLOR	128.00	135.70	86.14	14.72–226.82	240.5	1.0
	LIN	144.91	159.42	76.37	11.00–242.44	496.7	−15.5
	OFX	178.99	183.00	58.43	79.51–250.16	75.7	13.4
	OTC	150.93	156.84	72.41	13.73–217.86	186.4	7.1
	SFD	162.46	181.49	42.21	7.30–238.19	252.8	3.5
	TRM	131.42	159.83	117.6	14.39–218.82	721.5	−26.5
	Control	155.10	158.11	73.82	13.61–225.28	332.5	0.0
Mung bean (*V. radiata*)	AZI	219.38	203.13	100.44	71.04–346.36	−777.5	0.7
	CPX	208.10	192.47	118.92	41.28–315.07	−304.0	−19.6
	CHL	163.00	141.07	104.475	25.10–261.09	307.0	−59.0
	ERX	170.04	159.31	110.84	22.19–298.91	−693.9	10.8
	FLOR	158.66	145.56	116.07	13.05–290.92	−404.3	−12.1
	LIN	201.41	180.66	129.50	90.18–320.25	−130.8	−40.4
	OFX	200.59	186.47	120.63	21.78–365.35	−807.1	3.9
	OTC	155.15	176.39	152.93	12.06–296.20	−660.3	1.6
	SFD	191.64	221.2	142.72	22.31–329.39	−401.7	−16.5
	TRM	205.85	193.8	99.57	23.43–340.28	−479.9	−4.4
	Control	172.44	188.45	133.99	7.97–293.13	−747.9	0.0
Lettuce (*L. sativa*)	AZI	77.92	81.82	26.33	4.89–118.04	−12.7	38.2
	CPX	68.95	68.72	45.20	9.25–116.13	5.6	9.3
	CHL	72.85	78.01	40.53	8.20–138.19	11.1	−14.1
	ERX	63.01	74.64	56.89	3.16–118.54	−14.8	37.7
	FLOR	83.98	87.75	34.76	17.04–131.10	16.7	−21.5
	LIN	81.69	83.22	14.73	51.56–101.72	45.5	−91.2
	OFX	67.04	75.53	30.99	7.24–106.61	−2.8	8.2
	OTC	77.28	85.78	17.78	16.69–105.66	−0.4	9.3
	SFD	81.90	85.58	34.63	18.10–123.64	0.5	15.4
	TRM	79.20	83.15	40.34	11.16–119.10	−16.5	23.1
	Control	77.84	82.60	33.35	16.68–119.38	14.9	0.0
Rice (*O. sativa*)	AZI	66.68	63.21	35.86	22.34–106.97	112.7	−3.1
	CPX	69.36	74.57	25.71	12.03–97.95	143.8	−9.5
	CHL	51.35	46.27	26.45	6.79–98.61	74.6	3.4
	ERX	53.13	58.69	31.75	9.22–85.47	267.8	−24.6
	FLOR	65.83	67.62	20.37	21.56–99.34	197.5	−14.3
	LIN	54.85	57.51	14.31	17.71–82.20	79.6	0.5
	OFX	62.26	65.14	25.22	2.66–92.56	124.2	−3.5
	OTC	60.64	63.20	30.40	9.70–93.53	186.8	−12.7
	SFD	59.41	64.13	30.265	21.19–88.08	178.9	−16.2
	TRM	64.35	67.23	17.0075	16.30–95.35	68.9	4.3
	Control	43.43	46.65	35.89	2.74–77.86	56.9	0.0
Chinese cabbage	AZI	35.52	28.36	33.035	5.99–147.40	−25.3	10.3
(*B. rapa* subsp.*Pekinensis*)	CPX	30.87	30.8	19.765	9.37–97.20	10.1	−45.2
	CHL	27.4	21.2	22.115	3.33–106.46	−0.3	−86.4
	ERX	29.47	25.36	27.65	4.58–69.42	−12.2	−16.5
	FLOR	13.12	12.43	4.39	9.25–14.33	−58.5	100.0
	LIN	12.25	11.75	9.16	3.34–24.46	−12.3	−5.9
	OFX	33.8	31.32	24.76	5.63–93.65	−10	−5.4

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Table 3 (*Contd.*)

