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ORIGINAL PAPER

Application of municipal wastewater-derived *Escherichia coli* and *Enterococcus faecium* in mitigating ciprofloxacin phytotoxicity in crops

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Abstract

Ciprofloxacin (CIP) is frequently detected in environmental matrices due to incomplete removal during wastewater treatment, posing risks to sustainable agriculture through phytotoxicity and the promotion of antimicrobial resistance. This study isolated and characterised CIP-resistant bacteria from municipal wastewater in Lahore, Pakistan, and evaluated their potential for CIP bioremediation and phytotoxicity reduction in edible crops. Two potent isolates were identified through 16S rRNA sequencing as *Escherichia coli* and *Enterococcus faecium*. In biodegradation assays, the bacterial consortium demonstrated high removal efficiency, achieving up to 100% CIP degradation under optimal conditions (40 °C, pH 6.5), confirmed using HPLC. The process followed Michaelis–Menten kinetics, with *E. faecium* exhibiting a higher degradation rate (Vmax = 8.26 mg L^{-1} day^{-1}) than *E. coli* (Vmax = 6.37 mg L^{-1} day^{-1}). Phytotoxicity assays conducted using CIP-contaminated irrigation water (0.1–500 mg L^{-1}) showed that application of the consortium substantially improved seedling performance in carrot (*Daucus carota*), pea (*Pisum sativum*), radish (*Raphanus sativus*), lettuce (*Lactuca sativa*), and spinach (*Spinacia oleracea*). The consortium enhanced the germination index by 56–199% and increased the seedling vigour index by up to 337% compared with CIP-only treatments. All measured stress-tolerance indices (root and shoot length, fresh and dry weight) increased significantly, while the phytotoxicity index decreased in bacterially treated groups. Overall, the consortium effectively degraded CIP and mitigated its phytotoxic impacts, demonstrating strong potential as a sustainable biological strategy for treating antibiotic-contaminated water and protecting crop productivity.

Keywords Ciprofloxacin · Bioremediation · Municipal wastewater · Edible crops · Phytotoxicity · *Escherichia coli* · *Enterococcus faecium*

Introduction

The contemporary world's rapid industrialisation and urbanisation have led to the accumulation of pollutants in municipal wastewater, posing significant challenges to both human health and environmental sustainability (Khanam et al. 2022). Pharmaceutical residues, particularly those from antibiotics such as ciprofloxacin, have been a source of concern among these contaminants due to their persistence and ability to induce antimicrobial resistance (AMR) (Akhter et al. 2024). Due to its insufficient removal during standard wastewater treatment procedures and poor metabolism in humans, the commonly used fluoroquinolone antibiotic ciprofloxacin (CIP) is frequently found in municipal wastewater (Nas et al. 2021). Water bodies containing CIP pose a hazard to both agricultural productivity and aquatic ecosystems (Gauba And Saxena 2023). Reusing untreated

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or insufficiently treated wastewater for irrigation can have negative effects on plant development, soil microbiology, and potentially human health through the food chain (Khan et al. 2022). In order to address this problem, innovative and long-lasting solutions are needed to reduce the environmental impact of these pollutants and encourage resource recycling.

The use of biological treatment methods has become a viable strategy for eliminating persistent contaminants like CIP from wastewater (Aslam et al. 2023; Arya et al. 2025; Mohy-u-Din et al. 2024; Nas et al. 2021; Mays 2019). Specifically, by integrating the metabolic capacities of various microorganisms, the utilisation of bacterial consortia may provide benefits over single-strain systems. Therefore, the present study examines how a bacterial consortium made up of *E. coli* and *E. faecium* treats municipal wastewater contaminated with CIP so that it can be used again for irrigation. Both bacteria are good candidates for bioremediation applications because of their strong adaptability and capacity to break down or transform complex organic contaminants (Cheng et al. 2022; Maurya et al. 2021; Sharma et al. 2024). The purpose of this study is to evaluate the potential for creating treated water suitable for agricultural use and examine how well the *E. coli* and *E. faecium* consortium breaks down CIP in municipal wastewater. The goal of this research is to close the gap between sustainable irrigation techniques and wastewater management by integrating microbiological, biochemical, and agricultural perspectives. The findings of this study may provide two advantages: they may lessen the threats to the environment and public health posed by wastewater containing CIP and encourage the use of treated water for sustainable farming methods. Additionally, more efficient and scalable bioremediation techniques that target other emerging pollutants may also be made possible by an understanding of the synergistic interactions within the *E. coli* and *E. faecium* consortium.

Antibiotics have emerged as a new class of emerging contaminants, raising concerns in recent years (Boahen et al. 2025; Roose-Amsaleg And Laverman 2016). The extensive use of antibiotics in both medical and agricultural settings has resulted in significant environmental contamination, with pharmaceutical residues frequently detected in soil and water ecosystems (Pan And Chu 2016a and b; Tamtam et al. 2011; Tong et al. 2009). Among these contaminants, CIP, a commonly prescribed broad-spectrum antibiotic, has attracted attention due to its persistence in the environment and its potential to disrupt microbial communities (Al-Howri et al. 2025; Smedbol et al. 2018; Hénault-Ethier et al. 2017; Giroux 2015; Ebert et al. 2011; Martínez 2009). Previous researchers have identified CIP in a variety of environmental media, such as sediments, groundwater, and surface waters, frequently at levels ranging between nanograms and micrograms per litre (Al-Howri et al. 2025; Giger et al. 2003; Göbel et al. 2007). Ecological hazards include the emergence of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) due to the ongoing release of CIP into the environment, mostly through water treatment plants (WWTPs) (Martínez 2009). Irrigation with waters containing CIP results in the accumulation of this antibiotic in agricultural soils, which not only poses a risk of promoting antibiotic-resistant bacteria but also threatens soil health and crop productivity (Mohy U Din, et al. 2022; Jia et al. 2018; Xiong et al. 2017; Mohapatra et al. 2016; Janecko et al. 2016; Van Doorslaer et al. 2014; Heeb et al. 2011). Consequently, there is an urgent need to explore effective strategies for the bioremediation of CIP-contaminated environments.

The primary, secondary, and tertiary water treatment techniques that are often used are frequently insufficient to completely eradicate antibiotics such as CIP (Burch et al. 2019). Owing to its complicated molecular structure and high stability, conventional treatments, including activated sludge, membrane bioreactors (MBRs), and biofilm reactors, have demonstrated limited efficacy in degrading CIP (Carneiro et al. 2019; Radjenović et al. 2009; Michael et al. 2013). Advanced oxidation processes (AOPs) like ozonation (Westerhoff et al. 2005; Andreozzi et al. 2003), Fenton and photo-Fenton processes (Watts et al. 2005; Torrades et al. 2003), adsorption (Inyang et al. 2015; Ternes et al. 2004) and photocatalysis (Michael et al. 2012; Chong et al. 2010) have been used in the recent past, but these methods are not cost-effective. As an alternative, bioremediation, a process that utilises microorganisms to degrade or remove pollutants, offers a promising approach to mitigating the adverse effects of CIP contamination. *Escherichia coli* (*E. coli*) and *Enterococcus faecium (E. faecium*), two bacteria known for their robust metabolic versatility and resilience, have shown potential in degrading various organic pollutants, including antibiotics (Cheng et al. 2022; Maurya et al. 2021; Sharma et al. 2024; Garrity et al. 2005). Both bacteria were selected for this in vitro investigation due to their prevalence in wastewater, strong adaptability, and reported capacity to break down or transform complex organic contaminants, providing proof of concept for bioremediation using native wastewater flora (Mohy U Din et al. 2022; Alvarez et al. 2010). However, it is crucial to acknowledge that *E. coli* and *E. faecium* are also known opportunistic pathogens and key players in the dissemination of antimicrobial resistance (AMR). The significance of investigating these specific strains lies in understanding their dual role: their bioremediation potential must be weighed against their capacity to promote the horizontal gene transfer (HGT) of AMR genes to other bacteria (Mohy U Din, et al. 2022; Liu et al. 2022). Integrons and plasmids, which facilitate the acquisition and dissemination of resistance genes, contribute to this capacity, posing a significant risk to public health (Mohy U Din et al. 2022; Hu et al. 2022). Therefore, while this study

explores their degradative capabilities, their application in open-field settings requires extensive further risk assessment and mitigation strategies, potentially involving the use of non-pathogenic, genetically engineered, or contained systems in the future. Incorporating beneficial, non-pathogenic bacteria into agricultural practices could aid in detoxifying polluted soils and promote sustainable crop production. This dual benefit underscores the importance of studying the interactions between these microorganisms and plants in agricultural settings. Previous studies have examined the concentrations of other antibiotics in lettuce, tomato, maize, onion, and spinach (Qiu et al. 2019a, b; Azanu et al. 2016; Kang et al. 2013; Bassil et al. 2013; Grote et al. 2007; Boxall et al. 2006). By focusing on the impact of *E. coli* and *E. faecium* on the growth of carrots, peas, radish, lettuce, and spinach, this research seeks to provide comprehensive insights into how bioremediation can synergistically enhance agriculture.

Carrot, pea, radish, lettuce, and spinach are widely consumed vegetables that play a critical role in human nutrition due to their rich content of vitamins, minerals, and antioxidants (Afzal et al. 2025; Khalid et al. 2023; Axe 2023a, b, c; Healthline 2023; Verywell Fit 2023). Understanding the interplay between microbial activity and plant growth in these crops can have significant implications for food security and public health. The aim of this study is to assess the bioremediation efficiency of *E. coli* and *E. faecium* in CIP-contaminated municipal water and their influence on plant growth parameters after treatment, including germination rates, biomass production, and nutrient uptake. The novelty of this research lies in the innovative application of naturally occurring bacterial strains, *E. coli* and *E. faecium*, isolated from municipal water sources, to bioremediate CIP-contaminated waters. This study is among the first to demonstrate these bacterial strains' significant degradation of CIP, highlighting the potential of microbial-assisted remediation as a viable, in situ and cost-effective strategy for managing antibiotic contamination in agricultural systems. By doing so, this work contributes to the development of sustainable and resilient agricultural practices.

Materials and methods

Chemicals and materials

The chemicals used in this experiment were purchased from Sigma Aldrich, UK, Fisher Scientific, UK, and Biochem Chemopharma, France. The reagents for the CIP determination were chromatographically pure (Fisher Scientific, Waltham, MA, USA), and the others were analytically pure.

Before the experiment, all glassware, culture loops, needles, and growth media were sterilised. An infusion of antibiotic CIP (200 mg per 100 mL) was purchased from a pharmacy in Lahore. The stock and standard solutions were prepared by dissolving 100 mg L^{-1} of antibiotic in methanol, and they were then stored in amber glass vials at – 20 ºC. In accordance with established procedures (APHA 2005), municipally contaminated water samples were taken from drains at various locations throughout Lahore, Pakistan, and placed in sterile glass bottles (Fig. 1). To prevent any physicochemical changes, the glass bottles were labelled, transported, and stored at 4 ºC in an ice box.

Carrot (*Daucus carota* L. var. T-29), pea (*Pisum sativum* L. var. Mateor), radish (*Raphanus raphanistrum* L. var. Radish Minto Early), lettuce (*Lactuca sativa* L. var. Loose Leaves), and spinach (*Spinacia oleracea* L. var. Punjab) were selected as the target crops due to their unique edible portions, economic importance, and frequent use as raw vegetables. The seeds for these crops were obtained from the Punjab Seed Certification Department.

Enrichment, isolation, and selection of microbial strains

The enrichment of bacterial strains capable of degrading CIP was performed using a selective pressure strategy in Minimal Salt Medium (MSM). Approximately 15 mL of municipal wastewater effluent was inoculated into a 250 mL flask containing 150 mL of sterile MSM (pH 6.8–7.0) supplemented with 5 mg L^{-1} of CIP. The culture was incubated at 37 °C for ten days on a rotary shaker at 100 rpm to enrich for CIP-resistant and CIP-degrading bacteria. A sequential subculturing process was then employed to isolate strains with high degradation potential. Every week, 10 mL of the culture was transferred to fresh sterile MSM, and the CIP concentration was systematically increased in increments of 25–50 mg L^{-1}. This enrichment strategy, conducted over 6–8 weeks, successfully selected for bacterial strains capable of tolerating and degrading progressively higher concentrations of CIP, with the final subculture reaching 150 mg L^{-1}.

Following the final enrichment cycle, 150 µL of serial dilutions (10^{-1}–10^{-7}) from the culture exhibiting growth at 150 mg L^{-1} CIP were spread onto nutrient agar plates supplemented with the same concentration of CIP (150 mg L^{-1}) to isolate pure colonies. Distinct bacterial colonies exhibiting varied morphologies were selected and repeatedly streaked on fresh antibiotic-supplemented nutrient agar to obtain pure cultures. The purified isolates were stored on nutrient agar slants for further characterisation.

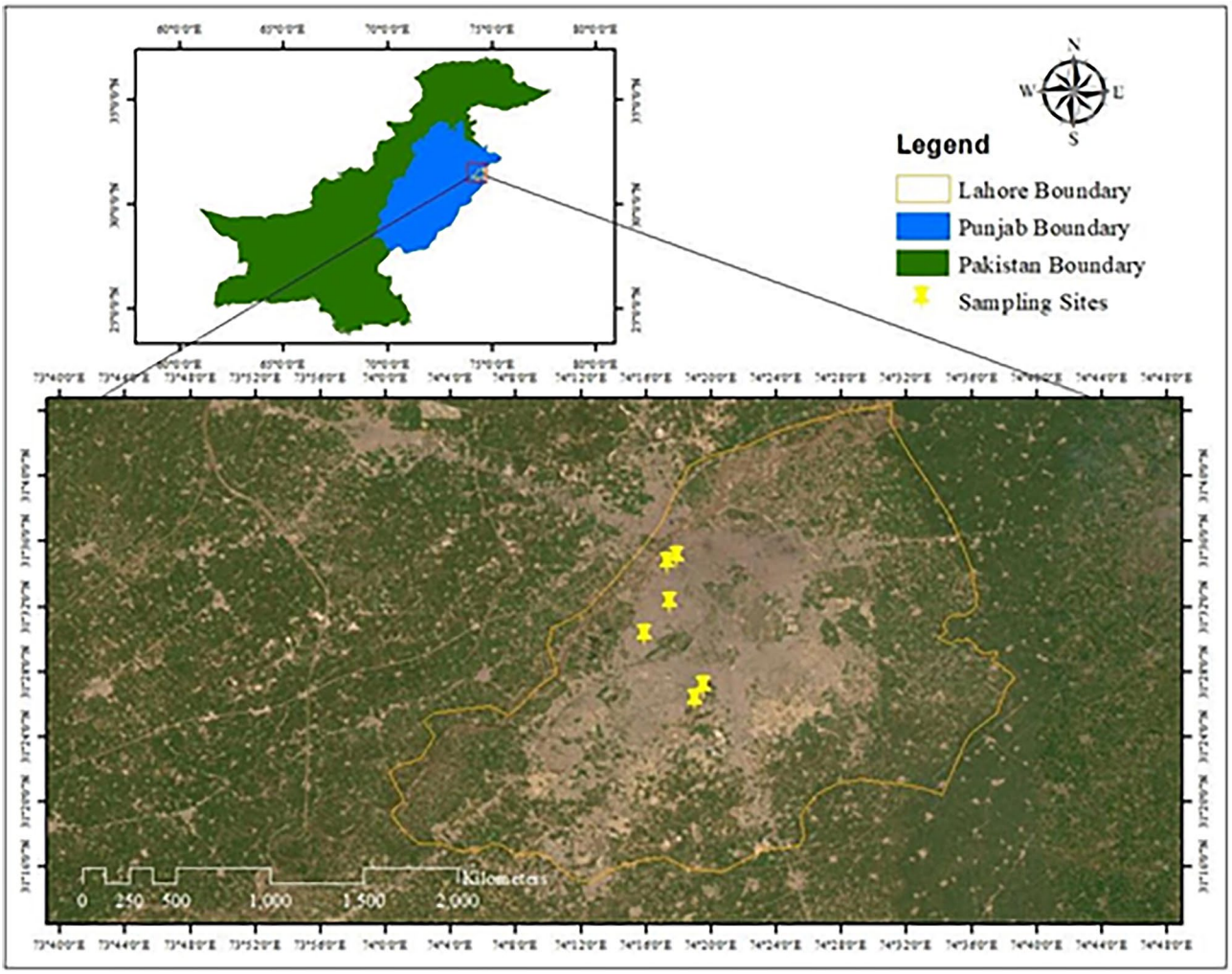


Fig. 1 GIS map showing sampling locations for municipal wastewater collection in Lahore, Pakistan

Identification and culture maintenance of the microbial strains

Bacterial isolates were identified using 16S rRNA gene sequencing. Genomic DNA was extracted from pure cultures, and the 16S rRNA gene was amplified via polymerase chain reaction (PCR) using the forward primer 27F (AGA GTT TGA TCM TGG CTC AG) and reverse primer 1492R (TAC GGY TAC CTT GTT ACG ACT T) as described (Mohy-u-Din et al. 2024). The DNA samples with the extension products were mixed with Hi-Di formamide, incubated for 5 min at 95 °C, and then placed on ice for 5 min. Sequencing was conducted using an ABI Prism 3730XL DNA analyser. Forward and reverse sequence chromatograms (abi files) were initially viewed with FinchTV version 1.5.0 and analysed further using MacVector version 17.5.4. The forward and reverse reads were imported into BioEdit version 7.2, and a consensus sequence for each strain was assembled using the contig assembler program, incorporating the forward read and the reverse complement of the reverse read. BLAST analysis of the assembled sequences was performed with deposited isolates in the GenBank repository. The selected isolates were aseptically transferred onto nutrient agar slants. For optimal growth, the slants were incubated for 48 h at 37 °C and then stored at 4 °C. Every two weeks, bacterial cultures were sub-cultured onto fresh agar slants to maintain their viability and prevent loss of viability (ISO 11133., 2014).

