



RESEARCH ARTICLE

Microbial bio-priming enhances germination, growth, and vigour indices in rice (*Oryza sativa*) varieties

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ABSTRACT

Seed priming is a vital pre-sowing strategy that enhances metabolic readiness and stress tolerance in crops. This study investigated the efficacy of bio-priming with *Pseudomonas fluorescens* on four paddy varieties (ADT 53, ASD 16, CO 51, and CO 55) using concentrations of 0%, 15%, 20%, and 25% for 12 hours. The impact was assessed on germination percentage, seedling growth, biomass accumulation, and seedling vigour indices. Results revealed that bio-priming significantly influenced early developmental parameters, though responses were variety-specific. Germination improved noticeably in ADT 53, CO 51, and CO 55 at 15% concentration, with CO 51 showing the greatest increase (from 75% to 95%). In contrast, ASD 16 performed best under control conditions (90%), indicating sensitivity to microbial load. Greater shoot length and root elongation were observed at 20% in ADT 53 and CO 51, whereas CO 55 responded strongly at 15%. Biomass accumulation was consistently higher in primed seeds, particularly at moderate concentrations. Seedling Vigour Index I (SVI-I) reached maximum values in ADT 53 at 20% (3741.33) and CO 55 at 25% (4349.17), confirming enhanced elongation under microbial priming. Seedling Vigour Index II (SVI-II) was highest in ASD 16 at 15% (14.80), followed by ADT 53 at 20% (10.54), reflecting robust dry matter accumulation. *P. fluorescens* bio-priming emerges as a cost-effective, eco-friendly strategy to improve germination, seedling vigour, and early establishment in rice. However, optimal concentration must be tailored to the varietal genetic background to avoid inhibitory effects, underscoring the importance of genotype-specific standardization in sustainable rice cultivation.

Keywords: Bio-priming, *Pseudomonas fluorescens*, Germination, Seedling growth, *Oryza sativa*, Seedling vigour, Sustainable Agriculture.

INTRODUCTION

Rice (*Oryza sativa* L.) is the most widely consumed staple cereal, contributing nearly 20% of global dietary energy and serving as the primary caloric source for more than half of the world's population (FAO, 2021). Its central role in food security underscores the importance of optimizing early growth stages, which are frequently constrained by uneven germination, poor seedling establishment, and vulnerability to both abiotic and biotic stresses (Farooq et al., 2019; Ramanathan et al., 2018). Abiotic stressors such as salinity, drought, and temperature extremes impair water uptake and metabolic activation (Ashokkumar et al., 2013; Kumar et al., 2013; Ashokkumar et al., 2023; Vivekanandhan et al., 2024), while biotic challenges, including blast (*Magnaporthe oryzae*) and sheath blight (*Rhizoctonia solani*), reduce seedling vigour and yield potential (Jabran et al., 2018). Addressing these constraints is critical for the sustainable intensification of rice production systems.

Seed priming has emerged as a physiological strategy to enhance seed performance under stress-prone environments (Ashokkumar et al., 2026). Defined by Heydecker (1973) as controlled hydration that initiates metabolic activity without radicle protrusion, priming accelerates germination, synchronizes seedling emergence, and improves resilience to environmental stressors. Conventional priming methods, such as hydro-priming and halo-priming, have been shown to improve seed vigour, nutrient mobilization, and stress tolerance (Singh et al., 2016; Bajpai et al., 2017). However, these approaches primarily address physiological activation and lack the biological dimension necessary to combat pathogens and enhance rhizosphere interactions.

Biopriming represents a significant advancement by integrating hydration with inoculation of beneficial microorganisms, thereby combining physiological activation with biological protection (Mahmood et al., 2016; Kanishka & Ashokkumar, 2026; Ashokkumar et al., 2026). Plant Growth-Promoting Rhizobacteria (PGPR) such as *Pseudomonas fluorescens* are particularly effective biopriming agents due to their multifaceted mechanisms of action. They colonize the rhizosphere, produce phytohormones such as indole-3-acetic acid (IAA), solubilize phosphorus and other nutrients, and secrete siderophores that limit pathogen growth (Glick, 2014). Moreover, PGPR trigger induced systemic resistance (ISR), priming the plant's immune system against major rice pathogens, including blast and sheath blight (Bhattacharyya & Jha, 2012). This dual role, enhancing seed physiology while fortifying disease resistance—positions biopriming as a sustainable alternative to chemical seed treatments.