Specie	Treatment	Mean (mm)	Median (mm)	IQR (mm)	Min – max (mm)	Δ mass (mg) ($m_{F_{Dry}} - m_i$)	RCI (%)
	OTC	34.77	31.64	40.885	3.27–89.50	−14.2	−39.3
	SFD	32.72	24.73	31.78	5.80–92.67	−7.3	−57.9
	TRM	19.65	13.93	12.975	3.26–43.75	−9.3	0.0
	Control	24.4	17.61	26.89	2.15–82.88	−25.2	0.0
Shasta daisy	AZI	19.57	19.63	17.15	9.4–30.22	−1.3	−14.4
(*Leucanthemum maximum*)	CPX	22.98	20.74	19.62	7.72–29.20	−6.8	11.7
	CHL	23.16	20.71	22.58	7.18–41.41	1.8	−34.2
	ERX	17.86	9.89	17.39	7.31–42.97	−3.5	4.5
	FLOR	11.7	7.49	4.36	6.24–10.60	−4.6	8.1
	LIN	13.81	10.9	13.69	4.04–23.60	−2.3	9.9
	OFX	21.64	21.15	21.08	7.61–35.76	0.5	−46.8
	OTC	19.13	11.94	24.97	3.51–28.48	1.8	−28.8
	SFD	26.48	34.89	26.83	10.60–37.43	−1.4	−5.4
	TRM	19.67	13.42	24.57	6.71–41.89	−2.9	5.4
	Control	24.56	24.79	32.93	9.26–43.52	−2.6	0.0
UK							
French bean (*P. vulgaris*)	AZI	NG	NG	NG	NG	NG	NG
	CPX	24.13	18.09	25.39	6.19–48.02	−1.2048	−4.1
	CHL	NG	NG	NG	NG	NG	NG
	ERX	37.57	29.58	31.96	11.07–79.33	−1.3605	−3.0
	FLOR	30.7	30.7	NA	30.7	−0.9461	−2.7
	LIN	NG	NG	NG	NG	NG	NG
	OFX	NG	NG	NG	NG	NG	NG
	OTC	NG	NG	NG	NG	NG	NG
	SFZ	21.83	29.45	NA	14.2–29.45	−1.4452	5.2
	TRM	NG	NG	NG	NG	NG	NG
	Control	22.7	22.7	NA	22.7	−1.1845	0.0
Pea (*P. sativum*)	AZI	17.08	14.99	10.24	4.78–48.53	−0.9375	1.5
	CPX	29.46	25.99	24.96	7.789–66.98	−0.4959	−14.4
	CHL	18.87	18.47	9.96	4.02–36.78	−1.0434	12.7
	ERX	27.94	24.43	14.14	10.84–59.08	−0.7206	6.3
	FLOR	22.47	24.18	27.23	2.78–40.63	−0.8992	0.9
	LIN	24.27	22.27	12.53	8.31–50.23	−0.932	27.3
	OFX	16.73	15.62	17.79	4.15–36.37	−0.9229	4.3
	OTC	16.55	15.41	9.55	7.48–26.39	−1.0244	2.6
	SFZ	14.59	12.36	6.53	6.59–39.05	−1.1888	4.1
	TRM	14.35	14.31	6.73	5.24–21.64	−1.3664	28.1
	Control	24.47	25.34	13.82	9.65–34.87	−1.3129	0.0
White clover (*T. repens*)	AZI	36.01	37.58	17.00	3.30–57.38	−0.0038	−14.3
	CPX	44.72	49.45	12.68	13.55–57.93	−0.0124	−76.0
	CHL	27.46	29.25	10.06	7.56–43.67	−0.0055	−4.5
	ERX	39.06	38.56	12.34	27.61–50.09	−0.0055	1.9
	FLOR	38.17	37.86	10.36	10.39–56.93	−0.0063	1.3
	LIN	33.93	34.76	13.03	1.16–48.84	−0.004	−3.2
	OFX	31.77	31.88	12.34	5.25–49.56	−0.0051	8.4
	OTC	33.11	33.30	11.36	16.51–49.02	−0.0054	−8.4
	SFZ	32.24	34.52	13.95	8.65–52.11	−0.0055	−6.5
	TRM	30.37	33.73	15.30	7.35–45.42	−0.0016	−24.0
	Control	35.31	36.82	14.15	6.10–47.84	−0.0059	0.0
Red clover (*T. pratense*)	AZI	47.05	51.01	29.52	7.97–74.96	−0.0100	−6.3
	CPX	33.52	31.40	12.29	13.19–51.86	−0.0075	63.8
	CHL	33.88	33.64	27.86	8.72–67.21	−0.0101	8.8
	ERX	37.83	37.27	23.63	6.77–55.54	−0.0092	17.5
	FLOR	45.61	51.64	32.69	9.65–67.46	−0.0086	−1.3
	LIN	39.69	41.31	36.11	6.48–70.25	−0.0101	−5.8
	OFX	46.13	49.24	16.03	4.11–70.18	−0.0129	12.7
	OTC	31.58	32.99	20.68	6.22–63.08	−0.0142	2.2
	SFZ	43.15	42.69	23.85	3.07–72.04	−0.0101	−1.0
	TRM	38.07	43.34	35.95	2.96–62.77	−0.0099	0.5
	Control	39.90	39.32	42.43	9.68–67.91	−0.0123	0.0
Oats (*A. sativa*)	AZI	29.62	25.32	39.35	4.15–65.62	−0.0539	3.0
	CPX	32.14	29.20	37.34	9.70–61.47	−0.1212	2.0

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Table 3 (*Contd.*)