For experimental use, a loopful of bacteria from a fresh slant was inoculated into 100 mL of sterile nutrient broth in a 500 mL flask and cultured for 24 h at 37 °C on a rotary shaker at 200 rpm. The resulting bacterial suspension was used as the inoculum, with cell density standardised using a haemocytometer to achieve the desired inoculum size for subsequent experiments (Mulla et al. 2018).

Bioremediation and biodegradation potential

The two identified bacterial strains, *E. coli* (isolate N6) and *E. faecium* (isolate N23), were evaluated both individually and as a consortium for their ability to degrade CIP.

Inoculum preparation

A single colony of each bacterial strain was inoculated into separate test tubes containing 10 mL of sterile Luria–Bertani (LB) broth and incubated for 24 h at 37 °C on a rotary shaker at 150 rpm to achieve active growth. For the consortium, equal volumes (1:1 ratio) of the 24-h-old cultures of *E. coli* and *E. faecium* were combined.

Biodegradation assay

The biodegradation potential was assessed in 250 mL Erlenmeyer flasks containing 100 mL of Minimal Salt Medium (MSM) supplemented with CIP as the sole carbon source. The effects of various critical parameters on degradation efficiency were investigated:

- *Initial Antibiotic Concentration*: A range of CIP concentrations (e.g., 10, 50, 100 mg L^{-1}) was tested.
- *Temperature*: Flasks were incubated at different temperatures (e.g., 25, 30, 37, and 40 °C).
- *pH*: The initial pH of the MSM was adjusted to different levels (e.g., 5.5, 6.5, 7.5, 8.5).
- *Inoculum Density*: Different inoculum sizes (e.g., 10^6, 10^7, 10^8 CFU mL^{-1}) were evaluated.

The flasks were inoculated with 2% (v/v) of the respective bacterial inoculum (pure cultures or consortium) and incubated on a rotary shaker at 150 rpm for a period of 7–10 days. Abiotic controls (MSM with CIP but no bacteria) were maintained under identical conditions to account for any non-biological removal, such as adsorption or photodegradation. All experiments were conducted in triplicate.

Analytical methods and kinetic analysis

Samples were withdrawn at regular intervals (e.g., 24h intervals). The samples were centrifuged at 10,000 × g for 10 min to separate the bacterial cells, and the supernatant was filtered through a 0.22 µm membrane filter. The residual concentration of CIP in the supernatant was quantified using High-Performance Liquid Chromatography (HPLC) equipped with a UV detector.

The degradation efficiency was calculated as follows:

$$\text{Degradation}(\%) = \left[\left(C_0 - C_e\right)/C_0\right] \times 100$$

Table 1 Treatments used in experiment

1	Control A	Distilled water	No Bacteria
2	Control B	0 mg L^{-1}+B	Added bacteria
3	T-1A	0.1 mg L^{-1}	No bacteria
4	T-1B	0.1 mg L^{-1}+B	Added bacteria
5	T-2A	1 mg L^{-1}	No bacteria
6	T-2B	1 mg L^{-1}+B	Added bacteria
7	T-3A	10 mg L^{-1}	No bacteria
8	T-3B	10 mg L^{-1}+B	Added bacteria
9	T-4A	100 mg L^{-1}	No bacteria
10	T-4B	100 mg L^{-1}+B	Added bacteria
11	T-5A	500 mg L^{-1}	No bacteria
12	T-5B	500 mg L^{-1}+B	Added bacteria

*T = Treatment; *A = Without bacteria only antibiotic; *B = Antibiotic with bacteria added

where C_0 is the initial concentration and C_e is the residual concentration of CIP at time *t*.

The degradation kinetics were modelled using the Michaelis–Menten equation to determine the maximum degradation rate (V_{max}) and the half-saturation constant (K_s).

Seed germination experiment

The seed germination and phytotoxicity experiments were conducted under controlled laboratory conditions to precisely assess the direct effects of CIP and the bacterial consortium on plant growth. For seed germination, phytotoxicity tests were conducted in triplicate, with a control for each antibiotic, using a completely randomised design (CRD). The Punjab Seed Certification Department provided seeds of selected crops. In a laminar flow hood, twenty seeds of each species were sterilised using a 4% (v/v) bleach solution. To prevent contamination during germination, the bleach solution was supplemented with 50 µL of Tween 20 detergent (Rashid et al. 2021). Stock solutions were prepared using deionised water at five different concentrations: 0.1, 0.10, 10.0, 100, and 500 mg L^{-1} for each antibiotic alone, and 0.1+B, 0.10+B, 10.0+B, 100+B, and 500+B mg L^{-1} with consortia for the chosen antibiotic CIP. Details of the treatments are given in Table 1. The American Society for Testing and Materials (ASTM) standard methodology for seed germination was followed using 10 cm Petri plates, each filled with 5 mL of solution (ASTM, 2003). Twenty seeds of each species were placed on Whatman No. 1 filter paper within each Petri plate, which had been moistened with 10 mL of the respective stock solution (treatments) (ASTM, 2003).

Three replicates were prepared for each treatment. White fluorescent tube lights with an intensity of 2500–3500 lx m^{-2} s^{-1} were used in the growth room set at a temperature of 25 ℃ for

the seed germination experiment. Daily, the water loss from the Petri plates was measured by subtracting the final weight of the Petri plates from the initial weight, accounting for any addition of distilled water, as required.

Measurements of length and weight of plants

Throughout the week, the observed values of the seed and growth parameters were recorded, and the germination index was calculated. A seed was considered germinated when its root was visible and measurable (i.e., > 0.5 mm); the root length of non-germinated seeds was recorded as zero. For each experimental group, the lengths of roots and shoots of germinated seeds were measured. Shoot length was measured by subtracting the length of the root from the overall length of the seedling in centimetres. Root length was measured from the tip of the root to the base of the hypocotyl in centimetres. The seedling length was computed by summing the lengths of the roots and the shoots (Hillis et al. 2011). Following overnight oven-drying at 65 °C, the dry weight of the seedlings, both root and shoot, was determined.

Germination index

The germination index (GI) is calculated by multiplying relative root growth (RRG) by the relative seed germination (RSG). A week later, the number of sprouted seeds and the length of their roots were measured to compute the GI. Antibiotic-induced phytotoxicity in seeds can be quantified by assessing the GI values under various treatment conditions. Equations (1–3) were used to calculate the seeds' RSG, RRG, and GI values (Hoekstra et al. 2002):

$$\text{Relative seed germination(RSG)} = \frac{\text{No.of seeds germinated in sample}}{\text{No.of seeds germinated in control}} \times 100 \quad (1)$$

$$\text{Relative root growth(RRG)} = \frac{\text{Mean root length in the sample}}{\text{Mean root length in control}} \times 100 \quad (2)$$

$$\text{Germination index(GI)} = \frac{\text{RSG} \times \text{RRG}}{100} \quad (3)$$

Stress tolerance indices

A valuable metric for evaluating the potential yield and stress tolerance of crop species is the stress tolerance index (STI), which comprises six indices: root length stress index (RLSTI), shoot length stress tolerance index (SLSTI), root fresh weight stress tolerance index (RFSTI), shoot fresh weight stress tolerance index (SFSTI), root dry weight stress tolerance index (RDSTI), and shoot dry weight stress tolerance index (SDSTI). These indices determine the seeds' tolerance levels to various treatments. Data on root and shoot lengths, as well as fresh and dry seed weights, establish the stress tolerance capacity of the plant under different antibiotic concentrations. Equations (4–9) were used to determine these indices (Rashid et al. 2021).

$$\text{RFSTI} = \frac{\text{Root fresh weight of stress plant}}{\text{Root fresh weight of control plant}} \times 100 \quad (4)$$

$$\text{SLSTI} = \frac{\text{Shoot length of stress plant}}{\text{Shoot length of control plant}} \times 100 \quad (5)$$

$$\text{RFSTI} = \frac{\text{Root fresh weight of stress plant}}{\text{Root fresh weight of control plant}} \times 100 \quad (6)$$

$$\text{SFSTI} = \frac{\text{Shoot fresh weight of stress plant}}{\text{Shoot fresh weight of control plant}} \times 100 \quad (7)$$

$$\text{RDSTI} = \frac{\text{Root dry weight of stress plant}}{\text{Root dry weight of control plant}} \times 100 \quad (8)$$

$$\text{RDSTI} = \frac{\text{Root dry weight of stress plant}}{\text{Root dry weight of control plant}} \times 100 \quad (9)$$

Seed vigour index

The seed vigour index (SVI) encompasses traits that influence the activity and productivity of seeds during germination and as they develop into seedlings. It is a critical parameter for characterising various germination traits linked to seed performance (Rashid et al. 2022a; Amin et al. 2013). SVI measures the germination potential of viable seeds during the experiment to assess seed vigour. The vigour index, determined by Eq. (10), combines seedling length and germination percentage (Zhao 2016).

$$\text{SVI} = \text{Germination Percentage} \times \text{Seed length} \quad (10)$$

A scatter plot matrix has also been generated using R software (v14.4; R Core Team 2021) to compare the SVIs of different crops for antibiotics with and without bacteria.

Phytotoxicity index

The phytotoxicity index (PI) can illustrate delays in seed germination, reduced plant growth, or lingering effects on seedlings caused by harmful pollutants. It also identifies any threats hindering plant development. According to

Tiquia et al. (1996), potential PI values range from 0 to 1, where a higher value indicates greater phytotoxicity, while a lower value indicates a non-toxic effect on seedlings. Equation (11), based on the root length values of the sample and control, can be used to compute PI (Rusan et al. 2015).

$$\mathrm{PI} = 1 - \frac{\text{Root length of stress plant}}{\text{Root length of control plant}} \tag{11}$$

Statistical analysis

Using SPSS version 16, a two-way analysis of variance (ANOVA) was conducted to analyse the data from the phytotoxicity studies, which involved parameters related to two different types of antibiotics and five different crop species. ANOVA was used to assess the homogeneity of variances at a significance threshold of 5%. The factors analysed in this study were the antibiotic concentrations with and without the bacterial consortium. Various statistical tests, including t-tests, Tukey's multiple tests, Brown-Forsythe test, Bartlett's test, and the two-tailed p-value calculation, were used to compare the plant performance data (e.g., germination indices, stress tolerance indices, phytotoxicity indices) for treated and untreated samples using GraphPad Prism software. For specific comparisons between treatment groups (e.g., with vs. without bacteria at the same CIP concentration), post hoc tests were conducted. The threshold for statistical significance was set at $p < 0.05$. In figures, significant differences are indicated by asterisks (*): one * shows $p < 0.05$, ** shows $p < 0.01$, *** shows $p < 0.001$, and **** shows $p < 0.0001$.

The low variability observed within treatment groups can be attributed to stringent experimental controls. Seeds were selected for uniform size and viability and sterilised under aseptic conditions (Sect. "Seed germination experiment"). Growth conditions, including light intensity (2500–3500 lx m^{-2} s^{-1}), temperature (25 °C), and humidity, were tightly regulated in a controlled growth room (Sect. "Seed germination experiment"). All measurements of root/shoot length and biomass were conducted using standardised protocols (Sect. "Measurements of length and weight of plants"). Error bars represent the standard deviation derived from triplicate biological replicates, each comprising 20 seeds per treatment, ensuring reproducibility and minimising within-group variation.

Results and discussion

Enrichment, isolation, selection and identification of isolates

In the initial phase of the study, bacterial isolates were screened from municipal water for their resistance to the antibiotic CIP and their ability to grow in the presence of CIP on Minimal Salt Medium (MSM). For CIP screening, a concentration range of 5–175 mg L^{-1} was used. Forty-five isolates were obtained that showed growth in the presence of CIP. Among these isolates, two isolates (N6 and N23) exhibited strong resistance, showing increased growth at 175 mg L^{-1} of CIP. In contrast, isolates such as N12 showed moderate growth at higher CIP concentrations.

Based on BLAST analysis, it was determined in our previous published research (Mohy-u-Din et al. 2024) that isolate N6 belongs to the genus *Escherichia*, sharing 100% homology with *Escherichia coli*. Phylogenetic analysis revealed a close resemblance to the genus *Shigella* (99.81%) and *Streptomyces* (99.60%) (Mohy-u-Din et al. 2024). However, the species under study formed a distinct outgroup, highlighting its phylogenetic difference from the BLAST reference sequence. BLAST analysis determined that isolate N23 belongs to the genus *Enterococcus* sharing 100% homology with *Enterococcus faecium*, (Mohy-u-Din et al. 2024). Phylogenetic analysis confirmed a close relationship with *Enterococcus faecium* (Mohy-u-Din et al. 2024). However, the species under study formed a distinct outgroup, illustrating its phylogenetic difference from the BLAST reference sequence (Mohy-u-Din et al. 2024).

Biodegradation of Ciprofloxacin by bacterial consortium

To directly quantify the antibiotic removal capability of the isolated strains, the biodegradation potential of *E. coli* (N6) and *E. faecium* (N23) for CIP was evaluated under controlled conditions. The influence of key environmental factors, including temperature, pH, and inoculum density, was investigated. *E. faecium* (N23) demonstrated a remarkable capacity to degrade CIP, achieving near-complete removal (100%) under its optimal conditions of 40 °C and pH 6.5, with an inoculum density of 10^8 CFU mL^{-1}. Under its optimal conditions (37 °C, pH 6.5, inoculum density of 10^8 CFU mL^{-1}), *E. coli* (N6) also exhibited strong degradation, removing up to 90% of CIP.