From an ecological perspective, biopriming reduces reliance on synthetic agrochemicals, thereby lowering production costs and mitigating environmental contamination (Jabran et al., 2018). It also promotes microbial diversity in the rhizosphere, enhancing soil health and nutrient cycling, which are essential for long-term productivity in rice-based cropping systems (Mahmood et al., 2016). In addition, biopriming aligns with global sustainability goals by supporting climate-resilient agriculture and reducing greenhouse gas emissions associated with fertilizer use (FAO, 2025).

Despite these advantages, systematic evaluations of biopriming efficiency across diverse rice varieties remain limited. Most previous studies have focused on generalized physiological improvements, with insufficient attention to variety-specific compatibility, optimal microbial dosage, and mechanistic insights into seedling vigour indices. Addressing this gap, the present study investigates the comparative effects of control (unprimed), hydro-priming, and biopriming with *Pseudomonas fluorescens* on rice seeds. Specifically, the objectives are to evaluate the influence of varying concentrations (15%, 20%, and 25%) of *Pseudomonas fluorescens* on the germination rates of four popular paddy varieties (ADT 53, ASD 16, CO 51, and CO 55), to determine the optimal bacterial dosage for maximizing seedling vigour, including shoot length, root development, and biomass accumulation, and to assess the variety-specific compatibility of biopriming to provide tailored agricultural recommendations. By bridging microbiology and traditional agronomy, this study introduces a novel, cost-effective protocol to enhance rice productivity through microbial bio-priming, thereby contributing to the sustainable intensification of rice cultivation.

MATERIALS AND METHODS

Materials

The laboratory experiment was carried out in March 2025 at the Genetics and Plant Breeding Laboratory, School of Agriculture and Animal Sciences, The Gandhigram Rural Institute – Deemed to be University, Gandhigram, Dindigul, Tamil Nadu, India. A completely randomized design (CRD) with three replications was

adopted to ensure statistical validity. Certified seeds of four high-yielding rice (*Oryza sativa* L.) varieties, ADT 53 (medium slender grain, 105–110 days duration), ASD 16 (short bold grain, 110–115 days duration), CO 51 (long slender grain, 105–110 days duration), and CO 55 (short bold grain, 115 days duration), were procured from the Tamil Nadu Rice Research Station, Aduthurai, Thanjavur and ICAR- KVK, Dindigul, Tamil Nadu. These varieties were selected to represent diverse genetic backgrounds and grain types, thereby allowing assessment of variety-specific responses to biopriming.

Preparation of biopriming solution and seed priming process

The biological agent used for priming was *Pseudomonas fluorescens*, obtained from the Department of Agricultural Microbiology, TNAU. Bio-priming solutions were prepared at three concentrations (15%, 20%, and 25%) by mixing the corresponding volumes of bacterial culture with sterile distilled water, while distilled water alone served as the hydropriming control. For each treatment, 50 g of seeds per variety were soaked in the respective solution for 12 hours to allow controlled hydration and microbial colonization of the seed coat. Following soaking, the seeds were air-dried for 1 hour to reduce surface moisture before sowing.

Experimental setup

Two experimental methods were employed to evaluate germination and seedling growth. In the Petri dish method, twenty seeds per treatment were placed in sterilized Petri dishes lined with moist blotting paper to determine germination percentage. In the roll towel method, twenty seeds per treatment were placed between moistened germination papers, rolled, and incubated to assess seedling growth parameters. The germination setup was maintained in a seed germinator at $25 \pm 1^\circ\text{C}$ with 95% relative humidity for 7 days.

Data collection and Analysis

Seedling parameters were recorded seven days after sowing, following the methods of Venothini et al. (2024), Ashokkumar et al. (2024), and Rajangam et al. (2024). Germination percentage, shoot length (cm), root length (cm), fresh weight (g), and dry weight (g) were measured. Fresh and dry biomass weights were determined for five seedlings per treatment, with dry weight measured after oven-drying at 70°C for 48 hours. Seedling vigour indices were calculated according to Abdul-Baki and Anderson (1973), where vigour index I was obtained by multiplying germination percentage by seedling length, and vigour index II was obtained by multiplying germination percentage by seedling dry weight. The overview of biopriming with *Pseudomonas fluorescens*, illustrating its effects on germination and early seedling vigour in four rice varieties, was presented in Figure 1. All recorded data were subjected to statistical analysis using the Web Agri Stat Package (WASP 2.0) (Jangam & Tahli, 2004). Treatment means were compared, and the critical difference was calculated at the 5% level of significance to interpret treatment effects.