Specie	Treatment	Mean (mm)	Median (mm)	IQR (mm)	Min – max (mm)	Δ mass (mg) ($m_{F_{Dry}} - m_i$)	RCI (%)
	CHL	60.20	72.69	45.34	3.43–91.32	−0.1404	18.0
	ERX	29.96	30.59	43.99	5.82–52.82	−0.0936	19.8
	FLOR	48.34	54.04	32.34	4.82–72.42	−0.1676	−2.1
	OFX	35.77	34.40	33.84	7.68–70.40	−0.1314	5.5
	OTC	14.59	12.95	16.86	5.62–26.85	−0.1179	9.6
	LIN	20.52	14.18	31.11	5.59–49.01	−0.1209	8.6
	SFZ	23.34	26.02	33.22	5.40–38.62	−0.1329	3.4
	TRM	27.89	22.25	25.48	6.25–67.16	−0.1277	14.1
	Control	22.57	23.81	25.09	3.41–43.66	−0.1385	0.0
Wheat (*T. aestivum*)	AZI	65.04	67.02	10.25	34.08–76.98	−0.1586	1.4
	CPX	58.06	64.68	22.54	13.41–79.69	−0.1708	21.5
	CHL	77.80	81.66	20.81	13.70–98.67	−0.2325	9.6
	ERX	63.66	67.47	12.94	22.35–80.46	−0.2044	9.8
	FLOR	61.29	66.14	17.69	14.52–82.21	−0.1954	−2.8
	LIN	62.29	66.83	13.22	18.81–81.74	−0.1657	17.8
	OFX	57.45	58.30	17.92	5.64–79.03	−0.2160	−9.9
	OTC	62.65	68.63	16.61	25.45–78.31	−0.2359	3.9
	SFZ	59.23	65.18	18.39	10.41–78.32	−0.2200	3.1
	TRM	56.01	58.99	19.69	6.29–83.23	−0.2346	15.5
	Control	54.63	55.03	16.96	13.32–81.49	−0.2214	0.0
Barley (*H. vulgare*)	AZI	76.04	73.76	13.79	59.49–96.90	−0.1651	−4.3
	CPX	63.55	69.21	21.95	13.37–89.77	−0.2104	4.3
	CHL	92.99	94.60	14.13	72.83–109.23	−0.282	11.1
	ERX	74.58	76.41	15.23	55.32–92.60	−0.2388	8.1
	FLOR	79.13	78.62	13.82	52.07–107.12	−0.229	11.3
	OFX	67.63	69.41	17.43	1.09–91.72	−0.2273	3.5
	OTC	72.09	72.11	14.29	49.32–89.87	−0.2444	11.9
	LIN	78.91	78.10	11.06	65.82–98.38	−0.2271	6.9
	SFZ	71.71	73.46	12.90	53.47–85.08	−0.2212	3.8
	TRM	78.04	76.85	12.25	61.24–96.28	−0.2756	8.3
	Control	67.00	67.46	22.64	34.37–92.22	−0.236	0.0
Sheeps fescue (*F. ovina*)	AZI	NG	NG	NG	NG	NG	NG
	CPX	37.64	37.64	NA	5.80–69.47	NA	NA
	CHL	8.00	8.00	NA	8.00	NA	NA
	ERX	3.08	3.08	NA	3.08	NA	NA
	FLOR	8.35	8.35	NA	8.35	NA	NA
	LIN	NG	NG	NG	NG	NG	NG
	OFX	NG	NG	NG	NG	NG	NG
	OTC	NG	NG	NG	NG	NG	NG
	SFZ	NG	NG	NG	NG	NG	NG
	TRM	NG	NG	NG	NG	NG	NG
	Control	NG	NG	NG	NG	NG	NG
Red fescue (*Festuca rubra*)	AZI	11.16	10.73	7.47	3.22–22.28	−0.0028	−12.5
	CPX	19.48	20.67	6.5	4.71–32.57	−0.0072	14.4
	CHL	11.98	12.65	7.78	2.57–22.28	−0.0031	22.1
	ERX	17.63	17.98	7.39	4.08–27.24	−0.0053	11.1
	FLOR	12.99	12.27	7.92	4.75–45.10	−0.0033	4.8
	LIN	13.26	12.77	8.08	3.67–.20.61	−0.0035	2.4
	OFX	14.32	15.46	4.3	3.14–21.86	−0.0039	0.0
	OTC	11.68	10.91	8.11	1.58–23.55	−0.0041	1.4
	SFZ	14.49	15.84	5.71	2.81–20.87	−0.0048	0.5
	TRM	11.38	10.82	8.6	2.84–19.67	−0.0037	−3.9
	Control	14.68	15.23	6.94	5.43–28.06	−0.0041	0.0
Quaking grass (*Briza media*)	AZI	NG	NG	NG	NG	NG	NG
	CPX	NG	NG	NG	NG	NG	NG
	CHL	2.24	2.24	NA	1.53–2.95	−0.0016	32.5
	ERX	6.52	6.52	NA	6.52	−0.0469	45.9
	FLOR	0.66	0.66	NA	0.66	−0.0017	24.7
	LIN	4.03	4.03	NA	4.03	−0.0021	17.3
	OFX	1.51	1.51	0.09	1.46–1.55	−0.0018	10.8
	OTC	NG	NG	NG	NG	NG	NG
	SFZ	NG	NG	NG	NG	NG	NG
	TRM	NG	NG	NG	NG	NG	NG
	Control	1.68	1.68	NA	1.40–1.96	−0.0031	0

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Table 3 (*Contd.*)