The degradation kinetics were analysed to further elucidate the process efficiency. *E. faecium* exhibited a maximum degradation rate (V ~ max ~) of 8.26 mg/L/day for CIP, with a half-saturation constant (K ~ s ~) of 49.52 mg/L. For *E. coli*, the kinetic parameters for CIP were a V ~ max ~ of 6.37 mg/L/day and a K ~ s ~ of 31.91 mg/L. The higher V ~ max ~ value for *E. faecium* indicates a faster rate of CIP degradation compared to *E. coli*. It was observed that the degradation efficiency decreased with increasing initial antibiotic concentration for both strains. Furthermore, the process was highly

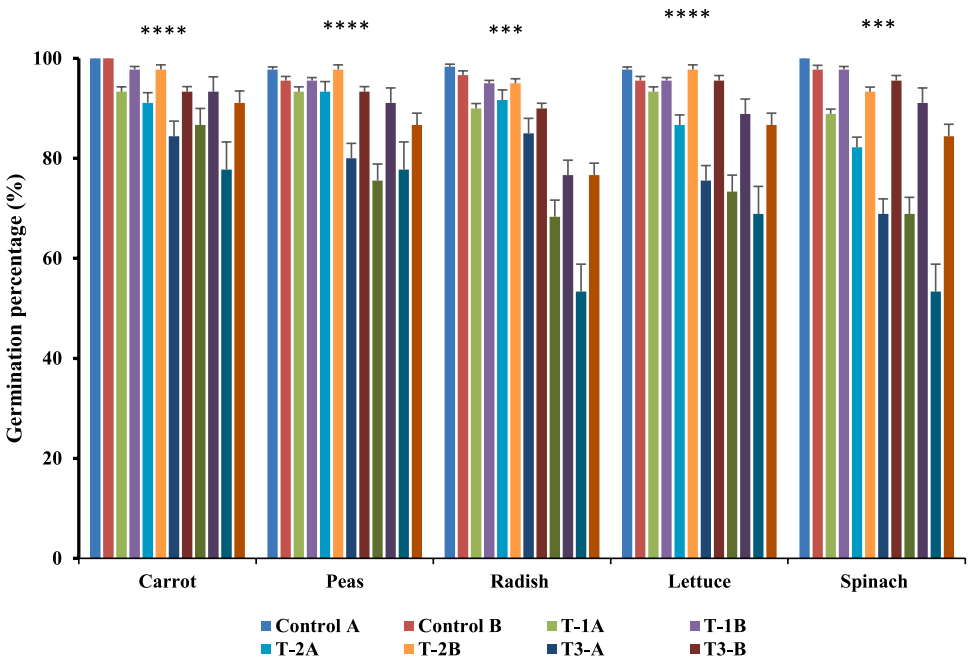


Fig. 2 Effect of ciprofloxacin (CIP) and bacterial consortium (CIP+B) on seed germination percentage in five edible crops. Seeds of carrot (*D. carota*), pea (*P. sativum*), radish (*R. raphanistrum*), lettuce (*L. sativa*), and spinach (*S. oleracea*) were germinated in Petri dishes containing CIP solutions (0.1–500 mg L^{-1}) with or without the bacterial consortium. Data represent mean ± SD (n = 3 replicates, 20 seeds per replicate). Asterisks indicate significant differences between CIP and CIP+B treatments at each concentration (***$p < 0.001$, ****$p < 0.0001$; two-way ANOVA with post hoc test)

dependent on temperature and pH, with significant reductions in efficiency observed outside the optimal ranges. These results provide direct analytical evidence that both *E. coli* (N6) and *E. faecium* (N23) possess a high inherent capability to degrade and remove CIP from solution, substantiating the hypothesis that their presence reduces antibiotic concentrations in the environment.

Phytotoxicity in the seed germination experiment

Germination percentage

For CIP, the percentage of seeds that germinated for each of the five crops ranged from 65 to 95% in those treatments containing the antibiotic CIP, and from 90 to 98% when combined with the bacterial consortium. *D. carota* (carrot), *L. sativa* (lettuce), and *R. raphanistrum* (radish) showed germination percentages (GPs) ranging from 90 to 100% across different concentrations of antibiotic-contaminated water (Fig. 2). Seeds of *S. oleracea* (spinach) and *P. sativum* (pea) showed a germination rate of 75% to 100% under all treatments (0.1,1, 10, 100, and 500 mg L^{-1}). All seed crops in this study thrived under the influence of antibiotic-treated bacterial consortia (as defined by Solomon 2013). For instance, seeds exhibited super-germination, with more than 90% germination percentage in laboratory conditions. Similarly, seeds with an 85% GP (excellent germination) in the lab are estimated to achieve a 50% GP rate in the field. On the other hand, seeds with germination rates of 75% or less in the lab (i.e., legal germination) are expected to exhibit at least 15% practical GP in agricultural settings. Germination is typically used as a metric to assess seed tolerance to toxins in studies examining the ability of crop species to germinate in the presence of contaminants (Bellino et al. 2018). The findings align with previous research (Bellino et al. 2018; Hillis et al. 2011; Pan And Chu 2016a, b; Gomes et al. 2019), which found no adverse effects on seed germination when exposed to various classes of antibiotics across multiple crops. This suggests that lettuce, spinach, radish, carrot, and peas are tolerant to CIP. However, other tests may reveal that such antibiotics can hinder germination or growth, as assessed by alternative formulas, even if they do not affect the germination rate.

Impact of CIP contamination on crop performance

Measurements of the fresh and dry weights, root lengths, and shoot lengths of all treated seeds, including *D. carota* (carrot), *P. sativum* (pea), *R. raphanistrum* (radish), *L. sativa* (lettuce), and *S. oleracea* (spinach) were taken after a week. Statistical analysis indicated significant differences in mean weights and lengths ($p < 0.0001$****, $p < 0.05$*, $p < 0.01$**, $p < 0.001$***). For each of the five crops, the effects of CIP under two treatments were compared for all seedling growth. The study compared (0.1, 1, 10, 100, 500 mg L^{-1}) with controls (distilled water, DW) and treatments with bacterial consortium (0.1 + B, 1 + B, 10 + B, 100 + B, 500 + B mg L^{-1}). Tables 2–5 list the controls: DW and no antibiotics with added consortium (0 mg L^{-1} + B).

The lengths of the seedlings (root and shoot) under CIP treatments were recorded for both control conditions. For

Table 2 Effect of the antibiotic ciprofloxacin (CIP) without (T-1A, T-2A, T-3A, T-4A, and T-5A) and with the bacterial consortium (T-1B, T-2B, T-3B, T-4B, and T-5B) on the seedling growth of *D. carota* (Carrot)

List of comparisons	Treatments	Root length (cm)	Shoot length	Seedling length	Root fresh weight (g)	Shoot fresh weight	Root dry weight	Shoot dry weight
	C-A (DW)	1.92 (±0.0004) *9 ****2–4,6,8,10,11	1.23 (±0.1029) **5 ****2,4,6,9,10	3.11 (±0.0007) ***3 ****1,2,4,6,8	0.015 (±0.0004) **10 ****6,7, 10	0.031 (±0.0002) ****2,3,5–11	0.007 (±0.0002) ***6,7	0.015 (±0.0003) ***6 ****7
1	C-B ($0mgL^{-1}$ + B)	1.88 (±0.0955) *9 ****1–3,5,7,9,10	1.22 (±0.0576) **4 ****1,3,5,7	3.11 (±0.0861) ***9 ****1–4,6–8	0.016 (±0.0001) **10 ****5,6	0.031 (±0.0002) ****1,2,3–10	0.008 (±0.0006) ***5,6	0.016 (±0.0001) ***5 ****6
2	T-1A ($0.1mgL^{-1}$)	1.42 (±0.0008) *9 **2 ****1,3,4–8	1.02 (±0.0098) **9 ***4 ****1,5,6,7,8	2.45 (±0.009) **2 ****1,3–9	(±0.0009) *7 **3 ****4,5	0.021 (±0.001) ****2,3,5–9	0.005 (±0.0005) *4,5	0.011 (±0.0011) *8 **4,5
3	T-1B ($0.1mgL^{-1}$ + B)	2.21 (±0.0856) ***2 ****1,3–8	1.19 (±0.0003) ***2 ****1,3,5,7	3.44 (±0.1002) ***4 ****1–3,5–8	0.011 (±0.0017) *6 **2 ****3,4	0.022 (±0.0006) ***4 ****1,2,5–8	0.007 (±0.0009)	0.0140 (±0.0008) *7 **3,4
4	T-2A ($1mgL^{-1}$)	1.27 (±0.0029) ****1–6	0.97 (±0.0064) *1 **2 ****3–7	2.25 (±0.0051) ****1–8	0.016 (±0.0025) **6 ****2,3	0.031 (±0.0016) ****1–7	0.008 (±0.0012) ***2,3	0.008 (±0.0012) ****2,3
5	T-2B ($1mgL^{-1}$ + B)	2.01 (±0.0816) ****1,3–6	1.08 (±0.0082) **2 ****1,3–5	3.09 (±0.0845) ****1,3,5,6	0.021 (±0.003) *3 **6 ****1,2,5	0.037 (±0.0016) ****1–6	0.009 (±0.0011) **1 ***2	0.009 (±0.0011) ****1,2
6	T-3A ($10mgL^{-1}$)	0.99 (±0.0004) ****1–5	0.86 (±0.01) ***2 ****1,3–5	1.85 (±0.0104) *4 ****1–3	0.012 (±0.0006) ****2–5	0.023 (±0.0007) *1 ****2–4	0.005 (±0.0003) ***2–5	0.004(±0.0009) ****2–5
7	T-3B ($10mgL^{-1}$ + B)	1.94 (±0.0165) **2 ****1,3,4	1.2 (±0.0072) ****1,3	3.15 (±0.0098) ****1,3,4	0.015 (±0.0005) ****1–4	0.008 (±0.0002) ****1–4	0.008 (±0.0002) ***1,2,4	0.014 (±0.0006) ****1–4
8	T-4A ($100mgL^{-1}$)	0.64 (±0.0002) ****1,3	0.72 (±0.0062) ****1,3	1.36 (±0.0059) ****1–3	0.013 (±0.0007)	0.006 (±0.0003)	0.006 (±0.0003) ****3	0.006 (±0.0003)
9	T-4B ($100mgL^{-1}$ + B)	1.78 (±1.7873) ****1,2	1.25 (±1.2544) *2 ****1	3.04 (±3.0418) ****1,2	0.018 (±0.0186) *2 ***1	0.008 (±0.0085)	0.008 (±0.0084)	0.008 (±0.0084)
10	T-5A ($500mgL^{-1}$)	0.54 (±0.5405) ****1	0.63 (±0.6317) ****1	1.17 (±1.1722) **1	0.006 (±0.0063)	0.007 (±0.0075)	0.003 (±0.0031)	0.003 (±0.0031)
11	T-5B ($500mgL^{-1}$ + B)	1.28 (±0.0578)	1.14 (±0.0154)	2.43 (±0.0157)	0.01 (±0.0051)	0.008 (±0.0003)	0.005 (±0.0025)	0.005 (±0.0025)
F- value		269.6	563.5	125.9	481.5	438.4	463	9.632

C-A = Control A; C-B = Control B; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$

D. carota (carrot) seedlings, root lengths were longer in both control treatments, DW (1.92 cm) and 0 mg L^{-1} + B (1.88 cm), compared to all treatments with and without bacterial consortia (Table 2). Shoot lengths were consistent across DW (1.23 cm) and 0 mg L^{-1} + B (1.23 cm) treatments. Seedling lengths were longer in bacterial consortium-treated water (root: 2.22, 2.02, 1.95, 1.79, and 1.29 cm; shoot: 1.20, 1.08, 1.20, 1.25 and 1.15 cm) compared to those in contaminated water (root: 1.43, 1.28, 0.99, 0.65, and 0.54 cm; shoot: 1.03, 0.98, 0.86, 0.72, and 0.63 cm). Additionally, root and shoot weights of *D. carota (*carrot) were higher in bacterial consortium-treated water.

For *P. sativum* (pea), seedling lengths were longer in bacterial consortium treatments (root: 3.96, 3.89, 3.84, 3.80 and 2.72 cm; shoot: 3.02, 3.08, 2.95, 2.83 and 1.85 cm) than those in contaminated water (root: 3.65, 3.42, 3.14, 2.93 and 1.92 cm; shoot: 2.92, 2.76, 2.55, 2.16 and 1.43 cm) (Table 3). Similarly, shoot and root weights were higher for *P. sativum* (pea) in treated water compared to contaminated water (root dry weight: 0.021, 0.020, 0.020, 0.017, and 0.011 g; shoot fresh weight: 0.032, 0.030, 0.018, 0.009 and 0.006 g; shoot dry weight: 0.020, 0.015, 0.013, 0.006, and 0.003).

The control treatments, DW and 0 mg L^{-1} + B, resulted in longer seedlings (root: 4.13 cm; shoot: 3.84 cm) and (root: 4.17 cm; shoot: 3.87 cm), respectively, when compared with the lengths of *R. raphanistrum* (Radish) seedlings under CIP across all treatments. Seedlings in the bacterial consortium treatment exhibited longer lengths (root: 3.72, 3.23, 3.07, 2.84, and 2.61 cm; shoot: 3.17, 2.22, 1.15, 0.97, and 0.84 cm) compared to those in contaminated water. Root and shoot weights of *R. raphanistrum* also increased at higher concentrations of the antibiotic in treated water (root fresh weight: 0.0404, 0.0478, 0.0719, 0.0127, and 0.0158 g; root dry weight: 0.0253, 0.0245) (Table 4).

The seedling length of *L. sativa* (lettuce) under CIP in both control treatments—DW and 0 mg L^{-1} + B—was longer (root: 8.58 cm; shoot: 4.53 cm) and (root: 8.48 cm; shoot: 4.53 cm) compared to all treatments, with and without bacterial consortia (Table 5). Seedling lengths increased with the application of the bacterial consortium (root: 8.92, 8.80, 7.65, 6.83, and 4.98 cm; shoot: 3.29, 2.36, 4.01, 3.15, and 3.03 cm) compared to those from contaminated water (root: 7.61, 6.41, 6.19, 5.74, and 3.96 cm; shoot: 4.27, 3.44, 3.31, 3.20, and 2.94 cm). Similarly, it was demonstrated that *L. sativa* roots and shoots had greater weights after treatment with the bacterial consortium.

The lengths of *S. oleracea* (Spinach) seedlings under CIP in the bacterial consortium treatment were greater (root: 1.32, 1.31, 1.27, 1.13, and 1.12 cm; shoot: 1.20, 0.98, 0.67, 0.81, and 0.66 cm) than those at the same concentrations of contaminated water (root: 1.55, 1.63, 2.09, 1.44, and 1.14 cm; shoot: 1.35, 1.31, 0.82, 1.10, and 0.81 cm) (Table 6). The study revealed that the root and shoot weights (both fresh and dry) of *S. oleracea* (Spinach) were higher in bacterially treated contaminated water (root fresh weight: 0.0291, 0.0284, 0.0278, 0.0275, and 0.0261 g; root dry weight: 0.0135, 0.0298, 0.0113, 0.0110, and 0.0114 g; shoot fresh weight: 0.0308, 0.1196, 0.0278, 0.0314, and 0.0284 g; shoot dry weight: 0.0218, 0.0202, 0.0162, 0.0159, and 0.0142) than in contaminated water.

The effect of treatments without bacteria and with bacteria added (0 mg L^{-1}, 0.1 mg L^{-1}, 1 mg L^{-1}, 10 mg L^{-1}, 100 mg L^{-1} and 500 mg L^{-1}) was compared in terms of seedling growth for the crops studied. Introducing bacteria to the treatments resulted in greater seedling lengths (both root and shoot) compared to treatments without bacteria. A similar trend was observed in seedling biomass (both fresh and dry weight), which decreased under antibiotic stress but increased when bacteria were added to the antibiotic treatments. Thus, the study demonstrated the beneficial impact of bacterial consortia on seedling length and biomass in the presence of antibiotics. Previous studies have also demonstrated a reduction in seedling biomass and length after exposure to hospital water containing antibiotic concentrations (Bao et al. 2022).

