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Research Article

Mitigation of Junglerice (*Echinochloa colona*) Allelopathic Stress in Rice (*Oryza sativa* L.) by *Bacillus subtilis*

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ABSTRACT

Rice (*Oryza sativa* L.) is one of the world's major staple crops due to its nutritional value and associated physiological and health benefits. However, rice production faces a serious threat from *Echinochloa colona* (junglerice), an aggressive and difficult-to-control weed. *E. colona* releases allelochemicals into the environment that suppress rice seed germination and seedling growth. In this study, we investigated the potential role of the beneficial bacterium *Bacillus subtilis* in alleviating allelopathic stress. As a plant growth-promoting rhizobacterium, *B. subtilis* enhances plant health by producing biologically active compounds and improving nutrient uptake. *In vitro* bioassays were conducted to evaluate the allelopathic effects of aqueous extracts prepared from different parts of *E. colona* (inflorescences, shoots, and roots) on rice. Extract concentrations ranging from 0.2% to 3.0% significantly inhibited seed germination, seedling biomass, and root and shoot lengths compared with the control. Application of *B. subtilis* significantly mitigated these adverse effects, improving seed germination by up to 73%, root length by 34.56%, shoot length by 20%, fresh biomass by 27.9%, and dry biomass by 6.8% relative to untreated stressed plants. Enhanced biomass production and seedling growth following *B. subtilis* application were further illustrated by heatmap analysis. A concentration range of 1.0% to 1.8% of *B. subtilis* was most effective in promoting overall seedling growth and reducing the negative effects of weed-induced allelopathic stress. These findings demonstrate that *B. subtilis* is a promising biocontrol agent for mitigating allelopathic interference from *E. colona*, thereby improving the growth and development of rice seedlings.

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Introduction

Weeds are among the most severe biological constraints to rice production, causing greater yield losses than most other pests. Under uncontrolled conditions, weed competition can reduce rice yields by as much as 94-96%, highlighting the critical need for effective weed management strategies. Among the diverse weed flora associated with rice ecosystems, *Echinochloa colona* is

recognized as one of the most widespread and destructive weed species globally. This annual grass weed competes aggressively with rice plants for essential resources, including nutrients, soil moisture, space, light, and carbon dioxide (CO_2). Such intense competition leads to substantial yield reductions and deterioration in grain quality, thereby threatening farmers' incomes and livelihoods, particularly in tropical and subtropical regions such as Pakistan.

Rice plays a pivotal role in global food security and serves as the staple food for more than half of the world's population (Batool et al., 2026). It is a primary source of calories and nutrition for billions of people, especially in Asia and Africa. Rice grains are rich in carbohydrates, proteins, essential amino acids, vitamins, and minerals, making them a vital component of human diets (Chaudhari et al., 2018). With the rapid growth of the global human population, the demand for rice is projected to increase substantially in the coming decades (Samal et al., 2022). Rising production costs and increasing yield variability pose serious challenges to global food security, particularly in sub-Saharan Africa (SSA), where rice consumption is increasing rapidly and the gap between domestic production and demand continues to widen (Tun, 2024). Effective weed management is therefore essential to ensure stable rice productivity and sustain global food systems.

Traditionally, chemical herbicides have been the primary tool for weed control in rice cultivation. Although herbicides provide rapid and effective weed suppression, their continuous and indiscriminate use has led to the evolution of herbicide-resistant weed populations, environmental contamination, and potential risks to human and ecosystem health. These concerns highlight the urgent need for sustainable and environmentally friendly alternatives for weed management in agricultural systems.

In this context, the use of beneficial microorganisms has emerged as a promising and eco-friendly strategy for weed suppression and crop improvement. Plant growth-promoting rhizobacteria (PGPR) offer a sustainable alternative by enhancing crop vigor while simultaneously reducing weed competitiveness. Among these PGPRs, *Bacillus subtilis* has gained considerable attention as a powerful tool in sustainable agriculture. First described by Ferdinand Cohn in 1872, *B. subtilis* is well known for its metabolic versatility and its ability to colonize the rhizosphere effectively (Akinsemolu et al., 2024). It functions as an efficient biofertilizer and biopesticide, promoting plant growth, improving nutrient uptake, and inducing systemic resistance against various plant pathogens (Kumar et al., 2024a; Azeem et al., 2025). These attributes suggest that *B. subtilis* may also play a role in sustainable weed management by indirectly enhancing crop competitiveness against invasive weed species such as *E. colona*.

The objective of this study was to evaluate the potential of *B. subtilis* as a sustainable and environmentally friendly alternative for the management of *E. colona* in rice, with the aim of reducing weed-induced yield losses and improving rice productivity under agro-ecological conditions relevant to Pakistan.

Materials and Methods

Weed collection

Fresh plants of *Echinochloa colona* (L.) were collected from cultivated fields. The collected weed samples were thoroughly washed with tap water to remove adhering soil particles and debris and then air-dried at room temperature. The roots, shoots, and inflorescences were separated and ground individually into a fine powder using an electric grinder. The powdered materials were stored in airtight containers until further use.