Specie	Treatment	Mean (mm)	Median (mm)	IQR (mm)	Min – max (mm)	Δ mass (mg) ($m_{F_{Dry}} - m_i$)	RCI (%)
Cos lettuce (*L. sativa*)	AZI	60.81	68.36	23.77	16.94–85.66	−0.0009	−10.2
	CPX	48.44	50.47	37.70	15.87–89.84	−0.0053	3.6
	CHL	60.27	63.52	19.52	6.07–85.21	−0.0043	12.7
	ERX	58.14	59.10	25.09	32.54–81.36	−0.0058	10.9
	FLOR	56.94	61.39	16.10	9.61–79.68	−0.0039	6.9
	LIN	52.10	53.71	33.24	7.76–91.22	−0.0033	0.4
	OFX	62.82	65.49	23.08	26.26–83.88	−0.0047	2.2
	OTC	43.40	46.23	27.19	5.41–79.29	−0.0037	7.3
	SFZ	43.72	44.29	22.04	16.92–64.59	−0.0044	5.8
	TRM	49.48	49.98	21.14	13.93–71.87	−0.0035	8.0
	Control	55.40	64.35	39.46	4.05–88.70	−0.0041	0.0
Oxeye Daisy (*L. vulgare*)	AZI	8.80	9.49	9.94	2.47–13.72	−0.0011	2.1
	CPX	13.45	12.32	14.31	5.83–23.48	−0.0021	5.6
	CHL	7.16	7.55	2.62	5.28–9.16	−0.0015	−0.7
	ERX	10.41	7.36	14.52	3.79–23.12	−0.0013	7.8
	FLOR	6.57	6.57	NA	4.55–8.59	−0.0012	9.2
	LIN	16.46	20.16	15.20	7.01–22.21	−0.002	7.0
	OFX	12.49	14.69	8.11	7.33–15.45	−0.0019	−9.9
	OTC	6.91	6.89	6.90	2.65–11.22	−0.0027	21.1
	SFZ	4.81	3.77	6.94	1.66–10.04	−0.0019	11.3
	TRM	0.46	0.46	NA	0.46	−0.0011	1.4
	Control	3.40	3.40	NA	3.4	−0.0017	0.0
Black Knapweed (*C. nigra*)	AZI	25.53	27.37	22.75	4.10–37.99	−0.002	−4.2
	CPX	18.89	18.89	NA	18.89	−0.005	−15.8
	CHL	8.04	7.15	3.81	6.59–10.39	−0.0045	−14.5
	ERX	10.83	10.83	NA	6.89–14.77	−0.0053	−33.7
	FLOR	12.04	12.04	NA	7.69–16.38	−0.0055	−26.8
	LIN	30.49	31.13	10.13	20.22–42.23	−0.0044	−12.3
	OFX	NG	NG	NG	NG	NG	−23.2
	OTC	13.81	13.81	NA	5.05–22.58	−0.0063	−24.8
	SFZ	6.17	6.17	NA	6.2	−0.0108	−17.6
	TRM	14.53	14.53	NA	3.24–25.83	−0.0057	−23.9
	Control	29.84	29.49	18.42	12.17–49.95	−0.0041	0.0
Cornflower (*C. cyanus*)	AZI	85.70	92.66	36.83	5.34–159.35	0.1479	3.1
	CPX	92.37	93.61	40.63	16.19–157.37	−0.0055	2.1
	CHL	97.78	103.20	48.38	14.38–142.28	−0.0092	14.4
	ERX	90.49	91.50	27.43	39.05–165.10	−0.0052	2.9
	FLOR	95.85	96.54	41.24	20.97–158.50	−0.0060	4.1
	LIN	72.83	75.47	13.06	32.13–91.61	−0.0032	4.2
	OFX	86.75	89.25	28.08	27.05–124.10	−0.0116	10.5
	OTC	75.97	71.41	41.57	34.13–152.55	−0.0100	9.8
	SFZ	75.92	79.75	36.93	21.90–128.37	−0.0085	6.7
	TRM	100.53	102.75	49.75	31.01–150.23	−0.0090	4.8
	Control	82.28	78.45	57.84	14.34–160.36	−0.0072	0.0
Mexico							
Tomate	AZI	5.97 ± 4.41	3.19	10.06	1.61–11.67	−1.30 ± 0.44	1.8 ± 4.8
(*S. lycopersicum*)	CPX	5.10 ± 2.68	4.19	5.62	2.23–11.41	−4.90 ± 0.98	14.6 ± 6.2
	OTC	5.25 ± 0.84	4.85	5.18	1.66–11.02	−1.33 ± 0.21	−2.7 ± 2.5
	TRM	3.91 ± 2.92	3.285	5.28	1.64–12.48	−1.43 ± 0.67	−5.2 ± 5.3
	Control	4.04 ± 1.06	3.18	4.35	1.03–8.28	−1.57 ± 0.42	0.0
Cempasuchil (*T. erecta*)	AZI	59.75 ± 7.94	54.18	51.94	4.41–144.59	−3.57 ± 2.00	−3.1 ± 11.1
	CPX	38.17 ± 7.52	33.01	32.20	5.02–89.77	−2.77 ± 2.55	−2.9 ± 9.8
	OTC	73.34 ± 21.83	81.61	55.33	4.09–159.56	−7.47 ± 1.21	−16.6 ± 7.7
	TRM	76.52 ± 19.13	69.04	73.85	5.14–161.05	−4.67 ± 0.29	−10.3 ± 3.9
	Control	61.20 ± 14.96	57.18	53.79	6.32–171.69	−4.72 ± 2.59	0.0
Carnation	AZI	4.77 ± 0.93	4.06	3.62	0.99–11.02	−2.57 ± 0.57	−2.9 ± 4.7
(*D. caryophyllus*)	CPX	4.99 ± 2.39	3.98	2.49	1.53–23.74	−1.50 ± 0.95	−10.6 ± 6.8
	OTC	5.85 ± 0.89	4.92	6.41	1.51–12.88	−1.00 ± 0.70	−19.4 ± 21.7
	TRM	8.48 ± 2.58	6.02	10.09	1.07–27.96	−2.70 ± 2.93	2.3 ± 11.0
	Control	5.02 ± 0.56	3.6	3.865	1.17–19.73	−2.57 ± 1.28	0.0

Table 3 (*Contd.*)

Specie	Treatment	Mean (mm)	Median (mm)	IQR (mm)	Min – max (mm)	Δ mass (mg) ($m_{F_{Dry}} - m_i$)	RCI (%)
Alfalfa (*M. sativa*)	AZI	23.79 ± 11.22	12.27	32.2	6.38–87.62	−8.23 ± 2.67	3.8 ± 4.8
	CPX	35.61 ± 6.20	18.8	55.57	3.28–85.58	−8.10 ± 1.13	−9.5 ± 8.9
	OTC	39.30 ± 2.49	38.045	37.24	5.91–77.31	−6.87 ± 0.60	−20.4 ± 2.6
	TRM	36.84 ± 5.88	30.64	50.63	6.1–93.61	−7.17 ± 0.55	−15.8 ± 6.1
	Control	19.91 ± 9.58	10.07	11.02	4.41–108.92	−8.82 ± 1.71	0.0

[a] NG: Not germinated, NA: Not applicable. [b] No data were collected for chicory seeds, as no germination occurred under any treatment, including the control.

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enhanced germination does not necessarily translate into increased early root growth. Notably, oxytetracycline exhibited divergent effects in alfalfa and soybean, promoting both germination and root elongation in the former while inhibiting root elongation in the latter. These contrasting responses highlight the importance of assessing sub-lethal endpoints beyond germination alone when evaluating early seedling establishment and potential impacts on plant development.

Following the germination stimulation observed under fluoroquinolone exposure in grasses, similar trends were observed for root elongation. In wheat (*Triticum aestivum*), median root length increased following exposure to fluoroquinolones (Fig. 4b). Likewise, rice (*Oryza sativa*) exhibited increased median root length under ciprofloxacin exposure (74.57 mm compared to 46.65 mm in the control; Fig. 4c), accompanied by a negative relative change index (RCI = −9.55; Table 3), indicating increased biomass. Comparable responses were observed in pea (*Pisum sativum*; Fig. 5a), where ciprofloxacin exposure resulted in a 50% increase in germination together with a negative RCI value of −14.4%, consistent with growth stimulation. These trends align with previous reports describing hormetic responses in plants exposed to low concentrations of fluoroquinolones and other antibiotics, where enhanced root growth has been associated with stress-related signalling pathways, including increased ROS production and activation of antioxidant defence mechanisms.[25] However, exceptions to this general trend were also observed. In red clover (*Trifolium pratense*; Fig. 5c), ciprofloxacin exposure resulted in increased germination, while the RCI value was positive (63.8%), indicating reduced biomass accumulation rather than growth stimulation. Similar response patterns have been reported in previous studies, where ciprofloxacin and related antibiotics promoted seed germination but inhibited subsequent biomass accumulation in plants.[35,49] These findings suggest that early seed activation does not necessarily translate into enhanced seedling growth and may reflect differential sensitivities of metabolic processes and stress responses during early development. In contrast, ciprofloxacin exposure in white clover (*Trifolium repens*; Fig. 5b) resulted in stimulation of both biomass accumulation (RCI = −76%) and median root length (increase of 12.7 mm). The divergent responses observed between closely related species, such as red and white clover, highlight pronounced species-specific physiological differences that influence antibiotic sensitivity and downstream growth outcomes.