To assess each crop's stress tolerance potential, STIs under specific treatments were compared. It was observed that all seeds exhibited higher STI values when irrigated with biologically treated water (with consortia) compared to contaminated water (without consortia). High STI values indicate greater crop resilience when exposed to stress (Zvobgo et al. 2018). Therefore, crops irrigated with biotreated water demonstrate robust growth and stress tolerance. Seeds grown in antibiotic solutions showed lower SVIs. The enhanced SVIs of all agricultural seeds germinated in treated water with added bacterial consortia indicate more dynamic growth in this scenario (Amin et al. 2013).

Germination index

The GI values of *D. carota* (carrot), *P. sativum* (peas), *R. raphanistrum* (radish), *L. sativa* (lettuce), and *S. oleracea* (spinach) increased in treatments involving the bacterial consortium as compared to those growing in different antibiotic concentrations. Adding a bacterial consortium with the same amount of antibiotics resulted in an increase in GI. However, higher GI values were noted in seeds grown in antibiotics with bacterial consortia, indicating a significant reduction in antibiotic concentrations after the treatment that promoted an increase in both RSG and RRG.

For *D. carota* (carrots) grown under various CIP conditions with a bacterial consortium, the GI value increased by 93–120 and 56–199. *P. sativum* (pea) cultivated in various antibiotic concentrations with bacterial consortia showed a GI value ranging from 10 to 56 for CIP, with the highest

Table 3 Effect of the antibiotic ciprofloxacin (CIP) without (T-1A, T-2A, T-3A, T-4A, and T-5A) and with the bacterial consortium (T-1B, T-2B, T-3B, T-4B, and T-5B) on the seedling growth of *P. sativum* (pea)

List of comparisons	Treatments	Root length (cm)		Shoot length	Seedling length	Root fresh weight (g)	Shoot fresh weight	Root dry weight	Shoot dry weight
	C-A (DW)	3.9 (+0.0422) ***4 ****6,8,10,11	3.9 (±0.0422) **5 ****2,3,4,6–11		3.14 (±0.0193) **3,5 ****2,4,6,8–11	7.05 (±0.0278) **2,4 ****1,3,5,7–10	0.037 (±0.0009) **2 ****3,5–11	0.024 (±0.0003) **4,5,9 ****3,6,8,10,11	0.018 (±0.0004) **3,9 ***8,10,11
1	C-B ($0mgL^{-1}$ + B)	3.97 (+0.0149) ***3 ****5,7,9,10	3.97 (±0.0149) **4 ****1,2,3,5–10		3.21 (±0.0358) *4 **2 ****1,3,5,7–10	7.08 (±0.0321)	0.037 (±0.0004) **1 ****2,4–10	0.024 (±0.0004) **2 ****3,5,7–10	0.019 (±0.0004) *2,5 **8 ***9,10 ****7
2	T-1A ($0.1mgL^{-1}$)	3.65 (+0.0333) ***4 ****6,8,9	3.65 (±0.0333) ***7 ****1–4,6,8,9		2.91 (±0.0049) **8,9 ****1,3,5	6.56 (±0.0378) *9 **8 ****1,3,5	0.031 (±0.0002) **1 ****4–9	0.028 (±0.0007) *3 **7 ***1,2 ****4,6,8,9	0.018 (±0.0003) **4 ***7 ****6,8,9
3	T-1B ($0.1mgL^{-1}$ + B)	3.95 (+0.0359) ***1 ****3,5,7,8	3.95 (±0.0359) *2,4 ****1,3,5–8		3.01 (±0.0006) ****1,3,5–8	6.97 (±0.0354) ****1,3,5–8	0.041 (±0.0017) ****1,3–8	0.031 (±0.0023) ****1,3,5–8	0.021 (±0.0006) *4 **1 ****3,5–8
4	T-2A ($1mgL^{-1}$)	3.41 (+0.0064) *5 **1,3 ***4 ****6,7	3.41 (±0.0064) **5 ****1–4,6,7		2.76 (±0.0195) *6,7 ****1,3	6.18 (±0.014) *6,7 ****1,3	0.03 (±0.0003 **1 ****2–7	0.026 (±0.0003) **2 ****1,3,4,6,7	0.016 (±0.0003) **5 ***6,7 ****4
5	T-2B ($1mgL^{-1}$ + B)	3.88 (+0.0087) ****1,3,5,6	3.88 (±0.0087) ****1–6		3.08 (±0.0225) ****1,3–6	6.96 (±0.0312) ****1,3–6	0.041 (±0.0022) ****1–6	0.029 (±0.0015) ****1,3–6	0.02 (±0.0006) ***1 ****3–5
6	T-3A ($10mgL^{-1}$)	3.14 (±0.022) **5 ****1,3,4	3.14 (±0.022) ****1–5		2.55 (±0.0082) *5 **4 ****1	5.69 (±0.0273) *4,5 ****1	0.03 (±0.0009) **1 ****2–5	0.014 (±0.0006) *2 **3 ****1,4,5	0.014 (±0.0004) *1
7	T-3B ($10mgL^{-1}$ + B)	3.83 (±0.0557) ****1,3,4	3.83 (±0.0557) ****1–4		2.95 (±0.0396) ****1–4	6.79 (±0.0748) ****4	0.039 (±0.0013) ****1–4	0.018 (±0.0007) ****4	0.019 (±0.0007) **2 ****1,3,4
8	T-4A ($100mgL^{-1}$)	2.92 (±0.0208) ***1,2	2.92 (±0.0208) ****1–3		2.15 (±0.0114)	5.08 (±0.0106)	0.028 (±0.0002) *1	0.005 (±0.0006) *3 ***2	0.012 (±0.0003)
9	T-4B ($100mgL^{-1}$ + B)	3.79 (±0.0013) ****1,2	3.79 (±0.0013) ****1,2		2.83 (±0.0149)	6.62 (±0.0154)	0.029 (±0.0006) **2	0.009 (±0.0007) ****2	0.016 (±0.0007)
10	T-5A ($500mgL^{-1}$)	1.92 (±0.0346) ****1	1.92 (±0.0346) ****1		1.42 (±0.0112)	3.35 (±0.0243)	0.027 (±0.0003)	0.006 (±0.0008)	0.01 (±0.0004)

Table 3 (continued)

List of comparisons	Treatments	Root length (cm)	Shoot length	Seedling length	Root fresh weight (g)	Shoot fresh weight	Root dry weight	Shoot dry weight
	C-A (DW)	3.9 (+0.0422) ***4 ****6,8,10,11	3.9 (± 0.0422) **5 ****2,3,4,6–11	3.14 (±0.0193) **3,5 ****2,4,6,8–11	7.05 (±0.0278) **2,4 ****1,3,5,7–10	0.037 (±0.0009) **2 ****3,5–11	0.024 (± 0.0003) **4,5,9 ****3,6,8,10,11	0.018 (±0.0004) **3,9 ***8,10,11
11	T-5B (500mgL^{-1}+B)	2.72 (± 0.3707)	2.72 (± 0.3707)	1.85 (±0.0171)	4.57 (± 0.3654)	0.027 (±0.0004)	0.005 (± 0.0003)	0.011 (±0.0005)
F- value		96.46	2732	76.61	74.34	315.7	152.5	25.56

C-A = Control A; C-B = Control B; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$

GI value noted at 100 mg L^{-1} (T-4B) for CIP. *R. raphanistrum* (radish) cultivated in various antibiotic doses with a bacterial consortium exhibited GI value ranging from 9 to 103 for CIP with a higher GI value observed at 500 mg L^{-1} (T-5B) (Fig. 3). *L. sativa* (lettuce) grown with a bacterial consortium and varying antibiotic concentrations showed a GI value ranging from 17 to 41 for CIP. *S. oleracea* (spinach) cultivated in various antibiotic concentrations with a bacterial consortium exhibited GI values ranging from 26 to 54 for CIP, with a higher GI value noted at 500 mg L^{-1} (T-4B) for CIP (Fig. 3).

The germination index, relative seed germination, and relative root growth contribute to PI, with root growth and germination index being more sensitive indicators (Rashid et al. 2021). All crop seeds in this study exhibited statistically significant PI values across various treatments. High PI values observed in seeds sown in contaminated water suggest a toxic or adverse effect. Seed coats traditionally shield embryos from the harmful effects of environmental contaminants such as antibiotics (An et al. 2009; Hillis et al. 2011; Rede et al. 2019). Seeds grown with bacterial consortia displayed the lowest PI values, indicating reduced antibiotic impact. Conversely, PI values were higher at higher antibiotic concentrations.

Stress tolerance indexes

Stress tolerance indices (STIs) (RLSTI, SLSTI, RFSTI, SFSTI, RDSTI, and SDSTI) were used to analyse each of the five crops for CIP: *D. carota* (carrot), *P. sativum* (pea), *R. raphanistrum* (radish), *L. sativa* (lettuce), and *S. oleracea* (spinach). These STIs were compared between antibiotic treatments without the bacterial consortium (T-1A, T-2A, T-3A, T-4A, and T-5A) and with bacterial consortium (T-1B, T-2B, T-3B, T-4B, and T-5B) (Table 7–11). It was observed that the STI values for antibiotic treatments in each crop treated without a bacterial consortium were lower compared to the values of STI values for antibiotic treatments in all targeted crops with a bacterial consortium. As antibiotic concentrations increased in both antibiotic treatments, the stress indices were found to decrease. The statistical F-value indicates a significant difference in the means of all the indices.

For *D. carota* (carrot), the RLSTI values for CIP were 74.18, 66.46, 51.73, 33.59, and 28.130, respectively (Table 7). When the bacterial consortium was introduced at the same concentrations, these values increased to 115.38, 104.95, 101.43, 93.02, and 66.91. Similarly, the SLSTI values increased to 103.09, 90.49, 100.64, 104.90, and 95.89 with the bacterial consortium under CIP treatments. It was also observed that the RFSTI, SFSTI, RDSTI, and SDSTI values increased when the bacterial consortium was involved in antibiotic treatments.

Table 4 Effect of the antibiotic ciprofloxacin (CIP) without (T-1A, T-2A, T-3A, T-4A and T-5A) and with bacterial consortium (T-1B, T-2, T-3B, T-4B and T-5B) on seedling growth of *R. raphanistrum* (Radish)

List of comparisons	Treatments	Root length (cm)	Shoot length	Seedling length	Root fresh weight (g)	Shoot fresh weight	Root dry weight	Shoot dry weight
	C-A (DW)	4.13 (±0.0007) ***4 ****5,7,9	3.83 (±0.0029) ****1,3,4	7.97 (±0.0036) ***2,5 ****4,6–11	0.042 (±0.0003) **6,9,11 ***7, 8, 10	0.157 (±0.0006) **3 ****2, 4–11	0.026 (±0.0037) ***2 ****6–11	0.056 (±0.0015) *3 ****2, 4–11
1	C-B (0mgL-1 + B)	4.16 (±0.1162) ***3 ****5,7,9	3.86 (±0.1159) ***2 ****1,3–10	7.96 (±0.0064) ***1,4 ****3,5–10	0.041 (±0.0007) **8, 10 ****5, 6, 7,9	0.156 (±0.0002) ***2 ****1, 3–10	0.025 (±0.0034) **1, 3, 9 ***6–8 ****4–5	0.055 (±0.0015) ***2 ****1, 3–10
2	T-1A (0.1mgL-1)	3.72 (±0.018) *4,7 ***6 ****8	3.16 (±0.0402) ****1–9	6.89 (±0.0332) *1 ****2,4–9	0.038 (±0.0008) *7 **6, 8–9 ***4 ****5	0.141 (±0.0008) ****1–9	0.016 (±0.0036) ****3–9	0.069 (±0.0005) ****1–9
3	T-1B (0.1mgL-1 + B)	4.28 (±0.0216) ****1,3,5,7	3.54 (±0.1588) ****1–8	7.6 (±0.1344) ****1,3–8	0.04 (±0.0013) *6 **7,8 ***3–5	0.159 (±0.0005) ****1–8	0.025 (±0.0023) ****2–8	0.059 (±0.001) ****1–8
4	T-2A (1mgL-1)	3.23 (±0.018) *6 **7 ***1 ****3,5	2.22 (±0.0589) ****1–7	5.45 (±0.068) ****1,2,4,6	0.044 (±0.0015) **2, 5 ***3, 4, 6, 7	0.021 (±0.0015) ***1, 5 ****2–4, 6, 7	0.022 (±0.001) ****1–6	0.006 (±0.0002) *2 **7 ****3
5	T-2B (1mgL-1 + B)	4.24 (±0.0484)	2.88 (±0.0315) ****1–6	6.99 (±0.1264) ****1–6	0.047 (±0.002) *1 **2 ***4 ****3, 5, 6	0.024 (±0.0004) ****1–6	0.024 (±0.0009) ****2–5	0.007 (±0.0004) **2
6	T-3A (10mgL-1)	3.06 (±0.0694)	1.14 (±0.058) *2 ***4	4.21 (±0.1275) **4,5 ****1–3	0.069 (±0.0015) ****2–5	0.014 (±0.0005) *1, 4 **3	0.036 (±0.0019) ****1–4	0.008 (±0.0007)
7	T-3B (10mgL-1 + B)	4.26 (±0.12)	1.16 (±0.0845) *1 ****3	5.42 (±0.1513) ****1,3	0.071 (±0.0022) ****1–4	0.016 (±0.0004) *1 ****3	0.04 (±0.0006)	0.011 (±0.0009) *2 **1 ***3
8	T-4A (100mgL-1)	2.83 (±0.1205)	0.96 (±0.0405)	3.97 (±0.1257) ***3 ****1	0.012 (±0.0108)	0.014 (±0.0004) *2 **1	0.006 (±0.0003)	0.007 (±0.0002)
9	T-4B (100mgL-1 + B)	4.33 (±0.1476)	1.13 (±0.0117) ***1	5.3 (±0.1828) ****1	0.019 (±0.0183)	0.017 (±0.0007) **2 ****1	0.007 (±0.0004)	0.008 (±0.0005)
10	T-5A (500mgL-1)	2.6 (±0.3133)	0.85 (±0.0226) *1	3.45 (±0.2936) ****1	0.013 (±0.0002)	0.012 (±0.0003) *1	0.006 (±0.0002)	0.007 (±0.0003)
11	T-5B (500mgL-1 + B)	4 (±0.5703)	1.04 (±0.0243)	5.04 (±0.5694)	0.015 (±0.0007)	0.014 (±0.0005)	0.007 (±0.0005)	0.009 (±0.0004)
F- value		269.6	29.6	1208	167.9	30.43	28,446	97.21

C-A = Control A; C-B = Control B; * = $p < 0.05$; **= $p < 0.01$; ***= $p < 0.001$; ****= $p < 0.0001$

For *P. sativum* (pea), without the bacterial consortium, the RLSTI values were 93.54, 87.51, 80.44, 74.94, and 49.25 (Table 8). When the bacterial consortium was added at the same concentrations, these was observed that the values increased (101.38, 99.58, 98.32, 97.24, and 69.74). Under CIP treatments, the values of SLSTI were also increased (95.79, 97.85, 93.77, 89.97, and 58.78). Additionally, when the bacterial consortium was added to CIP antibiotic

Table 5 Effect of the antibiotic ciprofloxacin (CIP) without (T-1A, T-2A, T-3A, T-4A, and T-5A) and with the bacterial consortium (T-1B, T-2B, T-3B, T-4B and T-5B) on the seedling growth of *L. sativa* (lettuce)