Collection of rice seeds

Seeds of rice (*Oryza sativa* L.) cv. Kainat PS-2 were obtained from the Punjab Seed Corporation. The seeds were healthy, uniform in size, disease-free, and free from mechanical damage.

Preparation of bacterial culture

B. subtilis strain ZMR4 was used as a plant growth-promoting rhizobacterium (PGPR) in this study. A pure culture of the bacterium was obtained from Minhaj University, Lahore. Nutrient broth was prepared by dissolving calcium carbonate (5 g), yeast extract (2.5 g), and dextrose (5 g) in 250 ml of distilled water. The medium was sterilized in an autoclave at 121°C and 15 psi pressure for 20 min. After cooling, bacterial inoculation was carried out under aseptic conditions in a laminar airflow cabinet to prevent contamination. The inoculated flasks were incubated at 30°C for 72 h. After incubation, the bacterial suspension was used for further experimentation. The bacterial population density was adjusted to 1.35×10^7 CFU ml^{-1}.

For clarification and sterilization, the culture medium was filtered using a sterile vacuum filtration unit fitted with a membrane filter (0.22 µm for sterilization or 0.45

μm for clarification). The filtrate was aseptically transferred into sterile bottles, properly labeled, sealed, and stored at 4°C until use.

Preparation of aqueous weed extracts

Aqueous extracts of *E. colona* roots, shoots, and inflorescences were prepared separately. Each powdered plant part was soaked in 100 ml of distilled water for 10 days to obtain a 50% (w/v) stock solution. After the extraction period, the mixtures were first filtered through muslin cloth and then through Whatman filter paper to obtain clear aqueous extracts.

From the stock solution, a range of concentrations (0-3%) was prepared to evaluate the allelopathic effects of different weed parts alone and in combination with PGPR. The final volume of each treatment solution was adjusted to 9 ml.

Preparation of set one (weed extracts alone)

Working solutions of *E. colona* inflorescence, root, and shoot extracts were prepared separately by diluting the stock solution with distilled water to obtain final concentrations of 3.0, 2.6, 2.2, 1.8, 1.4, 1.0, 0.6, and 0.2%. For example, a 3% solution was prepared by mixing 450 μl of stock extract with 8550 μl of distilled water to make a final volume of 9 ml. Similarly, 2.6% was prepared by mixing 390 μl of extract with 8610 μl of distilled water; 2.2% by mixing 330 μl extract with 8670 μl distilled water; 1.8% by mixing 270 μl extract with 8730 μl distilled water; 1.4% by mixing 210 μl extract with 8790 μl distilled water; 1.0% by mixing 150 μl extract with 8850 μl distilled water; 0.6% by mixing 90 μl extract with 8910 μl distilled water; and 0.2% by mixing 30 μl extract with 8970 μl distilled water. All solutions were prepared separately for each plant part.

Preparation of set two (weed extracts + PGPR)

In the second set, treatments were prepared by combining weed extracts with *B. subtilis* inoculum. Each treatment had a final volume of 9 ml containing 4500 μl of bacterial culture (1.35×10^7 CFU ml^{-1}). For example, a 3% concentration was prepared by mixing 225 μl of aqueous extract, 4275 μl of distilled water, and 4500 μl of bacterial culture. Similarly, 2.6% was prepared by mixing 195 μl extract, 4305 μl distilled water, and 4500 μl bacterial culture; 2.2% by mixing 165 μl extract, 4335 μl distilled water, and 4500 μl bacterial culture; 1.8% by mixing 135 μl extract, 4365 μl distilled water, and 4500 μl bacterial culture; 1.4% by mixing 105 μl extract, 4395 μl distilled water, and 4500 μl bacterial culture; 1.0% by mixing 75 μl extract, 4425 μl distilled water, and 4500 μl bacterial culture; 0.6% by mixing 45 μl extract, 4455 μl distilled water, and 4500 μl bacterial culture; and 0.2% by mixing 15 μl extract, 4485 μl distilled water, and 4500 μl bacterial culture. All treatments were freshly prepared before use.

Experimental setup

Sterile Petri plates were used for the germination bioassay. The plates were washed thoroughly and sterilized in a hot air oven to avoid contamination. A sterile filter paper was placed in each Petri plate, and ten rice seeds were arranged uniformly in each plate. Three milliliters of the respective treatment solution were added to moisten the filter paper. One experimental set included weed extract treatments alone, while the second set included weed extracts combined with PGPR. Control treatments (0%) received distilled water only. The experiment was conducted at room temperature and maintained for 15 days.

Data collection

Seed germination was recorded after 24 h and monitored daily. Radicle and plumule emergence was observed within 4-5 days. After 15 days, germination percentage, shoot length, root length, fresh weight, and dry weight were measured. Dry weight was determined after oven-drying the seedlings at 70°C until a constant weight was achieved.