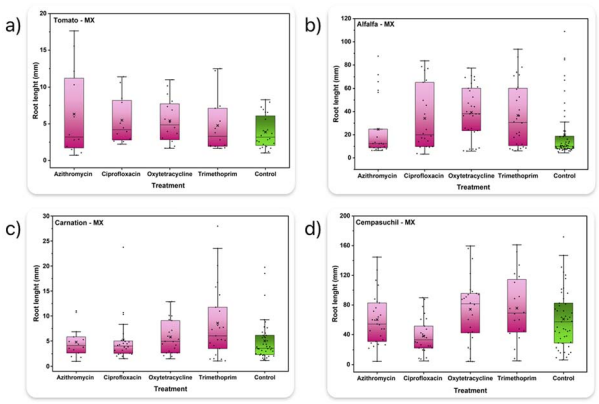

Fig. 3 Effect of antibiotic treatments on root length of four species from Mexico: (a) tomato (*S. Lycopersicum*), (b) cempasuchil (*T Erecta*), (c) carnation (*D. Caryophyllus*), and (d) alfalfa (*M. Sativa*).

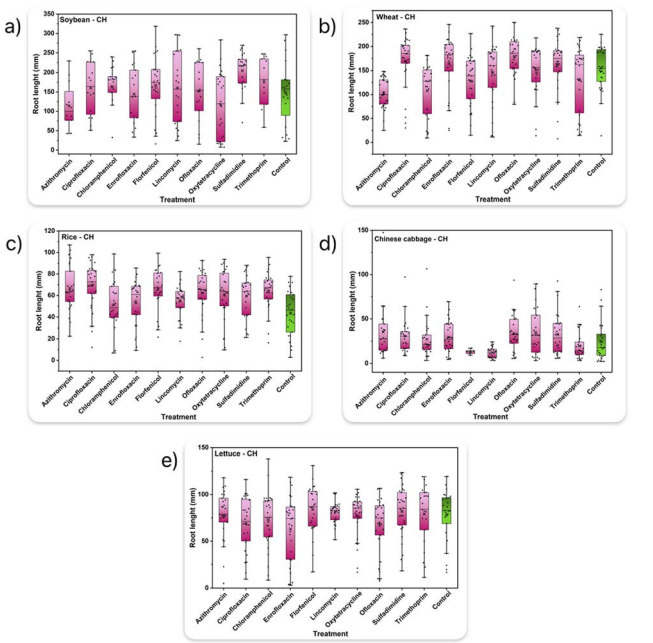

Fig. 4 Effect of antibiotic treatments on root length of five seed species from China: (a) soybean (*G. max*), (b) wheat (*T. aestivum*), (c) rice (*O. sativa*), (d) Chinese cabbage (*B. rapa subsp. Pekinensis*), and (e) lettuce (*L. sativa*).

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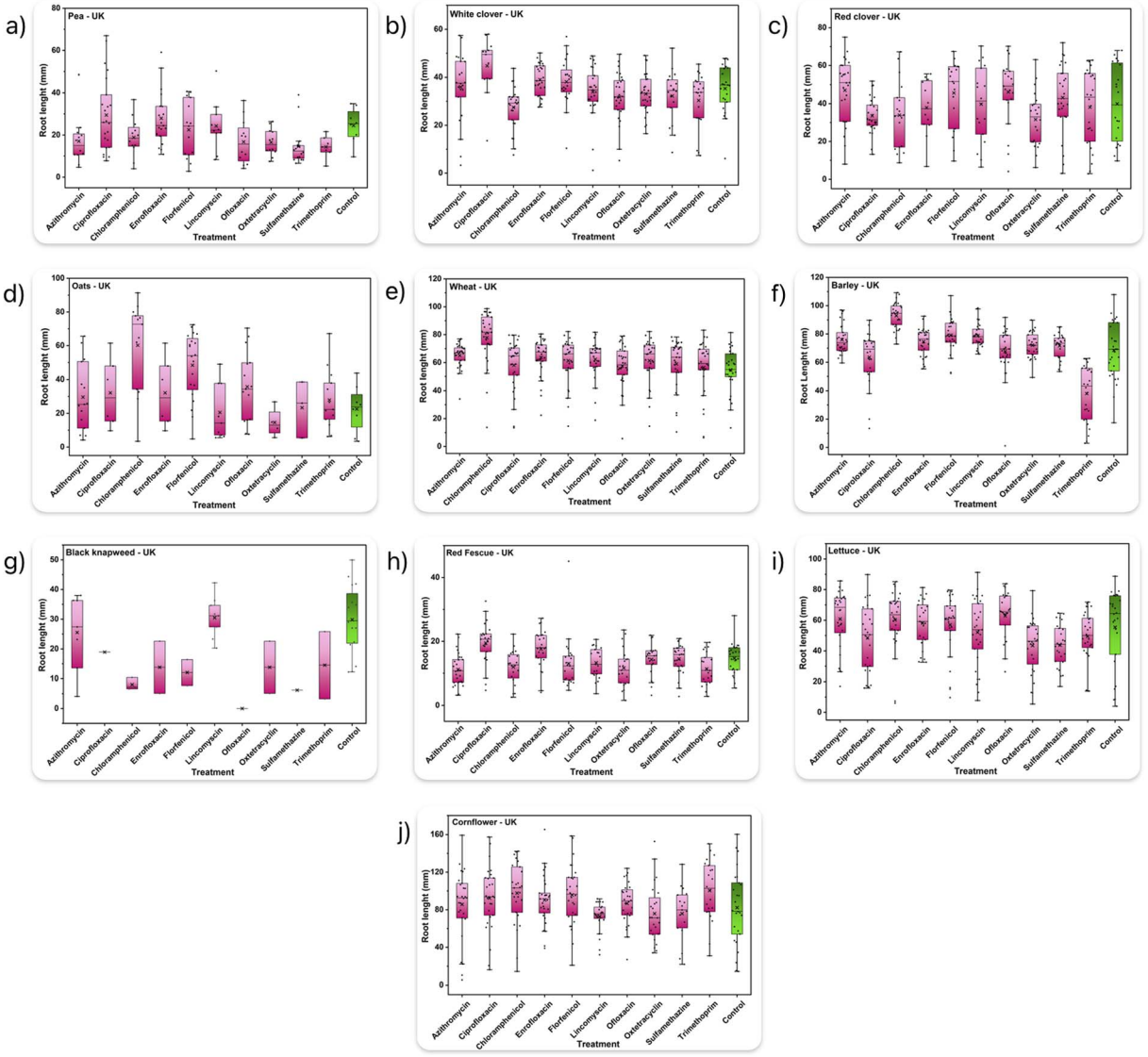