List of comparisons	Treatments	Root length (cm)	Shoot length	Seedling length	Root fresh weight (g)	Shoot fresh weight	Root dry weight	Shoot dry weight
	C-A (DW)	8.57 (±0.1002) **2 ****1,4,6,8,10, 11	4.52 (±0.0959) **4 ***3, 6 ****1, 5,	13.1 (±0.0081) ****1–11	0.009 (±0.0004) **3 ****1, 2, 4, 6, 8, 10, 11	0.005 (±0.0001) ****1	0.003 (±0.0002) *5, ***11 ****1, 2, 4, 6, 8, 10	0.002 (±0.0001) ***2 ****1, 4 6–11
1	C-B ($0mgL^{-1}$ + B)	8.47 (±0.1) ****1–10	4.62 (±0.0944)	13.1 (±0.0095) ****1–10	0.009 (±0.0002) ****1–10	0.004 (±0.0003) ****1–10	0.003 (±0.0005) ****1–10	0.002 (±0.0003) ****1–10
2	T-1A ($0.1mgL^{-1}$)	7.61 (±0.0352) **5 ***2, 3, 7 ****1, 4, 6, 8–9	4.27 (±0.0574)	11.88 (±0.0248) ***5 ****1–4, 6–9	0.005 (±0.0003) *4 ***9 ****1, 3, 5, 7	0.002 (±0.0004)	0.001 (±0.0001) **9 ****1, 3, 5, 7	0.001 (±0.0001) ****1
3	T-1B ($0.1mgL^{-1}$ + B)	8.91 (±0.0391) ****1, 3, 5, 7, 8	3.28 (±0.1308)	12.2 (±0.1018) ****1–8	0.008 (±0.0006) **2, 4 ****1, 3, 5, 7, 8	0.004 (±0.0005)	0.003 (±0.0001) **6 ****1–3, 5, 7	0.003 (±0.0001) **2 ****1, 3–8
4	T-2A ($1mgL^{-1}$)	6.4 (±0.0521) ****1,3,5,6	3.43 (±0.0271)	9.84 (±0.0406) ****1–4, 6–7	0.005 (±0.0002) **6 ****1, 3, 5	0.001 (±0.0005)	0.001 (±0.0001) *7 ****1, 3, 5	0.001 (±0.0002) **1
5	T-2B ($1mgL^{-1}$ + B)	8.79 (±0.0952) ****1,3, 5,6	2.35 (±0.14)	11.15 (±0.0454) ****1–6	0.009 (±0.0002) ****1, 3, 5, 6	0.004 (±0.0008)	0.004 (±0.0001) ***2 ****1, 3, 5, 6	0.001 (±0.0004) *4 **5 ***1 ****5
6	T-3A ($10mgL^{-1}$)	6.18 (±0.011) ****1,3–4	3.3 (±0.0109)	9.49 (±0.0068) ****1–5	0.004 (±0.0002) ***5 ****1, 3	0.001 (±0.0006)	0.001 (±0.0002) ***5 ****1, 3	0.001 (±0.0003)
7	T-3B ($10mgL^{-1}$ + B)	7.64 (±0.1392) ****1, 3, 4	4.01 (±0.1632)	11.65 (±0.0278) ****1–4	0.009 (±0.0002) ****1, 3, 4	0.002 (±0.0004)	0.003 (±0.0003) *2, 4 ****1, 3	0.001 (±0.0006)
8	T-4A ($100mgL^{-1}$)	5.74 (±0.016) ****1–2	3.19 (±0.597)	8.68 (±0.0527) ****1–3	0.004 (±0.0004) **3 ****1	0.001 (±0.0002)	0.001 (±0.0002) *3 ****1	0 (±0.0001)
9	T-4B ($100mgL^{-1}$ + B)	6.82 (±0.0228) ****1–2	3.15 (±0.5884)	9.85 (±0.0908) ****1–2	0.009 (±0.0002) ****1–2	0.003 (±0.0002)	0.004 (±0.0004) ****1, 2	0.001 (±0.0001)
10	T-5A ($500mgL^{-1}$)	3.96 (±0.5762) ****1	2.93 (±0.058)	7.11 (±0.0131) ****1	0.004 (±0.0002) ****1	0 (±0.0002)	0.001 (±0.0002) **1	0 (±0.0001)
11	T-5B ($500mgL^{-1}$ + B)	4.98 (±0.626)	3.02 (±0.0709)	8.17 (±0.0696)	0.006 (±0.0001)	0.001 (±0.0003)	0.002 (±0.0001)	0.001 (±0.0002)
F- value		269.6	29.6	252.7	39.34	7387	1136	25.88

C-A = Control A; C-B = Control B; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$

treatments, it was noted that the values for RFSTI, SFSTI, RDSTI, and SDSTI also increased across the treatments. The overall increase in STIs for *P. sativum* (pea) seeds indicates that the bacterial consortium mitigates the effects of antibiotics while promoting the efficacy of the bioremediation process for plant growth.

For *R. raphanistrum* (radish), the values of RLSTI in the CIP treatments without the bacterial consortia were

Table 6 Effect of the antibiotic ciprofloxacin (CIP) without (T-1A, T-2A, T-3A, T-4A, and T-5A) and with the bacterial consortium (T-1B, T-2B, T-3B, T-4B, and T-5B) on the seedling growth of *S. oleracea* (spinach)

List of comparisons	Treatments	Root length (cm)	Shoot length	Seedling length	Root fresh weight (g)	Shoot fresh weight	Root dry weight	Shoot dry weight
	C-A (DW)	8.57 (±0.1002) **2 ****1,4,6,8,10, 11	4.52 (±0.0959) **4 ***3, 6 ****1, 5,	13.1 (±0.0081) ****1–11	0.009 (±0.0004) **3 ****1, 2, 4, 6, 8, 10, 11	0.005 (±0.0001) ****1	0.003 (±0.0002) *5 ***11 ****1, 2, 4, 6, 8, 10	0.002 (±0.0001) ***2 ****1, 4 6–11
1	C-B ($0mgL^{-1}$ + B)	8.47 (±0.1) ****1–10	4.62 (±0.0944)	13.1 (±0.0095) ****1–10	0.009 (±0.0002) ****1–10	0.004 (±0.0003) ****1–10	0.003 (±0.0005) ****1–10	0.002 (±0.0003) ****1–10
2	T-1A ($0.1mgL^{-1}$)	7.61 (±0.0352) **5 ***2, 3, 7 ****1, 4, 6, 8–9	4.27 (±0.0574)	11.88 (±0.0248) ***5 ****1–4, 6–9	0.005 (±0.0003) *4 ***9 ****1, 3, 5, 7	0.002 (±0.0004)	0.001 (±0.0001) **9 ****1, 3, 5, 7	0.001 (±0.0001) ****1
3	T-1B ($0.1mgL^{-1}$ + B)	8.91 (±0.0391) ****1, 3, 5, 7, 8	3.28 (±0.1308)	12.2 (±0.1018) ****1–8	0.008 (±0.0006) **2, 4 ****1, 3, 5, 7, 8	0.004 (±0.0005)	0.003 (±0.0001) **6 ****1–3, 5, 7	0.003 (±0.0001) **2 ****1, 3–8
4	T-2A ($1mgL^{-1}$)	6.4 (±0.0521) ****1,3,5,6	3.43 (±0.0271)	9.84 (±0.0406) ****1–4, 6–7	0.005 (±0.0002) **6 ****1, 3, 5	0.001 (±0.0005)	0.001 (±0.0001) *7 ****1, 3, 5	0.001 (±0.0002) **1
5	T-2B ($1mgL^{-1}$ + B)	8.79 (±0.0952) ****1,3, 5,6	2.35 (±0.14)	11.15 (±0.0454) ****1–6	0.009 (±0.0002) ****1, 3, 5, 6	0.004 (±0.0008)	0.004 (±0.0001) ***2 ****1, 3, 5, 6	0.001 (±0.0004) *4 **5 ***1 ****5
6	T-3A ($10mgL^{-1}$)	6.18 (±0.011) ****1,3–4	3.3 (±0.0109)	9.49 (±0.0068) ****1–5	0.004 (±0.0002) ***5 ****1, 3	0.001 (±0.0006)	0.001 (±0.0002) ***5 ****1, 3	0.001 (±0.0003)
7	T-3B ($10mgL^{-1}$ + B)	7.64 (±0.1392) ****1, 3, 4	4.01 (±0.1632)	11.65 (±0.0278) ****1–4	0.009 (±0.0002) ****1, 3, 4	0.002 (±0.0004)	0.003 (±0.0003) *2, 4 ****1, 3	0.001 (±0.0006)
8	T-4A ($100mgL^{-1}$)	5.74 (±0.016) ****1–2	3.19 (±0.597)	8.68 (±0.0527) ****1–3	0.004 (±0.0004) **3 ****1	0.001 (±0.0002)	0.001 (±0.0002) *3 ****1	0 (±0.0001)
9	T-4B ($100mgL^{-1}$ + B)	6.82 (±0.0228) ****1–2	3.15 (±0.5884)	9.85 (±0.0908) ****1–2	0.009 (±0.0002) ****1–2	0.003 (±0.0002)	0.004 (±0.0004) ****1, 2	0.001 (±0.0001)
10	T-5A ($500mgL^{-1}$)	3.96 (±0.5762) ****1	2.93 (±0.058)	7.11 (±0.0131) ****1	0.004 (±0.0002) ****1	0 (±0.0002)	0.001 (±0.0002) **1	0 (±0.0001)
11	T-5B ($500mgL^{-1}$ + B)	4.98 (±0.626)	3.02 (±0.0709)	8.17 (±0.0696)	0.006 (±0.0001)	0.001 (±0.0003)	0.002 (±0.0001)	0.001 (±0.0002)
F- value		269.6	252.7	39.34	7387	1136	25.88	337.4

C-A = Control A; C-B = Control B; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$

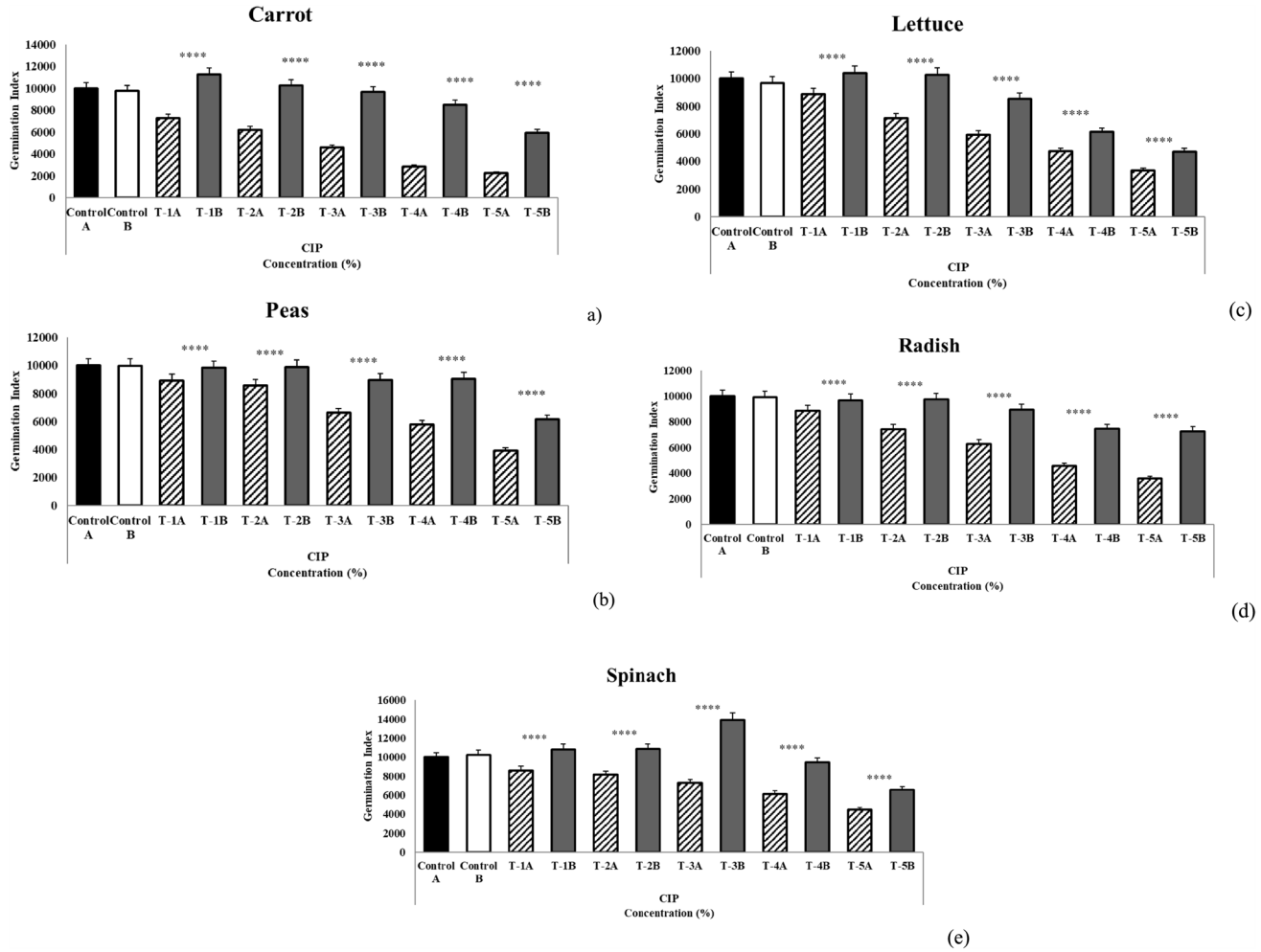


Fig. 3 Germination index of **a** carrot, **b** pea, **c** radish, **d** lettuce, and **e**) spinach under ciprofloxacin (CIP) alone and CIP with bacterial consortium (CIP + B). GI was calculated as (relative seed germination × relative root growth)/100. Data are means ± SD (n = 3). Treatments: T-1 to T-5 represent CIP concentrations of 0.1, 1, 10, 100, and 500 mg L^{-1}, respectively. Control "A" denotes CIP alone; "B" denotes CIP with bacterial consortium. (Asterisks denote significant differences between CIP and CIP + B at the same concentration (**$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; two-way ANOVA with post hoc test)

90.02, 78.19, 74.14, 68.64, and 63.09. However, when the bacterial consortium was introduced at the same concentrations, the values increased (103.76, 102.65, 103.01, 104.92, and 96.92) (Table 9). Under CIP conditions, the values of SLSTI also increased in the presence of the bacterial consortium (92.35, 75.04, 30.34, 29.67, and 27.10). Additionally, when the bacterial consortium was added to CIP antibiotic treatments, the values for RFSTI, SFSTI, RDSTI, and SDSTI also increased. The overall increase in STIs for *R. raphanistrum* (radish) seeds indicates that the bacterial consortium mitigates the impacts of antibiotics while enhancing the effectiveness of the bioremediation process for plant growth.

For *L. sativa* (lettuce), the values of RLSTI in treatments without the bacterial consortium were 88.74, 74.72, 72.11, 66.92, and 46.24 (Table 10). However, when the bacterial consortium was introduced at the same doses, the values increased (103.94, 102.55, 89.16, 79.58, and 58.13). Under CIP treatments, the values of SLSTI also increased when the bacterial consortium was added (72.63, 52.02, 88.61, 66.91, and 70.81). Moreover, the addition of the bacterial consortium to antibiotic treatments resulted in increased values for RFSTI, SFSTI, RDSTI, and SDSTI. The overall increase in STIs for *L. sativa* (lettuce) seeds indicates that the bacterial consortium mitigates the impacts of antibiotics while enhancing the effectiveness of the bioremediation process for plant growth. The significant increase in these indices suggests that bioremediation can improve seed growth in stressful environments.