Statistical analysis

The experiment was conducted in a completely randomized design (CRD) with three replications per treatment. Data were subjected to analysis of variance (ANOVA) to determine the significance of treatment effects. Means were compared using Tukey's Honestly Significant Difference (HSD) test at a 5% probability level. Statistical analyses were performed using SPSS statistical software. Percentage data were arcsine-transformed before analysis where necessary to normalize variance.

Results

Biological significance of *B. subtilis* on rice germination under the allelopathic influence of *E. colona* inflorescence, shoot, and root

The involvement of *B. subtilis* ZMR4 in enhancing rice germination under the allelopathic stress of dried inflorescence powder of *E. colona* was evident (Figure 1A). Statistical analysis using the LSD test at $P < 0.05$ indicated significant differences among treatments. The positive control (C^+) treated with *B. subtilis* ZMR4

exhibited 100% germination, compared with 91% in the negative control (C). All combined treatments (0.2%, 0.6%, 1.0%, 1.4%, 1.8%, 2.2%, 2.6%, and 3%) of *B. subtilis* ZMR4 with *E. colona* inflorescence significantly improved germination relative to the corresponding *E. colona*-only treatments, increasing germination from 11% to 71%.

Similarly, under allelopathic stress imposed by *E. colona* shoot powder, all treatments supplemented with *B. subtilis* ZMR4 resulted in significantly higher germination percentages than treatments containing *E. colona* shoot alone, with improvements ranging from 11% to 55% (Figure 1B).

Under root powder-induced stress, the application of *B. subtilis* ZMR4 also significantly enhanced rice germination compared with the respective negative treatments containing only *E. colona* root powder. Germination increased from 11% to 73%, as shown in Figure 1C.

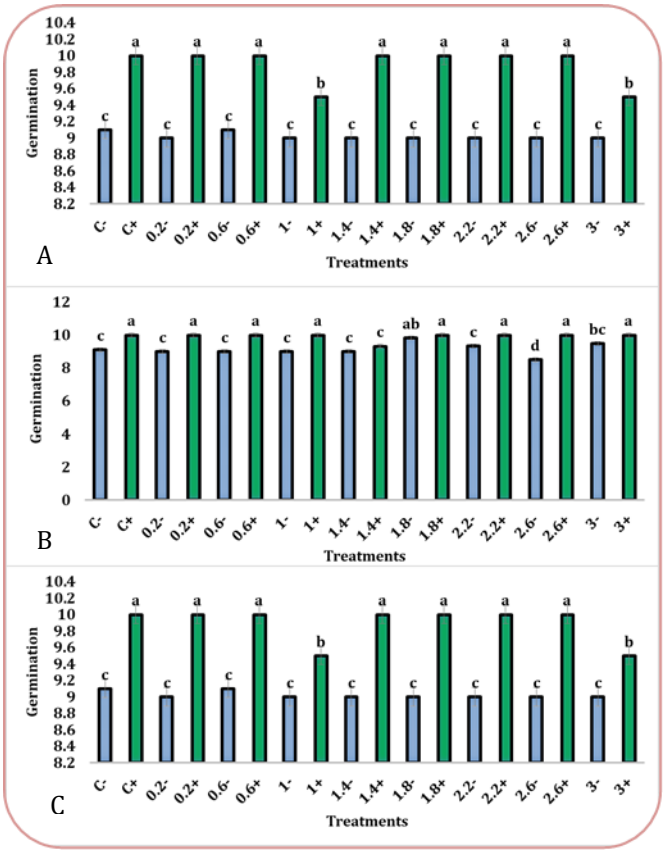

Figure 1. Allelopathic effects of aqueous extracts of *E. colona* inflorescence (A), shoot (B), and root (C), with and without PGPR inoculation, on rice seed germination. Vertical bars represent the standard error of the mean (n = 4). Bars sharing different letters indicate statistically significant differences at P ≤ 0.05 according to the LSD test.

Biological significance of *B. subtilis* on rice radicle length under the allelopathic Influence of *E. colona*

The effect of dried inflorescence powder of *E. colona* and *B. subtilis* strain ZMR4 on rice radicle length was evaluated at **P < 0.05**, as shown in Figure 2(A). Application of *B. subtilis* ZMR4 alone (C+) produced a pronounced promotive effect on radicle length (6.7 cm) compared with the untreated control (C−), where the radicle length was 4.93 cm, representing a 43% increase. Furthermore, the combined application of *B. subtilis* ZMR4 and *E. colona* inflorescence powder mitigated allelopathic inhibition and enhanced radicle elongation, shifting the response from moderate inhibition (-12.83%) to substantial promotion (52%). Statistical analysis indicated that the means of all treatments were significantly different from each other at **P < 0.05**.