Fig. 5 Effect of antibiotic treatments on root length of ten seed species from England: (a) pea (*P. sativum*), (b) wild white clover (*T. repens*), (c) red clover (*T. pratense*), (d) oats (*A. sativa*), (e) wheat (*T. aestivum*), (f) barley (*H. vulgare*), (g) black knapweed (*C. nigra*), (h) red fescue (*F. rubra*), (i) lettuce (*L. sativa*), and (j) cornflower (*C. cyanus*).

Contrasting responses to the same antibiotic were also observed for florfenicol. In Chinese cabbage (*Brassica rapa* subsp. *pekinensis*), germination was reduced by 70% under florfenicol exposure (Table 2), and this inhibitory effect persisted across additional endpoints, including root elongation (median root length of 12 mm compared to 17.61 mm in the control) and biomass accumulation, with an RCI value of 99.97%, indicating near-complete growth inhibition (Fig. 4d). In contrast, red clover (*Trifolium pratense*) exhibited increased median root length under florfenicol (51.64 mm) and azithromycin (51.01 mm) exposure compared to the control (39.32 mm; Fig. 5c), with corresponding RCI values of −1.3% and −6.3%, respectively. Similarly, oats (*Avena sativa*) displayed a marked increase in median root elongation under florfenicol exposure (54.40 mm compared to 23.81 mm in the control; RCI = −2.1%), although this response was less pronounced than that observed under chloramphenicol exposure (72.69 mm; RCI = 18%). Across these cases, increases in root elongation were not accompanied by proportional increases in biomass accumulation, as indicated by RCI values. This pattern suggests that, under antibiotic exposure, seedlings may preferentially allocate resources towards root elongation during early establishment, rather than overall biomass production. Similar response patterns have been discussed previously in the context of stress adaptation and resource allocation under chemical exposure.[35,54,56]

Consistent with the germination results, black knapweed (*Centaurea nigra*) exhibited inhibitory responses in root elongation under all antibiotics tested, with median root lengths markedly reduced relative to the control value of 29.49 mm (Fig. 5h). The most pronounced reductions were observed under sulfamethazine (6.17 mm), chloramphenicol (7.15 mm), and

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enrofloxacin (10.83 mm). These results further support the high sensitivity of *C. nigra* to antibiotic exposure across multiple early growth endpoints. Pea (*Pisum sativum*) was also identified as sensitive to antibiotic exposure. This species exhibited a median root length of 25.3 mm in the control, which decreased under exposure to several antibiotics, accompanied by reductions in biomass accumulation. The most notable effect was observed under trimethoprim exposure, where median root length decreased to 14.3 mm with a corresponding RCI value of 28.1%. Root length reductions and biomass inhibition were also recorded under lincomycin, azithromycin, oxytetracycline, and sulfamethazine exposure (Table 3 and Fig. 5a). In contrast, enrofloxacin exposure in *P. sativum* resulted in germination stimulation (36.6%), while the RCI value remained positive (6.3%), indicating that increased germination was not associated with enhanced biomass accumulation. This further illustrates that stimulation of early germination does not necessarily translate into improved seedling growth or establishment.

Chloramphenicol also exhibited marked effects on root elongation across cereal species. In wheat (*Triticum aestivum*), median root length increased substantially from 55.0 mm in the control to 81.7 mm under chloramphenicol exposure (Table 3 and Fig. 5e). Similarly, in barley (*Hordeum vulgare*; Fig. 5f), median root length increased from 67.5 mm in the control to 94.6 mm following chloramphenicol exposure, representing a markedly greater response than those observed under florfenicol (78.6 mm) and lincomycin (78.1 mm). The consistent stimulation of root elongation observed across cereal species under chloramphenicol exposure suggests recurring response patterns that merit further investigation using higher-powered and mechanistically focused experimental designs.

Standard toxicity test evaluation

Statistical power. An analysis of the relationship between statistical power, number of experimental runs, and replication level was carried out using the Mexico dataset, with results summarised in Table 4. This analysis illustrates the experimental effort required to reliably detect biologically meaningful effects under the variability observed in germination and early growth responses. For example, achieving a statistical power of 70–80%[57] to detect treatment effects in carnation (*Dianthus caryophyllus*) would require up to 35 experimental runs to compare four antibiotics with the control, with approximately seven replicates per treatment (Table 4). For other species, such as tomato (*Solanum lycopersicum*), cempasúchil (*Tagetes erecta*), and alfalfa (*Medicago sativa*), achieving 80% statistical power would require at least 23, 30, and 11 experimental runs, respectively, with replication levels ranging from five to six for tomato and cempasúchil, and three for alfalfa. These estimated replication requirements exceed those commonly applied in germination and phytotoxicity studies, where three to five replicates per treatment are frequently used.[29,43,58] Under conditions of high biological variability, such replication levels may be insufficient to detect real treatment effects, increasing the likelihood of Type II errors (false negatives). These findings highlight the need to carefully consider replication and statistical power in the design of future phytotoxicity studies, particularly when assessing subtle or sub-lethal effects.