For *S. oleracea* (spinach), RLSTI values in treatments without the bacterial consortium were determined to be 94.64, 94.14, 91.21, 81.21, and 80.28. However, when the

Table 7 Stress tolerance indices of *D. carota* (carrot) under application of the antibiotic ciprofloxacin (CIP) without (T-1A, T-2A, T-3A, T-4A, and T-5A) and with the bacterial consortium (T-1B, T-2B, T-3B, T-4B, and T-5B)

List of comparisons	Treatments	RLSTI	SLSTI	RFSTI	SFSTI	RDSTI	SDSTI
	C-B 0mgL^{-1} + B	97.91 (±4.95) *9 ****2–4,6,8,10,11	102.81 (±4.85) **5 ****2,4,6,9,10	103.64 (±2.7) **10 ****6,7	100.21 (±3.48) ****2,3,5–11	105.66 (±5.93) ***6,7	101.69 (±1.76) ***6 ****7
1	T-1A 0.1mgL^{-1}	74.18 (±0.06) ****1,3,5,7,9	85.74 (±0.79) ****1,3,5	68.41 (±4.36)	69.13 (±3.27)	61.67 (±5.11)	135.86 (±12.87)
2	T-1B 0.1mgL^{-1} + B	115.38 (±4.47) ****3	103.09 (±8.57) ****5,7	70 (±8.79)	71.57 (±2.17)	105.32 (±15.09) ****1,3,5	138.6 (±9.09) ****10
3	T-2A 1 mg L^{-1}	66.46 (±0.14)	81.72 (±0.56) ***3	103.37 (±13.27) ****1,3,5,7,9	99.47 (±5.3) ***7	103.37 (±13.27) ****7,9	51.69 (±8.98)
4	T-2B 1 mg L^{-1} + B	104.95 (±0.03) ****1,3,7,9	90.49 (±0.85) ***1	132.91 (±25.19) ****1,3,5–10	118.29 (±2.19) ****5,7	119.85 (±2.19) ****1,3,5,7	59.89 (±8.32)
5	T-3A 10 mg L^{-1}	51.73 (±51.73) **4	72.12 (±72.12) ***2	81.74 (±5.55)	75.58 (±5.74)	81.74 (±5.58)	89.48 (±8.74) ***3
6	T-3B 10 mg L^{-1} + B	101.43 (±0.83) ****1,3–9	100.64 (±0.64) ****5,7	60.64 (±5.23)	84.57 (±2.27) **3	98.71 (±7.15) **8	97.88 (±8.95) ****1
7	T-4A 100 mg L^{-1}	33.57 (±0.02) ***11	60.4 (±0.5)	83.49 (±3.54)	19.55 (±0.9)	83.49 (±3.54)	41.59 (±2.61)
8	T-4B 100 mg L^{-1} + B	93.02 (±2.48) ***10	104.9 (±2.8) ****1,3,8	118.29 (±11.21) ****11	26.94 (±3.5)	106.93 (±4.82) ****1,3,7	53.28 (±3.84)
9	T-5A 500 mg L^{-1}	28.13 (±0.01)	52.83 (±1.28)	40.83 (±34.28)	23.99 (±0.91)	40.83 (±4.28)	19.89 (±5.82)
10	T-5B 500 mg L^{-1} + B	66.9 (±3.01)	95.89 (±0.08) **2	65.64 (±26.15)	27.8 (±0.9)	64.55 (±7.14)	31.69 (±16.82)
F-value		1269.6	1269.6	7702.6	494.9	301.2	101.5

C-B = Control B; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$

bacterial consortium was introduced at the same concentrations, the values increased to 110.97, 116.59, 149.48, 103.44, and 81.84 (Table 11). Under CIP treatments, the values of SLSTI also increased when the bacterial consortium was added (52.43, 50.77, 31.84, 42.69, and 31.32). Additionally, the addition of the bacterial consortium to CIP treatments resulted in increased values for RFSTI, SFSTI, RDSTI, and SDSTI. The overall increase in STIs for *S. oleracea* (spinach) seeds indicates that the bacterial consortium mitigates the impacts of antibiotics while enhancing the effectiveness of the bioremediation process for plant growth. This significant rise in STI values for *S. oleracea* (spinach) demonstrates that the bioremediation process enhances the crops' ability to reduce seed stress levels and promote growth.

Seedling vigour index

SVI values were compared among control treatments, treatments (T-B) containing bacteria, as well as those without bacteria (T-A). When seeds were grown only with antibiotic concentrations, SVI was found to decrease. Specifically, CIP treatments without bacteria showed reduced SVI levels for *D. carota* (carrot) seeds (239, 210, 164, 115, and 93, respectively). However, compared to treatments without the bacterial consortium, SVI values in CIP treatments with a bacterial consortium were higher at 337, 302, 301, 277, and 216, respectively (Fig. 4), and were comparable to the control treatment. Similar trends of increasing SVI values were observed for additional seed types: *P. sativum* (pea), *R. raphanistrum* (radish), *L. sativa* (lettuce), and *S. oleracea* (spinach) in treatments with the bacterial consortium compared to their respective control values. This experiment demonstrates that seed growth is more dynamic during the bioremediation process due to the higher SVI values of seeds sprouted in various antibiotic doses with bacterial consortia.

The scatter plot matrix for SVI provides a visual representation of SVI values under various treatment conditions for different crop seeds. The control SVI values serve as a

Table 8 Stress tolerance indices of *P. sativum* (pea) under application of the antibiotic ciprofloxacin (CIP) without (T-1A, T-2A, T-3A, T-4A, and T-5A) and with the bacterial consortium (T-1B, T-2B, T-3B, T-4B, and T-5B)

List of comparisons	Treatments	RLSTI	SLSTI	RFSTI	SFSTI	RDSTI	SDSTI
	C-B (0mgL^{-1} + B)	101.78 (± 1.2) ***3 ****1,3,5,7,9,11	102.13 (± 1.1) ***2 ****1,3,7,9,11	100.63 (± 1.61) ****1,3,7	101.79 (± 1.04) ****1,3,5,7,11	104.5 (± 1.57) ****1,3,5,7	104.58 (± 2.99) ****1,3,5,7,11
1	T-1A (0.1mgL^{-1})	93.54 (± 1.72) ****5	92.61 (± 0.42) ***1 ****2,4,6,8	84.51 (± 2.58) ****4,6	117.4 (± 4.22) ****1,3,5	101.84 (± 3.67) ****4	115.48 (± 3.78) ****1,3,5
2	T-1B (0.1mgL^{-1} + B)	101.31 (± 1.69) ****1,3,7	95.79 (± 0.6) ****6	111.22 (± 5.15) ****2,4,8	131.95 (± 11.36) ****7	115.7 (± 4.9) ****6	158.54 (± 4.34) ****7,9
3	T-2A (1 mg L^{-1})	87.51 (± 0.81) ***4	87.8 (± 0.84) ***9	82.96 (± 1.28) ****6	107.61 (± 2.83) ****8	88.5 (± 2.07)	101.89 (± 3.06) ****5,9
4	T-2B (1 mg L^{-1} + B)	99.58 (± 1.27) ****7,9	97.85 (± 0.12) ****11	110.33 (± 6.68) ****8	122.68 (± 7.8) ****1,4,6,8,10	110.07 (± 2.55) ****8	125.71 (± 6.68) ****1,3,5,9
5	T-3A (10 mg L^{-1})	80.44 (± 1.38)	81.08 (± 0.43) ****4	83.28 (± 4.3) ***3	60.02 (± 3.22) **2	78.25 (± 2.21)	58.25 (± 0.81)
6	T-3B (10 mg L^{-1} + B)	98.32 (± 1.55) ***4	93.77 (± 1.78) ****1,3,7	104.98 (± 5.41) ****4	76.44 (± 3.84) ****7	107.53 (± 1.45) ****9	106.33 (± 48.67) ****5,7
7	T-4A (100 mg L^{-1})	74.94 (± 0.5) **11	68.5 (± 0.1)	77.77 (± 1.94) ****1	23.88 (± 2.81)	69.1 (± 2.84)	23.03 (± 2.37)
8	T-4B (100 mg L^{-1} + B)	97.24 (± 1.06) ***2	89.97 (± 0.25) ****8	80.28 (± 2.32) ****3	37.68 (± 3.35)	90.09 (± 3.03)	45.48 (± 10.14)
9	T-5A (500 mg L^{-1})	49.25 (± 0.66)	45.32 (± 0.08)	73.2 (± 2.05)	27.02 (± 3.28)	56.81 (± 1.24)	26.87 (± 4.14)
10	T-5B (500 mg L^{-1} + B)	69.74 (± 9.41)	58.78 (± 0.24)	74.46 (± 2.49) ***3	22.77 (± 1.75)	60.59 (± 2.07)	28.19 (± 3.15)
F-value		1269.6	151	35.34	121	10.2	6.18

C-B = Control B; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$

baseline for comparison. SVI values for seeds grown only in antibiotic concentrations (T-A) are significantly reduced compared to the control. Conversely, SVI values for seeds grown in CIP treatments with a bacterial consortium (T-B) are higher and comparable to the control values. Specifically, without the bacterial consortium (T-A), SVI values are 239, 210, 164, 115, and 93, whereas with the bacterial consortium (T-B), SVI values increase to 337, 302, 301, 277, and 216. The presence of the bacterial consortium notably enhances SVI in carrot seeds across all antibiotic concentrations. Similar trends are observed across different crops, where SVI values increase in the presence of a bacterial consortium compared to antibiotic-only treatments. The scatter plot matrix demonstrates a positive correlation between the presence of a bacterial consortium and higher SVI values across various crops.

The higher SVI values observed in treatments with a bacterial consortium indicate more dynamic growth, suggesting that the consortium helps to mitigate the negative effects of antibiotics on seedling vigour. SVI values in bioremediation treatments approach or match those of the control, indicating effective bioremediation.

The scatter plot matrix is expected to show trend lines illustrating the relationship between antibiotic concentration and SVI. For T-A treatments, the trend line would slope downward, indicating a decrease in SVI with increasing antibiotic concentrations (Fig. 5). In contrast, for T-B treatments, the trend line would slope upward, showing an increase in SVI with the addition of the bacterial consortium. Data points for T-B treatments would cluster closer to those of the control, suggesting similar SVI values. On the other hand, data points for T-A treatments would cluster lower than the control, reflecting reduced SVI values.

Overall, the scatter plot matrix visually confirms that the presence of a bacterial consortium in CIP treatments enhances seedling vigour across various crop seeds. The bioremediation process, facilitated by the bacterial consortium, leads to SVI values comparable to those of the control, demonstrating its effectiveness in promoting dynamic growth despite the presence of antibiotics.

Table 9 Stress tolerance indices of *R. raphanistrum* (radish) under application of the antibiotic ciprofloxacin (CIP) without (T-1A, T-2A, T-3A, T-4A, and T-5A) and with the bacterial consortium (T-1B, T-2B, T-3B, T-4B, and T-5B)

List of comparisons	Treatments	RLSTI	SLSTI	RFSTI	SFSTI	RDSTI	SDSTI
	C-B ($0mgL^{-1}+B$)	100.78 (±2.79) ****1,3,5,7,9,11	100.82 (±2.97) ***3 ****1,3,5,7	98.58 (±0.86) ****1,3,7	99.55 (±0.32) ***5 ****1,3,9	97.78 (±1.1) ****3,5	98.22 (±0.05) ****1,3–9
1	T-1A ($0.1mgL^{-1}$)	90.09 (±0.42) ****1,3,9	82.55 (±1.08) ****4,6	90.11 (±1.85)	90.21 (±0.88) ****8	62.65 (±5.23)	123.41 (±3.6) ****1,3,9
2	T-1B ($0.1mgL^{-1}+B$)	103.76 (±0.5) **5 ****1,3,7,9	92.35 (±4.08) ***10	95.14 (±3.31)	101.72 (±0.75) ****10	97.39 (±6.87) ***2,3	104.96 (±3.79) ****1,10
3	T-2A ($1\ mg\ L^{-1}$)	78.19 (±0.42) ****2,4	57.95 (±1.55)	104.08 (±3.76) ****1,3,5	13.48 (±1.05)	87.05 (±10.29)	11.23 (±0.54)
4	T-2B ($1\ mg\ L^{-1}+B$)	102.65 (±1.17) ****2,6,8	75.04 (±0.82) ***11	112.49 (±5.51) ****11	15.41 (±0.36)	94.82 (±11.46)	13.78 (±0.95)
5	T-3A ($10\ mg\ L^{-1}$)	74.14 (±1.68) **5	29.92 (±1.52)	163.51 (±2.3) ***1 ****3,5,7	9.4 (±0.28)	144.32 (±30.26) ****7	15.76 (±0.9)
6	T-3B ($10\ mg\ L^{-1}+B$)	103.08 (±2.89) ****1,3–9	30.34 (±2.22) ***5	169.15 (±4.05) ****1,3,5,7	10.76 (±0.27)	157.76 (±26.68) ****6	19.9 (±1.18)
7	T-4A ($100\ mg\ L^{-1}$)	68.64 (±2.91) **2	25.2 (±1.04)	30.1 (±25.79)	9.4 (±0.28)	25.25 (±4.14)	13.6 (±0.74)
8	T-4B ($100\ mg\ L^{-1}+B$)	104.91 (±3.55) ****7,9,11	29.67 (±0.32)	44.94 (±43.79)	11.29 (±0.43)	28.4 (±6.31)	15.09 (±1.36)
9	T-5A ($500\ mg\ L^{-1}$)	63.09 (±7.59)	22.18 (±0.57)	32.7 (±0.47)	8.23 (±0.22)	26.85 (±4.82)	12.42 (±0.94)
10	T-5B ($500\ mg\ L^{-1}+B$)	96.92 (±13.8) **3	27.1 (±0.64)	37.25 (±1.67)	9.53 (±0.35)	30.44 (±6.46)	16.56 (±1.14)
F-value		1269.6	126.3	115.7	119.2	53.8	4.12

C-B = Control B; * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; **** = $p<0.0001$

Phytotoxicity index

PI, which measures the ratio of RRG to RSG, is a critical indicator in this study. Relative root growth and seed germination are considered more sensitive predictors of phytotoxicity than relative germination alone. All PI values for the seeds of the five different crops investigated in this study were found to be significant. Higher PI values were observed in treatments containing antibiotic concentrations, indicating a detrimental or unfavourable impact on seed growth under antibiotic-affected conditions.

However, all five crops showed lower PI values when the contaminated water used for irrigation was treated with the bacterial consortium, except for *D. carota* (carrot) in T-5B. Notably, for *L. sativa* (lettuce) and *P. sativum* (peas), when the same seeds were grown in CIP-contaminated treatments, a different pattern emerged, demonstrating a positive or stimulating effect on seedling growth. Positive PI values indicate that antibiotic levels in contaminated water negatively affect the growth of agricultural seedlings (Fig. 6).

Previous studies have shown that antibiotics exert a stronger phytotoxic effect on root and shoot elongation than on seed germination, primarily due to their hydrophobicity (Pan And Chu 2016a, b, 2017a). Both antibiotic type and crop species influence the extent of phytotoxicity. This study demonstrated significant reductions in phytotoxicity across all seeds during the bioremediation treatment.

Bioremediation, evidenced by lower PI values, promotes the growth of *D. carota* (carrot) seeds in water contaminated with antibiotics. The seedlings treated with the bacterial consortium showed CIP values of − 0.15, − 0.04, − 0.014, 0.06, and 0.33. These negative numbers indicate a significant stimulatory effect on seed growth after irrigation with biologically treated water. Conversely, positive values suggest detrimental effects on seedling growth (Fig. 6a).