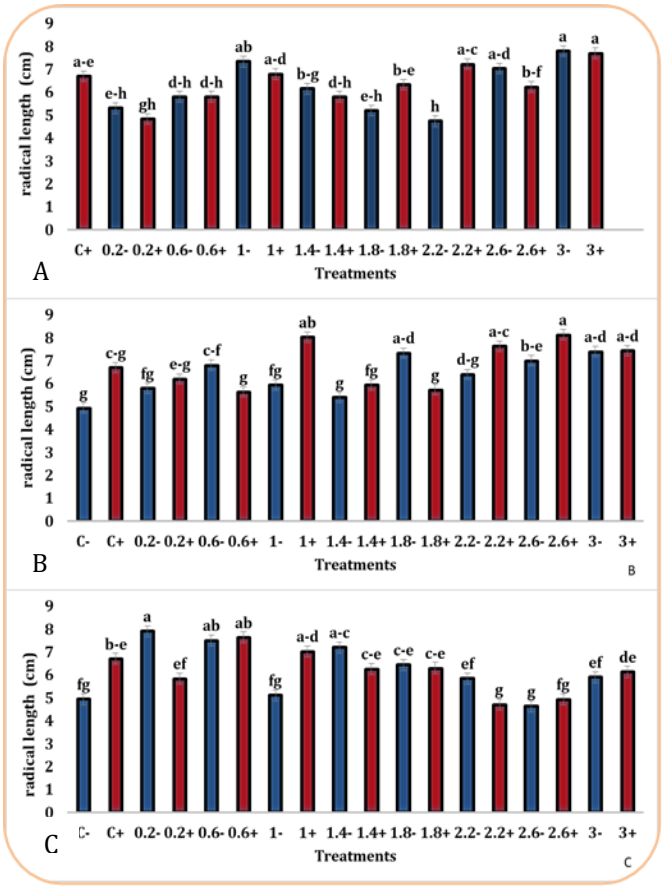

Figure 2. Allelopathic effect of *E. colona* (inflorescence (A), shoot (B) and root (C)) aqueous extract and PGPR on the radical length of rice. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

Similarly, the combined application of *B. subtilis* ZMR4 and dried shoot powder of *E. colona* consistently influenced rice radicle development. The observed improvement ranged from 6% to 37% compared with treatments receiving *E. colona* alone.

Under stress imposed by dried root powder of *E. colona*, the presence of *B. subtilis* ZMR4 consistently modulated radicle length. Positive treatment effects ranged from 12% to 40%. Analysis using the Least Significant Difference (LSD) test confirmed that all treatment means differed significantly from one another at **P < 0.05**.

Biological significance of *B.subtilis* on rice plumule length under the allelopathic influence of *E. colona* inflorescence, shoot, and root

Statistical analysis using LSD test revealed significant differences among treatment means at $P < 0.05$. The effect of *B. subtilis* strain ZMR4 on rice plumule elongation under stress induced by *E. colona* inflorescence powder is presented in Figure 3A. Treatment with *B. subtilis* ZMR4 (C+) significantly increased plumule length (7.0 cm) compared with the untreated control (C−), which recorded a plumule length of 6.37 cm.

Across all tested concentrations (0.2%, 0.6%, 1.0%, 1.4%, 1.8%, 2.2%, 2.6%, and 3.0%), application of *B. subtilis* ZMR4 consistently enhanced rice plumule length under inflorescence powder-induced allelopathic stress. The observed increase ranged from 11% to 45%, as illustrated in Figure 3A.

Similarly, under shoot powder-induced stress, *B. subtilis* ZMR4 significantly promoted plumule elongation across all concentrations (0.2%-3.0%), with maximum enhancement reaching 42.68%. Furthermore, under root powder-induced stress, all bacterial treatments markedly improved rice plumule length, showing up to 37% enhancement compared with weed root-stressed treatments without bacterial inoculation (Figure 3C).

Biological significance of *B. subtilis* on the fresh weight of rice seedlings under the allelopathic influence of *E. colona* inflorescence, shoot, and root powders

The effect of *B. subtilis* strain ZMR4 on the fresh weight of rice seedlings exposed to allelopathic stress from *E. colona* inflorescence powder was evaluated (Figure 4A). Inoculation with *B. subtilis* ZMR4 (C+) exhibited a significant growth-promoting effect, resulting in a mean seedling fresh weight of 3.98 g compared with 3.90 g in the uninoculated control (C−). This corresponded to a 48% increase in biomass accumulation relative to the weed-stressed treatments. Across all tested concentrations, *B. subtilis* ZMR4 significantly enhanced rice seedling fresh weight compared with the respective weed-stressed treatments, with increases ranging from 14% to 66%. Statistical significance was determined using LSD test at $P < 0.05$.