Underpowered studies may therefore underestimate subtle or variable phytotoxic or stimulatory effects, particularly when assessing complex stressors such as antibiotics. These considerations highlight the practical and financial challenges associated with evaluating seedling toxicity across a broad range of species and chemical stressors.[43,59] When applied to the UK and China datasets, the replication and experimental effort estimated in this analysis remains directly relevant. In cases where no statistically significant effects were observed (*e.g.*, lettuce or oxeye daisy), the absence of significance may reflect limitations in experimental power rather than a true absence of biological effects. To robustly distinguish between these possibilities, future studies should plan replication levels that meet or exceed those recommended in Table 4 for each species. One practical strategy to address these constraints is the use of screening-level assays, such as the approach adopted in this study, where broad patterns and potentially sensitive species–compound combinations can be identified initially and subsequently investigated using higher replication to achieve the statistical power required for confirmatory testing. This tiered approach minimises the risk of overlooking real effects while maintaining experimental feasibility.

Table 4 Required number of experiments and replicates per treatment for each plant species, statistical power analysis results

	Statistical power	Experiment number	Replicates per treatment
Tomato (*S. lycopersicum*)	70%	19	4
	80%	23	5
	90%	27	6
Cempasuchil (*T. erecta*)	70%	25	5
	80%	30	6
	90%	36	8
Carnation (*D. caryophyllus*)	70%	35	7
	80%	45	9
	90%	48	12
Alfalfa (*M. sativa*)	70%	11	3
	80%	11	3
	90%	12	4

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Pharmaceutical mass balance. The experiments were conducted using a commonly adopted hydroponic filter-paper approach, consisting of a spiked aqueous solution (nominal antibiotic concentration of 1 mg L^{-1}) in Petri dishes. However, this experimental configuration raises potential concerns regarding pollutant removal *via* sorption processes. To address this, changes in antibiotic concentrations in the exposure solutions were quantified (Table 5).

Across the antibiotics tested, the concentrations decreased during the exposure period, ranging from 12.66 ± 9.41% for trimethoprim to 58.40 ± 8.33% for ciprofloxacin. These reductions are most plausibly attributed to sorption onto the filter paper. Consistent with this interpretation, the apparent partition coefficients ($K_{d,app}$) calculated after 7 days followed the order: ciprofloxacin (≈1.40) > azithromycin (≈0.87) > oxytetracycline (≈0.46) > trimethoprim (≈0.15). This ranking mirrors the magnitude of concentration losses observed in the aqueous phase. These $K_{d,app}$ values are specific to the experimental water–filter paper system and are used here solely for comparative purposes. Because the mass-to-volume ratio (m V^{-1}) remained constant across experiments, differences in sorption behaviour primarily reflect intrinsic physicochemical properties of the antibiotics, including acid-base speciation, hydrogen bonding capacity, and electrostatic interactions with cellulose. Understanding these partitioning dynamics is critical for estimating the fraction of antibiotics that remained freely available for seed exposure (Table 5). Sorption to the filter paper effectively reduces dissolved antibiotic concentrations, which may lead to underestimation of phytotoxic effects and complicates direct comparison with environmental scenarios, where sorption dynamics depend strongly on soil properties and organic matter content.

Separately, and beyond sorption processes, the potential role of root exudates should also be considered. For example, tomato root exudates are known to contain organic acids, amino acids, sugars, and phenolic compounds that might modify local chemical conditions and enhance the desorption and bioavailability of certain pollutants.[60] In the absence of supporting water-quality measurements (*e.g.*, pH, electrical conductivity, total organic carbon), however, this interpretation remains speculative and is therefore presented as a plausible mechanism rather than a demonstrated process. This hypothesis is consistent with the observed behaviour of azithromycin and ciprofloxacin, which exhibit a higher cationic or neutral fraction at near-neutral pH, compared to oxytetracycline and trimethoprim, which are more likely to occur as zwitterionic, anionic, or neutral species with lower affinity for cellulose-based filter paper. Such differences in speciation may influence both sorption and desorption dynamics within the experimental system. This framework also provides a potential explanation for the occurrence of negative seed-removal values, defined as the net difference in antibiotic loss between dishes with seeds and control dishes without seeds. Rather than reflecting direct uptake by seeds, these negative values suggest that seed-associated processes, including the release of root exudates or other abiotic interactions, may have promoted desorption of antibiotics from the filter paper, resulting in higher aqueous concentrations after initial sorption.[61] Consequently, the seed-removal metric does not exclusively represent plant uptake but instead integrates all seed-associated processes that may either decrease or increase antibiotic availability in solution.

In summary, sorption to filter paper can reduce effective antibiotic exposure concentrations, potentially leading to underestimation of phytotoxic effects in controlled assays. Conversely, seed- and root-associated processes, such as exudate-mediated interactions, may locally modify pollutant bioavailability and influence early seedling development. Direct assessment of exudate-mediated desorption would require additional water-quality measurements and should be addressed in future hypothesis-driven studies. These interactions highlight the complex relationship between antibiotic chemistry and processes at the root surface environment, underlining the importance of considering both physicochemical fate and biological responses in phytotoxicological assessments.[61]

Test conditions and species tested. Three species were excluded from further results analysis because germination across all treatments, including controls, consistently remained ≤ 20%: chicory (*Cichorium intybus*) in China, as well as sheep's fescue (*Festuca ovina*) and quaking grass (*Briza media*) in the UK. This outcome highlights a limitation of standardized phytotoxicity testing protocols, which prioritise uniform experimental conditions at the expense of accommodating species-specific germination requirements.