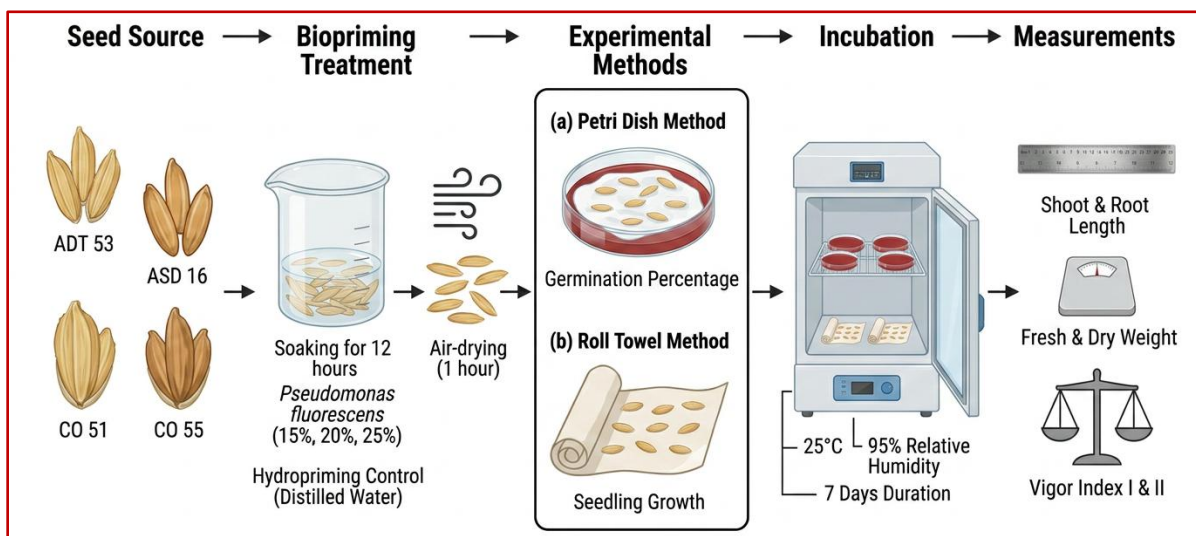


Figure 1. Diagrammatic overview of bio-priming with *Pseudomonas fluorescens* illustrating its influence on germination and early seedling vigour in four rice varieties.

RESULTS AND DISCUSSION

The experimental data reveal a complex relationship between *Pseudomonas fluorescens* concentration and the physiological responses of different rice varieties. The results are categorized into germination efficiency and seedling growth parameters, including root length, shoot length, and biomass accumulation. Bio-priming significantly influenced germination, but the effect was dosage-dependent. At 15% concentration, most varieties showed peak germination (Table 1). For example, CO 51 increased from 75% (control) to 95%, while ADT 53 improved from 85% to 95%. Similarly, CO 55 recorded 90% germination at 15% compared to 80% in the control. In contrast, ASD 16 was an outlier, achieving its highest germination (90%) in the control group, with a decline under bio-priming treatments (85% at 15% and 80% at 25%). This suggests a potential inhibitory interaction between ASD 16 and the bacterial inoculum. Across all varieties, increasing concentrations to 20% and 25% led to a gradual decline in germination, indicating that while low concentrations stimulate metabolic repair, higher concentrations may induce osmotic stress or microbial saturation, hindering radicle emergence (Mitra et al., 2020; Sharma et al., 2022).

Table 1. Effect of *Pseudomonas fluorescens* based seed biopriming on germination percentage in four rice varieties

Variety	Control	15%	20%	25%
ADT 53	85 ^b	95 ^a	90 ^{ab}	85 ^b
ASD 16	90 ^a	85 ^b	85 ^b	80 ^c
CO 55	80 ^c	90 ^a	85 ^b	80 ^c
CO 51	75 ^c	95 ^a	90 ^{ab}	90 ^{ab}
CV	5.10	5.12	5.08	5.05
CD (≤ 0.05)	4.20	4.32	4.25	4.18

Note: Means followed by the same superscript letter within a row are not significantly different according to DMRT at 5% probability level. CD = Critical Difference; CV = Coefficient of Variation.