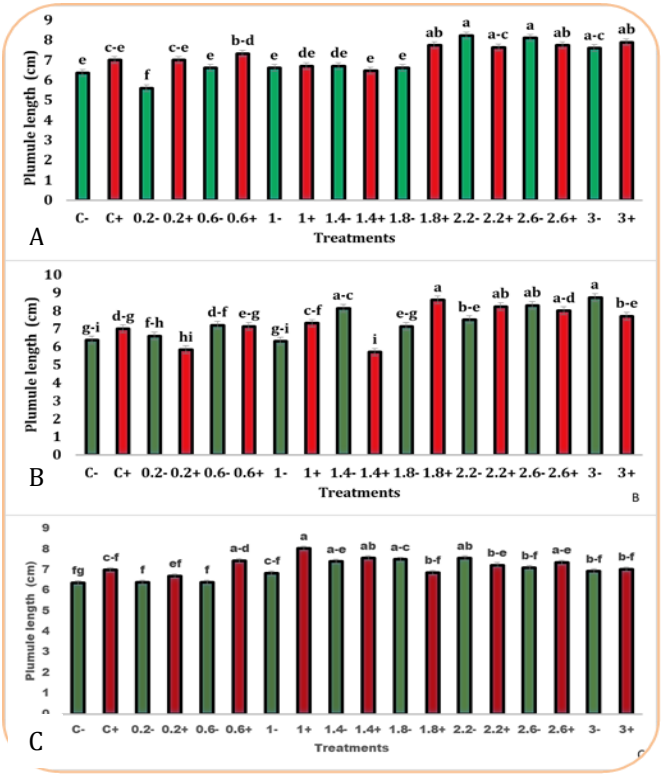

Figure 3. Allelopathic effect of *E. colona* aqueous extracts from inflorescence (A), shoot (B), and root (C), with or without PGPR, on rice plumule length. Vertical bars represent the standard error of the mean of four replicates. Values with different letters above the bars indicate significant differences (P ≤ 0.05) according to LSD test.

Similarly, under stress induced by *E. colona* shoot powder (Figure 4B), all positive (inoculated) treatments significantly improved rice seedling fresh weight compared with the corresponding negative (uninoculated) treatments. Biomass improvement ranged from −24.2% to 42%, indicating a mitigating effect of *B. subtilis* ZMR4 against shoot-derived allelopathic stress. Differences among treatments were statistically analyzed using LSD test at $P < 0.05$.

Under root powder-induced stress (Figure 4C), inoculation with *B. subtilis* ZMR4 also significantly increased the fresh weight of rice seedlings compared with the respective negative controls, with improvements ranging from 10% to 38%. These findings collectively demonstrate the role of *B. subtilis* ZMR4 in

alleviating allelopathic stress caused by different plant parts of *E. colona* and enhancing rice seedling biomass.

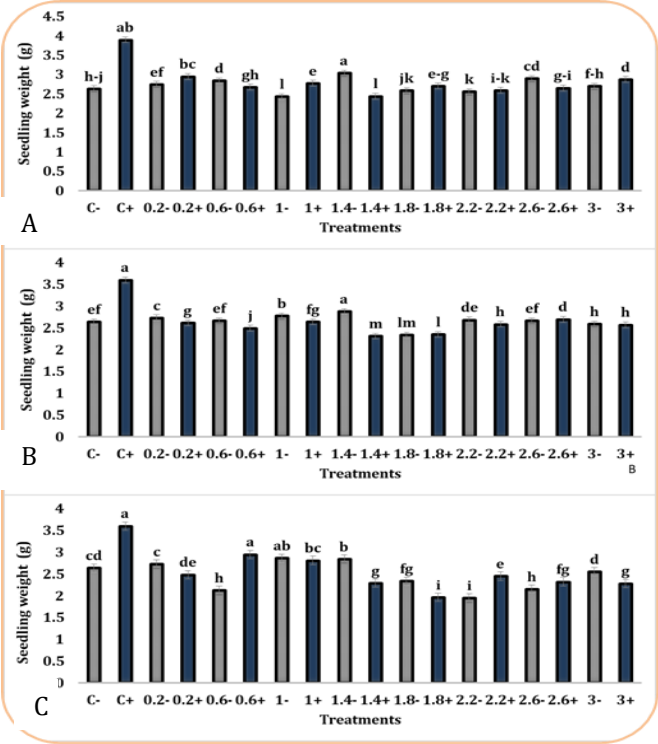

Figure 4. Allelopathic effects of *E. colona* aqueous extracts from inflorescence (A), shoot (B), and root (C), with and without PGPR treatment, on the fresh weight of rice seedlings. Vertical bars represent the standard error of the mean (n = 4). Different letters above the bars indicate significant differences at P ≤ 0.05, as determined by LSD test.

Biological significance of *B. subtilis* on the dry weight of rice seedlings under the allelopathic influence of *E. colona* inflorescence, shoot, and root

Control-positive treatments (C^+) increased the dry weight of rice seedlings to 0.89 g compared with 0.72 g in control-negative treatments (C^-), representing a 24% increase over the negative control. All positive treatments enhanced the dry weight of rice seedlings relative to their corresponding negative treatments, with changes ranging from –15% to 39% (Figure 5A). These results indicate the involvement of *B. subtilis* strain ZMR4 in promoting rice growth under stress induced by dried powder of *E. colona* inflorescences.

Similarly, under allelopathic stress caused by dried shoot powder of *E. colona*, all positive treatments increased seedling dry weight by 13% to 44% compared with the respective negative controls (Figure 5B), further demonstrating the growth-promoting role of *B. subtilis* ZMR4.

Moreover, in the presence of dried root powder of *E. colona*, control-positive treatments enhanced the dry weight of rice seedlings by 13% to 68% over control-negative treatments (Figure 5C). This substantial improvement confirms the effectiveness of *B. subtilis* ZMR4 in mitigating allelopathic stress and enhancing rice seedling growth.