Short-term assays (<7 days) conducted under fixed temperature, light, and hydroponic conditions may fail to capture germination responses in species that require longer

Table 5 Antibiotic removal in tomato, cempasuchil, carnation, and alfalfa after 7 days: concentration loss (Δ*C*%), seeds removal (%), and abiotic loss in no-seed controls

	Tomato		Cempasuchil		Carnation		Alfalfa		No seeds
	Δ*C*%	Seeds removal (%)	Δ*C*%	Seeds removal (%)	Δ*C*%	Seeds removal (%)	Δ*C*%	Seeds removal (%)	Δ*C*%
AZI	84.67 ± 4.19	38.03 ± 2.86	84.66 ± 2.86	38.02 ± 3.44	56.17 ± 9.08	9.53 ± 6.05	57.44 ± 5.96	10.8 ± 4.57	46.64 ± 7.38
CPX	75.52 ± 4.65	17.12 ± 4.33	79.94 ± 7.80	21.54 ± 5.64	58.74 ± 12.30	0.34 ± 7.87	53.73 ± 12.37	−4.67 ± 7.91	58.40 ± 8.33
OTC	30.32 ± 14.05	−1.19 ± 8.92	17.89 ± 7.85	−13.62 ± 5.86	32.70 ± 2.16	1.19 ± 3.91	20.82 ± 7.74	−10.69 ± 5.81	31.51 ± 9.09
TRM	5.26 ± 3.16	7.4 ± 4.25	22.66 ± 5.40	10.00 ± 4.95	14.47 ± 6.68	−17.04 ± 5.44	13.35 ± 1.81	0.69 ± 3.98	12.66 ± 9.41

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germination periods or specific environmental cues, such as soil-based substrates, distinct temperature ranges, or light exposure. The three species showing no germination in this study are terrestrial plants for which germination is typically recommended in moist, well-drained soils over extended periods. Consequently, the observed low germination likely reflects a mismatch between their ecological requirements and the uniform hydroponic conditions applied here, rather than an absence of viability. For example, optimal germination of sheep's fescue (*F. ovina*) has been reported at approximately 15 °C, with reduced germination occurring at temperatures between 20 and 30 °C.[62] Similarly, quaking grass (*B. media*) exhibits specific requirements for light exposure and an optimal germination temperature of 28.9 °C; its germination is also influenced by sowing depth, with higher rates reported at shallow depths of 0.5–2 mm.[63] These ecological requirements may explain the low germination observed under the fixed temperature (22 °C) and hydroponic conditions used in this study. Rather than indicating an inappropriate experimental design, the exclusion of these species underscores the need for adaptable ecotoxicological testing protocols that account for intra- and inter-species variability. Incorporating species-specific germination requirements is particularly important when assessing non-cultivated or regionally relevant species, whose responses may be overlooked under standardised conditions.

Standard phytotoxicity guidelines often require a minimum of 70% germination under fixed hydroponic, temperature, and light conditions. While this criterion was met for several species tested in Mexico, the UK, and China (including French bean, pea, oats, barley, oxeye daisy, black knapweed, chicory, Shasta daisy, and tomato), such requirements may inadvertently restrict the inclusion of lesser-studied or ecologically important species. Expanding phytotoxicity testing frameworks to allow customised, species-specific germination conditions would enhance both the inclusiveness and ecological relevance of future terrestrial risk assessments.

Conclusions

This study presents a broad Tier I screening of germination and early seedling phytotoxicity for ten antibiotics across 23 plant species, identifying both sensitive species and compounds that warrant further, hypothesis-driven investigation. In addition, this work provides practical guidance on experimental design requirements needed to achieve adequate statistical power in future phytotoxicity studies. A central finding is that antibiotic effects on germination and early growth are highly species- and compound-specific. While fluoroquinolones generally stimulated germination and root elongation in grasses and legumes, notable exceptions highlight the complexity of plant–antibiotic interactions and caution against generalisation across taxa or antibiotic classes. Importantly, the identification of non-cultivated species, such as black knapweed (*Centaurea nigra*), as particularly sensitive to antibiotic exposure underscores the need to extend phytotoxicity assessments beyond agriculturally or commercially relevant crops. Such species represent promising bioindicators for environmental monitoring of antibiotic contamination. This study also demonstrates that sublethal endpoints, including changes in root elongation and biomass, provide critical information beyond simple germination inhibition or stimulation. Incorporating these endpoints alongside a geographically diverse selection of cultivated and non-cultivated species is essential for a more comprehensive understanding of antibiotic impacts on terrestrial plant communities. Lastly, the results highlight limitations of standard phytotoxicity testing frameworks, as many environmentally relevant species fail to germinate under fixed light, temperature, and hydroponic conditions. More flexible, species-adapted protocols are therefore required to improve ecological relevance. Accurate quantification of antibiotic concentrations throughout experiments is equally critical, as processes such as sorption, degradation, and seed-associated interactions directly influence effective exposure levels and the interpretation of phytotoxic effects. Together, these findings support the need for adaptive, tiered phytotoxicity assessments to better capture the environmental risks posed by antibiotics in terrestrial ecosystems.

Author contributions

RSCL: conceptualization, data curation, formal analysis, investigation, methodology, software validation, visualization, writing–original draft. AGJ: conceptualization, data curation, methodology, writing–original draft, project administration, supervision. JCDA: supervision, writing–review & editing, resources, funding acquisition. FE: conceptualization, methodology, writing–review & editing. WSB: conceptualization, investigation, formal analysis, data curation, resources. JG: investigation, software, formal analysis, data curation, writing-review & editing. JN: investigation, writing–review & editing. FZ: conceptualization, investigation, formal analysis, data curation, resources, funding acquisition. LC: conceptualization, funding acquisition, methodology, project administration, resources, writing–review & editing

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting the findings of this study, including all statistical summaries and results presented in the main text and figures, are included within the article and its supplementary information (SI). The complete raw measurements for root length and seedling growth are available as part of the supplementary information. Supplementary information: Tables (antibiotic details, seed sterilisation, environmental concentrations reported in irrigation water and soil pore-water compared with the nominal concentration used in this study, MRM quantification parameters, and method validation parameters) and Figures (germination and root length data for

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China and UK species; detailed protocols, and raw data). See DOI: https://doi.org/10.1039/d6em00053c.

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