Shoot Length

Shoot growth varied among varieties. ASD 16 exhibited the most aggressive elongation, reaching 12.7 cm at 15%, followed by CO 51 at 10 cm. ADT 53 showed moderate growth (8.4 cm at 15%), while CO 55 improved significantly from 5.12 cm (control) to 9.38 cm at 15% (Table 2). However, shoot length declined at higher concentrations, reflecting a threshold beyond which microbial activity may inhibit apical growth. These findings align with earlier reports that PGPR priming enhances shoot elongation by stimulating meristematic activity and nutrient assimilation (Kavitha et al., 2019).

Table 2. Effect of *Pseudomonas fluorescens* based seed biopriming on the shoot length of rice

Variety	Shoot length (cm)				Root length (cm)			
	Control	15%	20%	25%	Control	15%	20%	25%
ADT 53	8.3 ^b	8.4 ^{ab}	7.8 ^b	7.7 ^b	4.04 ^b	4.78 ^a	2.94 ^c	2.20 ^c
ASD 16	10.7 ^b	12.7 ^a	10.3 ^b	7.9 ^c	6.66 ^a	5.80 ^b	5.36 ^b	4.40 ^c
CO 55	5.12 ^c	9.38 ^a	9.2 ^a	7.14 ^b	3.50 ^c	4.56 ^a	3.26 ^b	3.00 ^b
CO 51	8.3 ^b	10.0 ^a	9.2 ^{ab}	8.7 ^{ab}	3.88 ^b	4.46 ^a	4.44 ^a	4.10 ^{ab}
CV	4.85	5.12	5.44	5.20	4.25	4.10	4.32	4.28
CD (≤ 0.05)	0.72	0.74	0.70	0.69	0.68	0.72	0.70	0.69

Note: Means followed by the same superscript letter within a row are not significantly different according to DMRT at 5% probability level. CD = Critical Difference; CV = Coefficient of Variation.

Root Length

Root architecture was most responsive at 20% concentration. ADT 53 recorded 4.78 cm, while ASD 16 reached 5.8 cm (Table 2). CO 51 showed balanced growth across treatments, peaking at 4.44 cm under 20%. CO 55 improved from 3.5 cm (control) to 4.56 cm at 15%. The enhanced root depth and branching provide superior

anchorage and nutrient uptake, granting seedlings resilience under stress conditions. Excessive concentrations (25%) reduced root length, suggesting phytotoxic effects of microbial overload (Saharan & Nehra, 2011).

Total Seedling length

The analysis of total seedling length revealed that bio-priming with *Pseudomonas fluorescens* significantly enhanced elongation in most rice varieties, particularly at moderate concentrations (15–20%) (Table 3). ASD 16 recorded the longest seedlings (18.50 cm at 15%), while CO 55 showed a dramatic improvement from 8.62 cm (control) to 13.94 cm at 15%, representing a 62% increase. These results highlight the role of microbial priming in stimulating root and shoot elongation by enhancing nutrient mobilisation and hormonal regulation. Similar findings were reported by Gull et al. (2021), who demonstrated that rhizobacterial priming enhances seedling vigour by modulating auxin and gibberellin pathways, thereby promoting elongation and early establishment.

However, varietal differences were evident. ADT 53 showed modest gains at 15% but declined at higher concentrations, while ASD 16 exhibited sensitivity to microbial load beyond 15%, with seedling length dropping sharply at 25%. This aligns with the observations of Kavitha et al. (2019), who emphasized that excessive microbial concentrations can impose osmotic stress, reducing elongation despite initial gains. The consistency of CO 51 across treatments further supports the idea that varietal genetic background determines the threshold for optimal microbial stimulation. Collectively, these findings reinforce the conclusion that bio-priming is a cost-effective and eco-friendly strategy, but the concentration must be carefully calibrated for each genotype to maximise seedling growth and vigour.

Table 3. Effect of *Pseudomonas fluorescens* based seed biopriming on the total seedling length

Variety	Total seedling length (cm)			
	Control	15%	20%	25%
ADT 53	12.34 ^b	13.18 ^{ab}	10.74 ^b	9.90 ^b
ASD 16	17.36 ^a	18.50 ^a	15.66 ^b	12.30 ^c
CO 55	8.62 ^c	13.94 ^a	12.46 ^b	10.14 ^b
CO 51	12.18 ^b	14.46 ^a	13.64 ^a	12.80 ^{ab}
CV	4.85	5.12	5.44	5.20
CD	1.20	1.32	1.28	1.25

Note: Means followed by the same superscript letter within a row are not significantly different according to DMRT at 5% probability level. CD = Critical Difference; CV = Coefficient of Variation.