Fluoroquinolones, as highlighted by Walters et al. (2010) and Girardi et al. (2011), have a strong tendency to persist in soil and are resistant to breakdown by abiotic factors. Bacteria can enhance antibiotic degradation and reduce uptake when used with water (Riaz et al. 2019). Early spikes in

Table 10 Stress tolerance indices of *L. sativa* (lettuce) under application of the antibiotic ciprofloxacin (CIP) without (T-1A, T-2A, T-3A, T-4A, and T-5A) and with the bacterial consortium (T-1B, T-2B, T-3B, T-4B, and T-5B)

List of comparisons	Treatments	RLSTI	SLSTI	RFSTI	SFSTI	RDSTI	SDSTI
	C-B ($0mgL^{-1}+B$)	98.82 (±0.01) ****1,3,5	102.2 (±0.07) ****1,2,4–9	94.92 (±6.1) ****1,3,5,6,9	90.82 (±7.85) ****1,2,4,6,8	82.15 (±10.85) ****4	87.8 (±7.95) ****1,3,5
1	T-1A ($0.1mgL^{-1}$)	88.74 (±1.42) ****4,6	94.35 (±3.01) **4 ****7,9,11	58.52 (±5.59)	45.59 (±6.62) ****1,3–6	35.08 (±6.11) **9	43.92 (±5.28)
2	T-1B ($0.1mgL^{-1}+B$)	103.94 (±1.62) ****4	72.63 (±3.67) ****2–6	85.89 (±9.45) ****4,6	89.41 (±7.7) ****1,6,8,11	83.83 (±9.21) ***8	109.75 (±5.63) ****4
3	T-2A ($1 mg L^{-1}$)	74.72 (±1.36) ****6	75.9 (±2.18) ***3	57.35 (±1.91) **2	29.51 (±9.73) ****11	43.21 (±4.79) ****4,6,8	28.76 (±9.4)
4	T-2B ($1 mg L^{-1}+B$)	102.55 (±1.67) **2 ****5,7,9,11	52.02 (±3.38)	99.36 (±2.95) ****1,3,9	86.25 (±13.24) ****4	125.96 (±8.42) ****1,3,5,7	69.77 (±13.8) ****7
5	T-3A ($10 mg L^{-1}$)	72.11 (±0.86)	73.07 (±1.68)	46.83 (±0.66)	22.34 (±11.72)	32.07 (±4.91) ****5	22.13 (±12.52)
6	T-3B ($10 mg L^{-1}+B$)	89.16 (±2.61) ***3	88.61 (±5.27) ****7,9,11	99.34 (±1.59) *5	42.06 (±9.44) ***3	88.09 (±8.6) ****4	41.6 (±27.73)
7	T-4A ($100 mg L^{-1}$)	66.92 (±0.93)	64.94 (±2.58)	48.31 (±5.12) **3	25.88 (±4.59)	42.48 (±7.92)	25.17 (±3.5)
8	T-4B ($100 mg L^{-1}+B$)	79.58 (±1.02) ****7	66.91 (±2.42)	94.83 (±3.69) ****5,8,9	62.93 (±4.13) ***5	114.49 (±19.22) ****5	36.64 (±4.11)
9	T-5A ($500 mg L^{-1}$)	46.23 (±7.29)	69.82 (±14.29)	42.39 (±3.53)	12.91 (±4.77)	36.55 (±3.72)	12.49 (±4.2)
10	T-5B ($500 mg L^{-1}+B$)	58.12 (±8)	70.8 (±14.49)	63.73 (±3.78)	22.79 (±5.44)	65.12 (±0.58)	39.48 (±7.61)
F-value		1269.6	305.3	291.5	111.25	57.26	33.1

C-B = Control B; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$

antibiotic concentrations largely determine their accumulation in plants, which can affect germination rates and other variables (Lillenberg et al. 2010), as observed in this study. The application of bacterial consortia significantly reduced CIP, with consortium isolates showing enhanced antibiotic degradation, consistent with previous research (Alexandrino et al. 2017; Riaz et al. 2019).

Lower PI values for *P. sativum* (pea) grown in bacterial consortium treatments also suggest that bioremediation enhances seed growth in antibiotic-contaminated water. The seeds treated with bacterial consortium showed CIP values of − 0.013, 0.004, 0.016, 0.02, and 0.302. These negative numbers indicate a substantial stimulatory effect on seed growth following irrigation with biologically treated water. Conversely, positive values suggest negative effects on seedling growth (Fig. 6b).

Similarly, lower PI values for radish grown in bacterial consortium treatments indicate that bioremediation promotes seed growth in water containing antibiotics. The seeds treated with bacterial consortia showed CIP values of − 0.03, − 0.002, − 0.03, − 0.04, and 0.03. These negative values indicate a significant stimulatory effect on seed growth after irrigation with the biologically treated water. Conversely, positive values suggest detrimental effects on seedling growth (Fig. 6c).

Lower PI values demonstrate that bioremediation promotes seed growth in lettuce grown in bacterial consortium treatments. Compared to treatments containing only antibiotics, the seedlings treated with the bacterial consortium showed decreased CIP values of − 0.03, − 0.02, − 0.10, 0.20, and 0.41. These negative values indicate a significant stimulatory effect on seed growth after irrigation with biologically treated water. Conversely, positive values indicate detrimental effects on seedling growth (Fig. 6d).

Lastly, lower PI values for spinach cultivated in bacterial consortium treatments suggest that bioremediation encourages seed growth. The seeds treated with the bacterial consortium exhibited CIP values of − 0.10, − 0.16, − 0.49, 0.03, and 0.18. These negative values demonstrate a potent stimulatory effect on seed growth after irrigation with biologically treated water. Conversely, positive values indicate detrimental effects on the growth of seedlings (Fig. 6e).

Table 11 Stress tolerance indices of *S. oleracea* (spinach) under application of the antibiotic ciprofloxacin (CIP) without (T-1A, T-2A, T-3A, T-4A, and T-5A) and with the bacterial consortium (T-1B, T-2B, T-3B, T-4B, and T-5B)

List of comparisons	Treatments	RLSTI	SLSTI	RFSTI	SFSTI	RDSTI	SDSTI
	C-B ($0mgL^{-1}+B$)	104.68 (±4.11) ***9 ****1,3,5–8	97.38 (±2.22) ****1,3–9	99.07 (±2.28) ***1 ****3,5,7	99.19 (±0.85) ****1,3	98.88 (±3.31) ****1,3,5,7–10	97.57 (±2.42) ***3 ****1,5,9
1	T-1A ($0.1mgL^{-1}$)	94.64 (±0.1) ***6	46.34 (±0.09) **2	99.32 (±1.47) ****8	64.45 (±18.55) **3	100.62 (±3.5) ****2,6,10	98.09 (±2.1) ****4
2	T-1B ($0.1mgL^{-1}+B$)	110.97 (±4.83) ****3,5,8	52.43 (±5.98) **5	105.23 (±2.99) ****9	68.37 (±19.99) ***1	118.07 (±3.81) ****5	113.56 (±3.22) ****5
3	T-2A ($1 mg L^{-1}$)	94.14 (±0.65) ***5	38.19 (±8.4)	96.81 (±1.02) ****5	63.77 (±18.03)	96.8 (±0.44)	96.17 (±0.26) ****6
4	T-2B ($1 mg L^{-1}+B$)	116.59 (±7.18) ****11	50.77 (±4.48) ***6	101.29 (±1.53) ****7	66.92 (±19.71) ***5	103.03 (±5.95) ****7,9	105.56 (±3.47) ****7
5	T-3A ($10 mg L^{-1}$)	91.21 (±0.57) ***6	26.08 (±2.28)	94.77 (±1.26) ****9	48.2 (±13.63) ****8	89.28 (±4.08) ****6	75.65 (±7.24) ****9
6	T-3B ($10 mg L^{-1}+B$)	149.47 (±0.67) ****7	31.84 (±0.12)	94.99 (±2.65) **3	49.49 (±14.57)	98.53 (±1.04)	84.71 (±2.71) ****10
7	T-4A ($100 mg L^{-1}$)	81.21 (±0.69) ****9	31.56 (±0.26)	93.75 (±1.31) ***9	46.91 (±13.53)	90.46 (±4.39) **10	78.09 (±1.57)
8	T-4B ($100 mg L^{-1}+B$)	103.44 (±30.91) ****10	42.69 (±29.45) ***8	107.3 (±4.6) ****5	48.75 (±14.24) ****8	95.91 (±1.1)	82.96 (±2.3)
9	T-5A ($500 mg L^{-1}$)	80.28 (±1.49)	25.64 (±0.51)	44.83 (±2.69)	89.2 (±13.17)	45.69 (±0.94)	92.14 (±3.18)
10	T-5B ($500 mg L^{-1}+B$)	81.84 (±2.38)	31.32 (±0.64)	96.81 (±1.86)	45.02 (±13.5)	99.67 (±2.21)	74.08 (±1.02)
F-value		1269.6	176.45	209.15	106.3	89.15	4.12

C-B = Control B; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$

Accumulation and phytotoxic effects of CIP

For *D. carota* (carrot), *P. sativum* (pea), *R. raphanistrum* (radish), *L. sativa* (lettuce), and *S. oleracea* (spinach), the highest percentages of inhibition for shoot elongation were 48.8%, 54.7%, 77.8%, 30.4%, and 58.1%, respectively (Fig. 7a–e). Similarly, the highest percentages of inhibition for root elongation were 50.7%, 71.8%, 36.9%, 53.4%, and 19.7%. There was a noticeable increase in the inhibition of shoot and root elongation as the antibiotic concentrations in the treatments increased.

Previous studies have demonstrated minimal effects of antibiotics on plant crops such as lettuce, carrot, peas, and tomatoes (Afzal et al. 2025; Khalid et al. 2023; Jin et al. 2009; Liu et al. 2009; Hillis et al. 2011; Pino et al. 2016). CIP negatively affected root and shoot length and development but had no impact on seed germination in tomato seeds exposed to up to 300 mgL^{-1} of norfloxacin (Pan And Chu 2016b; Bellino et al. 2018).

The R2 values ranged from 0.87 to 0.97 for root elongation and from 0.71 to 0.99 for shoot elongation. This observation indicates that, among the crops tested, antibiotics had a stronger phytotoxic effect on shoot elongation compared to root elongation. As antibiotic concentrations increased, it was observed that the inhibition rates for both shoot and root elongation also increased. However, it was found that root elongation was more strongly inhibited than shoot elongation in *D. carota* (carrot) and *L. sativa* (lettuce). These findings demonstrate that higher concentrations of antibiotics in water have detrimental effects on seed and plant growth, particularly inhibiting root elongation. While there was minimal variation in the inhibitory effects of the antibiotics across all five crops, differences in regression values were observed between shoot and root elongation.

Antibiotic sensitivity follows the order: root elongation > shoot elongation > seed germination, consistent with earlier research (An et al. 2009). However, some crops in the study exhibited greater shoot inhibition, as also noted by Jin et al. (2009). The study suggests that excessive CIP concentrations in water can inhibit the growth of carrots, peas, radishes, lettuce, and spinach (Pan And Chu 2016b). While antibiotic accumulation in all agricultural seeds was generally low, high concentrations in water could persist and pose significant environmental and terrestrial ecosystem risks.

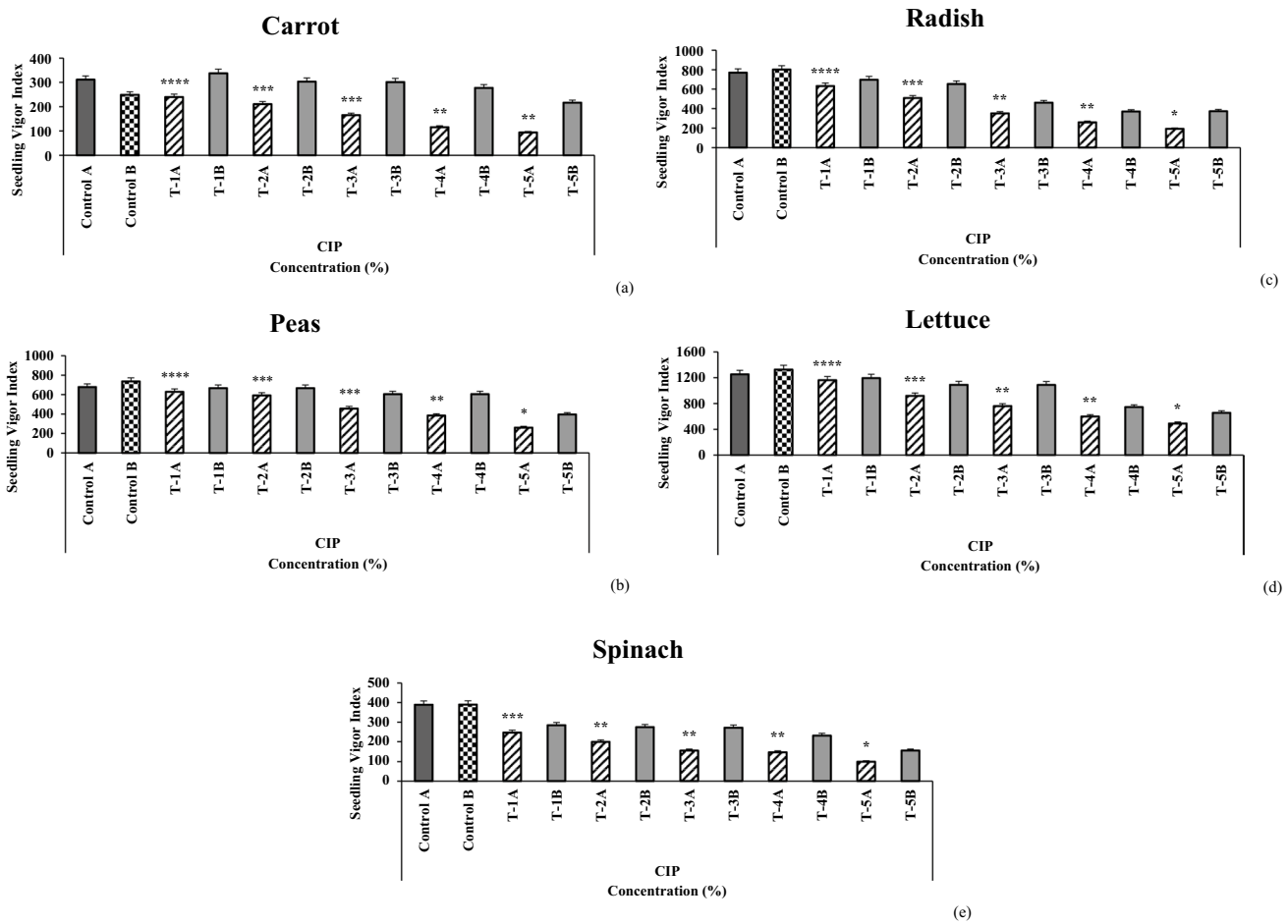


Fig. 4 Seedling vigour index (SVI) for **a** carrot, **b** pea, **c** radish, **d** lettuce, and **e** spinach under ciprofloxacin (CIP) without bacteria (CIP) and with bacterial consortium (CIP+B). SVI was calculated as germination percentage × seedling length. Data are means ± SD (n = 3). Asterisks denote significant differences between CIP and CIP+B at the same concentration (**$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; two-way ANOVA with post hoc test)

Apart from exposure concentration, factors influencing antibiotic hazard to plant growth include tissue sensitivity, crop species, physiology, and antibiotic type (Grossmann 2010). Except for spinach, which is leafier, roots showed a strong linear relationship with antibiotics in the crops studied (Pan And Chu 2016b). Antibiotics do not penetrate hard seeds; instead, they migrate to roots and shoots post-emergence. However, the present research has some limitations, including the lack of direct quantification of antibiotic degradation, potential scale-up of the bacterial isolates, use of opportunistic pathogens in remediation efforts, and the potential for AMR spread and HGT.