Significant differences among treatments are indicated by different letters above the bars, as determined by the LSD test at $P < 0.05$.

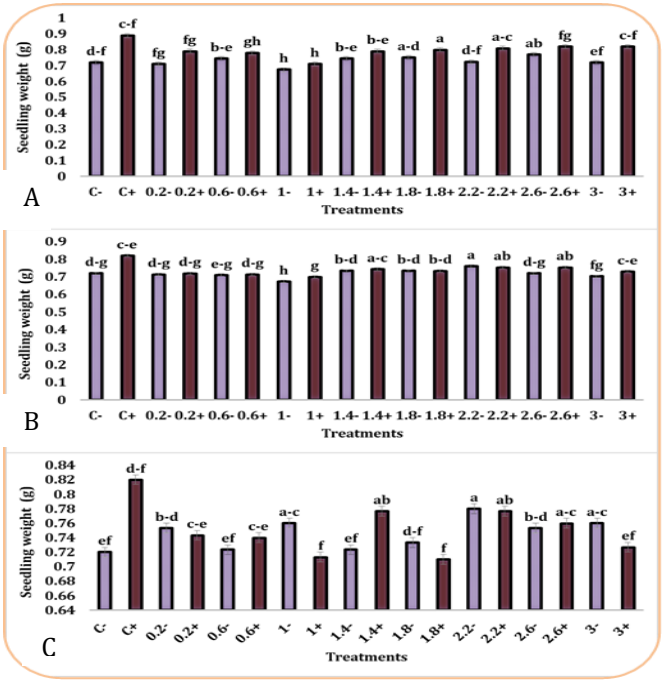

Figure 5. Allelopathic effects of *E. colona* aqueous extracts from inflorescence (A), shoot (B), and root (C), and the influence of PGPR on the dry weight of rice seedlings. Vertical bars represent the standard errors of the mean from four replicates. Different letters above the bars indicate statistically significant differences (P ≤ 0.05) according to LSD test.

Cluster heat map

Hierarchical clustering along the vertical axis grouped the treatments into two distinct clusters. The first cluster comprised the *B. subtilis* ZMR4-treated sets (with and without weeds), which were predominantly green, indicating enhanced growth performance. The second cluster included the non-*B. subtilis* ZMR4 treatments, where the weed-free control (C–) exhibited

neutral/yellow values, while the weedy-stressed control (C+) showed predominantly red/orange values, reflecting severe growth inhibition as shown in Figure 6.

Plant components (flower, shoot, and root) generally exhibited values close to the maximum range (9-10 out of 10) under *B. subtilis* ZMR4 treatments. Highly responsive parameters included radicle length, plumule length, and seedling fresh and dry weight. A significant promotive effect was observed, particularly under weed stress. Treatments such as 1.8+ (8.6 cm shoot length), 3– (8.73 cm shoot length), and 1– (8.23 cm flower length) produced the longest plumules, frequently exceeding both control groups (C– and C+). Root growth was also markedly enhanced. The 0.2%– treatment produced the longest roots (7.9 cm), while other treatments such as 0.6%+ (7.63 cm root length) and 1%+ (8.03 cm shoot length) also demonstrated substantial positive effects.

Seedling fresh weight, an indicator of tissue hydration and overall vigor, showed considerable variation among treatments. Notably, only specific PGPR treatments achieved equal or superior performance: 1.4%+ (3.03 g shoot fresh weight) and 0.6%+ (2.95 g root fresh weight). In contrast, dry weight values were tightly clustered (0.67–0.78 g), indicating limited variation across treatments and plant organs.

The most pronounced color shifts were observed in biomass-related traits. Deep green coloration in the *B. subtilis* ZMR4 + *E. colona* treatments indicated that inoculation most effectively mitigated the adverse effects of weed competition on biomass accumulation and photosynthetic capacity. Moderately responsive parameters, including root length, shoot length, and seedling vigor index, exhibited moderate to strong green coloration in *B. subtilis* ZMR4 treatments, demonstrating substantial improvement in seedling architecture and overall vigor.

In contrast, germination percentage was the least responsive parameter, showing minimal variation across treatments. Although PGPR application markedly improved post-germination growth and vigor, its effect on final germination count was comparatively limited. The intense red coloration observed in biomass and growth parameters under weedy stress confirmed the strong inhibitory impact of weed competition. Conversely, treatments combining *B. subtilis* ZMR4 and *E. colona* displayed clear shifts toward green, indicating a pronounced protective effect, with PGPR inoculation largely compensating for growth suppression caused by weed stress.

Correlation patterns

The heat map further indicated strong positive correlations among biomass and growth parameters, as they clustered closely and exhibited similar treatment responses. This pattern suggests that the growth-promoting effect of PGPR is systemic, simultaneously enhancing multiple physiological and morphological aspects of plant development.