Limitations and considerations for environmental application

While the results of this study demonstrate the promising potential of a bacterial consortium comprising *E. coli* and *E. faecium* to degrade CIP and mitigate its phytotoxicity, several critical limitations and risks must be addressed before any field-scale application can be considered.

First and foremost is the inherent risk associated with the deliberate introduction of these particular bacterial species into the environment. Both *E. coli* and *E. faecium* include strains that are opportunistic human pathogens. Their application in agriculture, especially for food crops, raises justifiable concerns regarding public health safety. Furthermore, the isolates used in this study were selected for their antibiotic resistance, which is a double-edged sword. While necessary for their survival and function in CIP-contaminated environments, these resistance traits are often located on mobile genetic elements such as plasmids and integrons [Santos et al. 2024]. The environmental release of these strains could therefore act as a potential reservoir for HGT, facilitating the spread of AMR genes to indigenous soil and water microbiota, including other potentially pathogenic bacteria, thereby exacerbating the global AMR crisis.

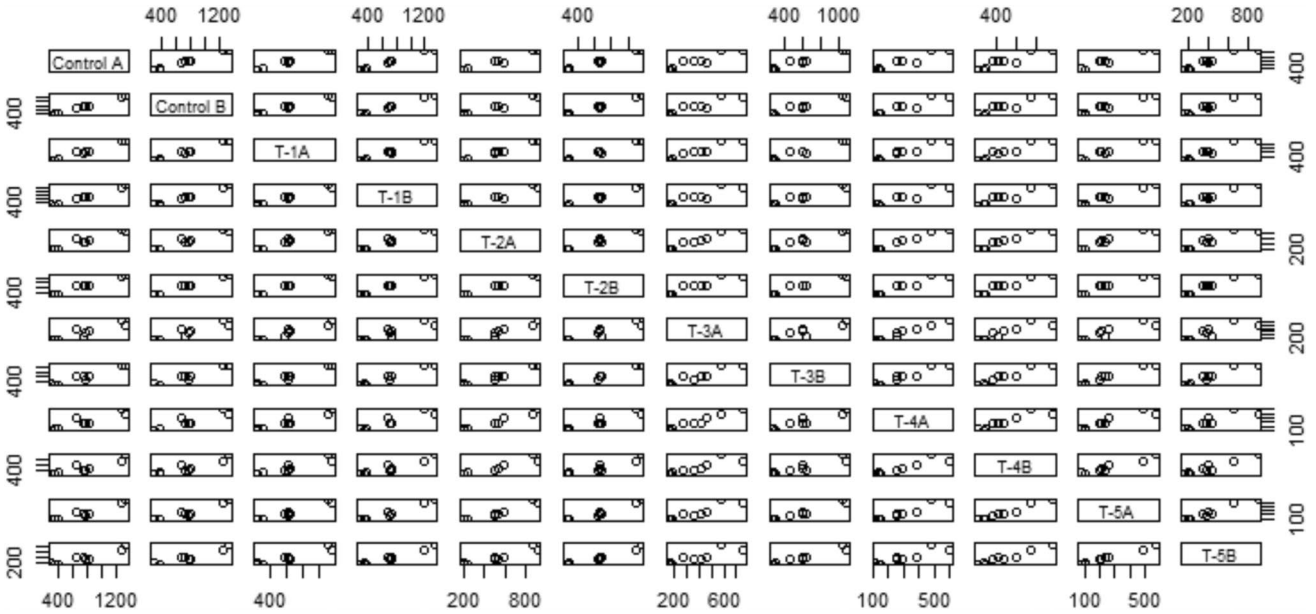


Fig. 5 Scatter plot matrix of seedling vigour index (SVI) for five crops under ciprofloxacin (CIP) treatments without and with bacterial consortium. Each panel compares SVI across CIP concentrations (0.1–500 mg L^{-1}). The diagonal shows histograms of SVI distribution. Generated using R software (v14.4)

Another significant limitation of this study is the lack of direct quantification of the extent of CIP degradation by the consortium. While the phytotoxicity assays and plant growth metrics provide strong indirect evidence of reduced antibiotic stress, future work must employ analytical techniques such as HPLC or LC–MS to precisely quantify the degradation efficiency and identify any potentially toxic intermediate products.

While this study demonstrates the high potential of the E. coli and E. faecium consortium for CIP degradation and phytotoxicity mitigation, it is important to note that all experiments were conducted under controlled laboratory conditions. These simulated environments, while essential for establishing proof-of-concept and isolating the specific bacterial capabilities, lack the complexity of a real-world agricultural ecosystem. Factors such as competition with indigenous soil microbiomes, adsorption of CIP to soil organic matter and clay particles, varying pH and temperature regimes, and the presence of other contaminants could significantly influence the efficiency, survival, and activity of the introduced bacterial strains. Therefore, these findings represent a crucial first step. Future research must focus on validating these results in more complex systems, including soil microcosms and ultimately field trials, to assess the practical applicability and ecological impact of this bioremediation strategy.

Consequently, future research should focus on: (i) identifying the specific catabolic genes and enzymes responsible for CIP degradation, (ii) cloning these genes into safer, non-pathogenic host organisms (e.g., *Pseudomonas putida* or plant growth-promoting rhizobacteria), (iii) developing contained bioreactor systems for treating wastewater before irrigation, and (iv) conducting comprehensive risk assessments, including studies on the survival, persistence, and gene transfer potential of any candidate strains in complex ecosystems. This study serves as a foundational step, highlighting the metabolic potential present in wastewater microbiomes, but it also underscores the imperative for rigorous safety evaluations in the development of bioremediation strategies.

Conclusion

The findings of this study underscore the dual role of *E. coli* and *E. faecium* in bioremediation and agricultural enhancement. These bacterial strains effectively degrade CIP in contaminated water, thereby reducing the antibiotic's phytotoxic effects and creating a more favourable environment for plant growth. Improved germination rates following treatment with bacteria reached up to 85%, increased root and shoot lengths up to 73%, and enhanced biomass up to 65% were observed in carrot, pea, radish, lettuce, and spinach, demonstrating the potential of these bacteria to enhance agricultural productivity. The findings of this in vitro study demonstrate that a consortium of municipal wastewater-derived *E. coli* and *E. faecium* can effectively mitigate CIP phytotoxicity and enhance the growth of edible crops. However, as discussed, the translational application of these specific strains in open-field agriculture is fraught with significant risks related to pathogenicity and AMR spread. Therefore, this research primarily underscores the potential of

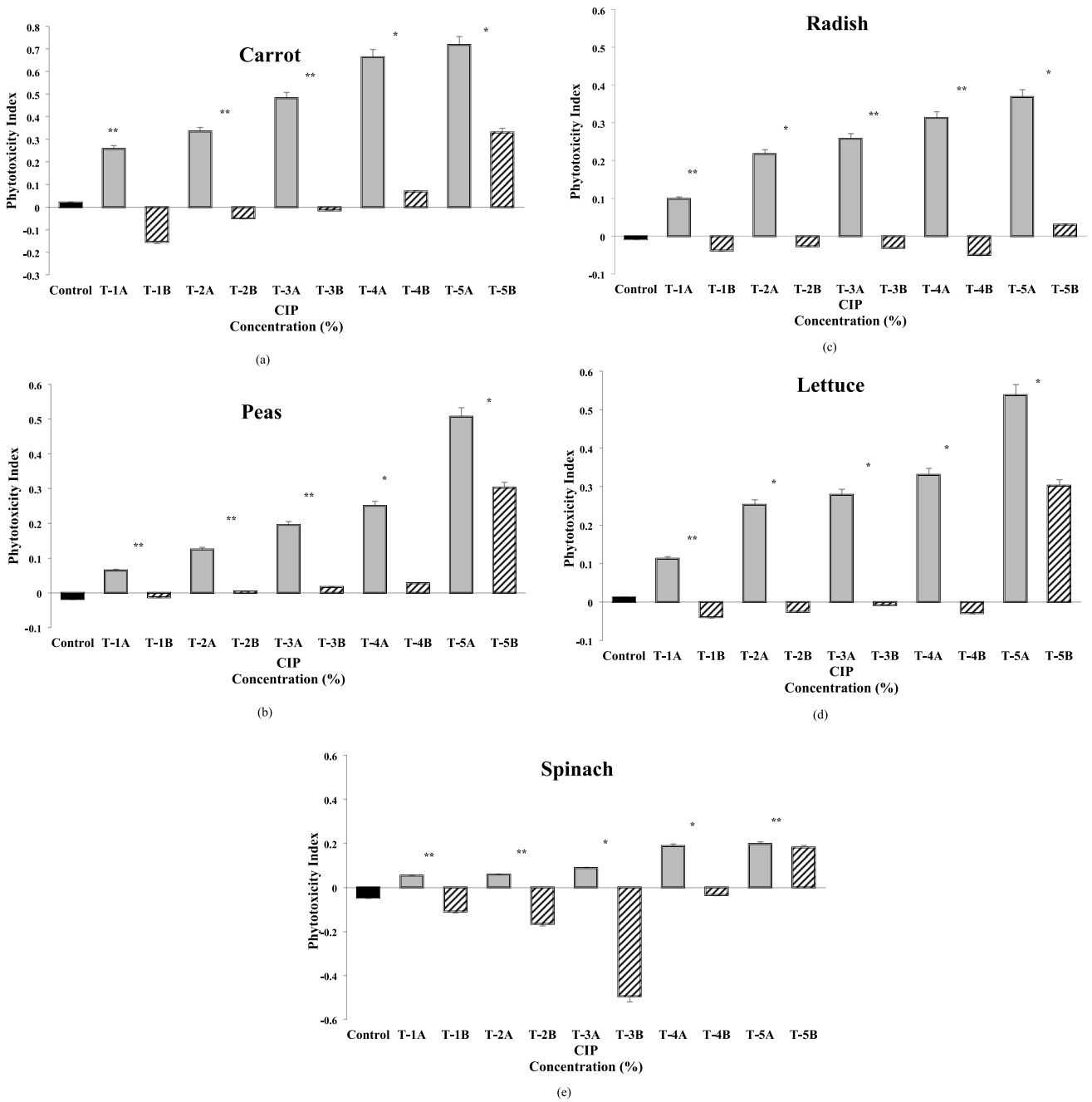


Fig. 6 Phytotoxicity index (PI) for **a** carrot, **b** pea, **c** radish, **d** lettuce, and **e** spinach nder ciprofloxacin (CIP) alone and with bacterial consortium (CIP + B). PI was calculated as 1−(root length in treatment / root length in control). Negative values indicate growth stimulation; positive values indicate phytotoxicity. Data are means ± SD (n = 3). Treatments as in Fig. 5

leveraging microbial communities for bioremediation, but it also strongly advocates for a cautious approach. Future work must focus on harnessing this degradative capability through safer means, such as employing non-pathogenic chassis organisms or closed treatment systems, to develop environmentally responsible and safe bioremediation technologies for antibiotic-contaminated water. Future research should prioritise field trials and long-term assessments to further validate the practical applications of this bioremediation approach across diverse agricultural settings.

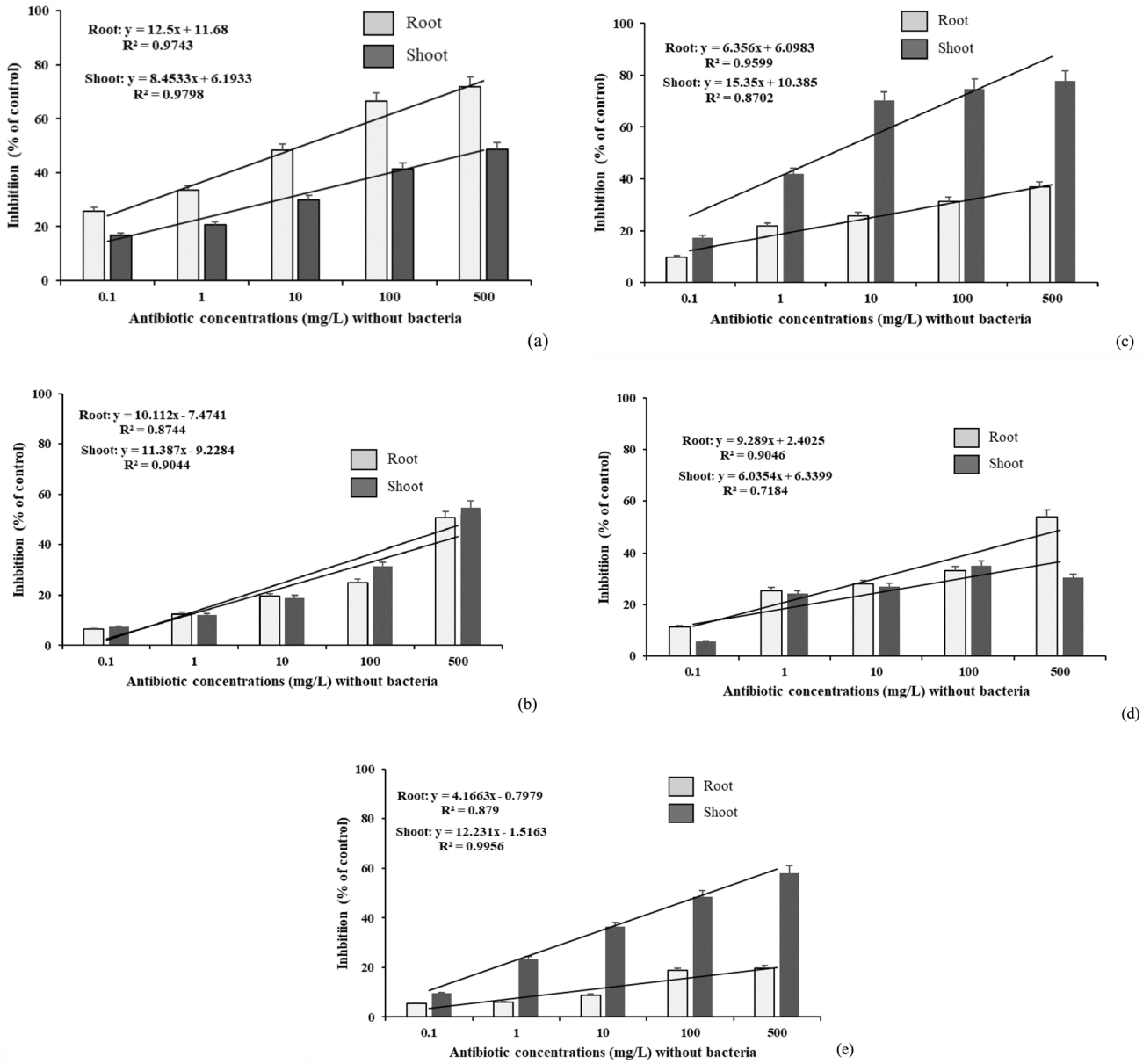


Fig. 7 CIP-induced inhibition of root and shoot elongation in **a** carrot, **b** pea, **c** radish, **d** lettuce, and **e** spinach seedlings. Inhibition percentage was calculated relative to control. Data are means ± SD (n = 3). Solid lines represent root inhibition; dashed lines represent shoot inhibition. Regression coefficients (R^2) are shown for each trend line

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Authors contributions NMD and MF planned this research. NMD, AR, and MMD conducted this research. NMD, A.R, MMD, M.F, C.K., and L.C.C. analysed the data. All of the authors contributed to the research paper writing.

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Data availability Data will be made available on reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest.

Consent to participate This study did not involve human participants, and therefore, consent to participate was not required.

Consent to publish This study does not contain any identifiable human data or images, and therefore, consent to publish was not required.

Ethical approval This study did not involve human participants, human data, or animals, and therefore did not require ethical approval.

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