Discussion

The present study demonstrates that allelopathic extracts of *E. colona* significantly inhibited rice germination and early seedling growth. Marked reductions in root and shoot length, as well as fresh and dry biomass, were observed in seedlings treated with powdered extracts derived from the root, shoot, and inflorescence of *E. colona* (Trang et al., 2024). These findings are consistent with previous reports identifying *E. colona* as a highly competitive and toxic weed capable of releasing phytotoxic compounds that interfere with cell division and disrupt essential physiological processes in neighboring plants (Chauhan and Johnson, 2011; Mahajan and Chauhan, 2023a).

Published literature confirms that aqueous extracts of *E. colona* suppress rice germination, biomass accumulation, and root and shoot elongation. This inhibition is primarily attributed to allelochemicals such as phenolics, flavonoids, and alkaloids released from decomposing weed residues, which impair cell division, nutrient uptake, membrane integrity, and enzyme activity in recipient plants (Chauhan and Johnson, 2011; Mahajan and Chauhan, 2023b). The observed concentration-dependent inhibition further substantiates the allelopathic nature of the stress imposed by *E. colona*.

In contrast, inoculation with *B. subtilis* ZMR4 markedly alleviated the deleterious effects of allelopathy. Notably, germination was fully restored to 100% under bacterial treatment, suggesting that the strain may produce enzymes capable of degrading or detoxifying allelochemicals. The significant improvements in root and shoot growth and biomass indicate classical PGPR activity. Such effects likely arise from multiple mechanisms, including phytohormone production, enhanced nutrient solubilization, and modulation of stress-related pathways (Kumar et al., 2024b; Iqbal et al., 2025). The ability of *B. subtilis* ZMR4 not only to mitigate allelopathic stress but also to promote growth beyond stressed conditions represents a significant outcome of this study.

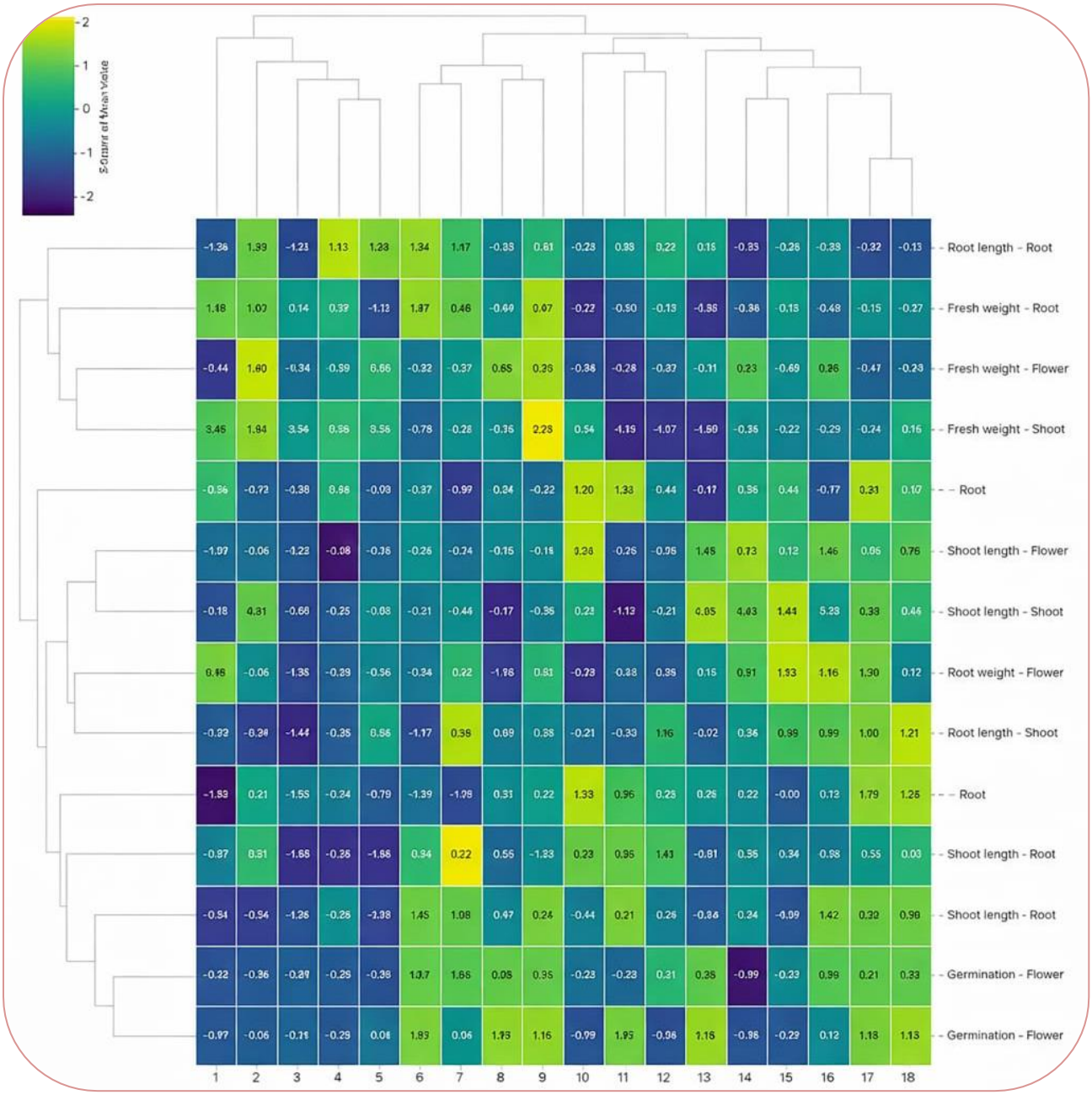

Figure 6. Overall impact of PGPR treatment on rice seedling growth under weed-induced stress. The color gradient, from deep red (strongest inhibition) to dark green (strongest promotion), illustrates treatment efficacy and highlights the growth parameters most affected.

The enhancement of root length, shoot length, and biomass under stress following ZMR4 application may be attributed to the production of indole-3-acetic acid (IAA), which stimulates root proliferation and branching (Kumar et al., 2019; Zia et al., 2024). Moreover, the bacterium likely increases the bioavailability of essential nutrients such as phosphorus, potassium, and iron through siderophore production and nutrient solubilization mechanisms (Sethi et al., 2025). Improved nutrient acquisition has been consistently associated with increased biomass production (Kumar et al., 2024a). Seed biopriming with *B. subtilis* may also activate plant defense responses, enhancing tolerance to allelopathic stress (Singh et al., 2024).

The heatmap analysis further highlights the concentration-dependent action of *B. subtilis* ZMR4. Mid-

range inoculum concentrations (1.0-1.8%) produced the most consistent and significant growth enhancements, particularly in radicle and plumule length. This response aligns with the hormesis principle, whereby moderate levels of a biological stimulus elicit beneficial effects, while suboptimal or excessive levels may be ineffective or suppressive (Calabrese and Mattson, 2017). Optimal bacterial concentrations likely ensure effective rhizosphere colonization and metabolite production without triggering excessive plant defense responses or competitive stress (Vejan et al., 2016).

A hierarchical response among growth parameters was evident. Root and shoot elongation showed the greatest responsiveness to PGPR treatment, whereas dry biomass accumulation exhibited comparatively stable changes. This pattern corresponds with known PGPR-mediated auxin activity, particularly IAA, which primarily regulates cell division and elongation (Glick, 2012). Enhanced root architecture likely improves resource foraging capacity, conferring a competitive advantage under weed pressure (Kang et al., 2019). The relatively moderate changes in dry weight suggest that structural biomass accumulation may require longer periods to reflect significant differences compared to rapid cell expansion and water uptake reflected in fresh weight.

The ability of *B. subtilis* ZMR4 to improve rice growth under *E. colona*-induced stress underscores its potential as a biocontrol and bioenhancement agent. Allelopathic effects of *E. colona* through root exudates and decaying residues are well documented (Chauhan and Johnson, 2011), and the present findings demonstrate that PGPR application significantly mitigates this inhibition. PGPR are known to activate stress-responsive genes and reduce stress-induced ethylene levels through the production of ACC deaminase, thereby preventing growth retardation under adverse conditions (Yang et al., 2009; Glick, 2014). Certain strains also degrade or transform soil allelochemicals, neutralizing weed-derived phytotoxins (Kaur et al., 2020).

Importantly, the most effective PGPR treatments under weed stress (ZMR4+) surpassed the unstressed control (C–) in several growth indices. This suggests that *B. subtilis* ZMR4 provides benefits beyond simple stress alleviation, potentially enhancing competitive fitness through improved root architecture and accelerated shoot growth. These findings have significant implications for sustainable agriculture, indicating that PGPR-based strategies can be integrated into weed management programs to reduce reliance on synthetic herbicides and promote environmentally friendly crop production systems (Kumar et al., 2021).

Conclusion

The present investigation confirms that extracts of *E. colona* significantly suppress rice germination and early seedling growth through allelopathic effects. However, inoculation with *B. subtilis* ZMR4 effectively mitigated this stress, restoring germination to 100% and significantly improving root length, shoot length, and biomass accumulation (up to 34.56%).

Optimal bacterial concentrations (1.0-1.8%) produced the most consistent growth enhancement, indicating a concentration-dependent physiological response. These results demonstrate that Plant Growth-Promoting Rhizobacteria represent an environmentally friendly and sustainable strategy for improving crop establishment under weed-induced allelopathic stress.

Overall, *B. subtilis* ZMR4 shows strong potential as a bio-agent for alleviating allelopathy and enhancing rice resilience against *E. colona*, offering a promising component for integrated and sustainable weed management systems.

Author Contribution

NJ, BT, SS, SN, SG, SSA, and MH collectively contributed to the conceptualization and design of the study. They were actively involved in the implementation of the experimental work, data collection, and laboratory/field investigations. All authors participated in data analysis and interpretation of the results. Furthermore, they contributed to drafting, reviewing, and critically revising the manuscript for important intellectual content. All authors read and approved the final version of the manuscript prior to submission.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

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