Biomass Accumulation

Biomass analysis further confirmed the efficacy of bio-priming with *Pseudomonas fluorescens* (Table 4). Fresh biomass weight values remained relatively stable across varieties, but notably higher fresh biomass weights were observed in ASD 16 (0.40 g) and CO 51 (0.40 g) under the 25% treatment, reflecting enhanced hydration and tissue expansion. In contrast, ADT 53 and CO 55 maintained lower ranges (0.20–0.30 g), suggesting limited water uptake. However, fresh weight alone can be misleading, as it primarily reflects tissue hydration rather than true structural growth (Mitra, 2020).

Dry biomass weight, a more reliable indicator of physiological maturity, revealed clearer varietal differences. In ADT 53, dry matter increased significantly from 0.05 g (control) to 0.08 g at 15%, representing a 60% improvement in structural biomass. This highlights efficient conversion of seed reserves into solid tissues, likely mediated by microbial stimulation of nitrogen metabolism and carbohydrate translocation (Glick, 2014). ASD 16, despite consistently high fresh weight, maintained stable dry weight values (0.04–0.08 g), indicating that its biomass gains were largely water-driven rather than structural. CO 55 showed progressive improvement, peaking at 20% (0.07 g), while CO 51 remained relatively stable across treatments (0.04–0.05 g). Together, these results demonstrate that bio-priming enhances biomass accumulation, particularly at moderate concentrations (15–20%), where structural growth is maximized. The distinction between fresh and dry weight underscores the importance of evaluating both parameters: fresh weight reflects hydration status, while dry weight confirms true physiological development and seedling vigour.

Table 4. Effect of *Pseudomonas fluorescens* based seed biopriming on the fresh and dry biomass weight of rice.

Variety	Fresh biomass weight (g)				Dry biomass weight (g)			
	Control	15%	20%	25%	Control	15%	20%	25%
ADT 53	0.3 ^b	0.3 ^b	0.2 ^b	0.2 ^b	0.05 ^a	0.08 ^a	0.03 ^b	0.05 ^a
ASD 16	0.4 ^a	0.4 ^a	0.2 ^b	0.4 ^a	0.04 ^b	0.08 ^a	0.05 ^{ab}	0.05 ^a
CO 55	0.2 ^c	0.2 ^c	0.2 ^b	0.2 ^b	0.04 ^b	0.06 ^b	0.07 ^a	0.04 ^{ab}
CO 51	0.3 ^b	0.3 ^b	0.3 ^a	0.4 ^a	0.05 ^a	0.05 ^{bc}	0.05 ^{ab}	0.04 ^{ab}
CV	4.80	5.10	4.95	5.05	4.20	4.35	4.25	4.30
CD (≤ 0.05)	0.05	0.06	0.05	0.06	0.01	0.02	0.02	0.02

Note: Means followed by the same superscript letter within a row are not significantly different according to DMRT at 5% probability level. CD = Critical Difference; CV = Coefficient of Variation.

Seedling Vigour Index I (SVI-I)

Seedling Vigour Index I, which integrates germination percentage and seedling length, showed clear varietal and treatment-specific differences (Table 5). In ADT 53, the highest SVI-I values were recorded at 15% (3707.20) and 20% (3741.33), both significantly superior to the control (2193.25). This indicates that moderate concentrations of *Pseudomonas fluorescens* effectively enhance seedling vigour by promoting elongation and balanced germination. In ASD 16, SVI-I peaked at 20% (3994.52) and 25% (4020.00), while the control also maintained a high baseline (3378.50). This suggests that ASD 16 responds better to higher concentrations, though the 15% treatment was comparatively less effective. For CO 55, the maximum SVI-I was observed at 25% (4349.17), followed by 15% (4041.18), both significantly higher than the control (3415.87). This demonstrates a strong positive response to microbial priming, particularly at higher concentrations. In CO 51, SVI-I values were highest at 15% (3994.52), followed by 20% (3611.87). The control (2893.23) and 25% (3415.87) were comparatively lower, highlighting the optimal effect of moderate concentrations. These findings are consistent with Gull et al. (2021), who reported that rhizobacterial priming significantly enhances seedling vigour by modulating nutrient uptake and hormonal signalling pathways.

Seedling Vigour Index II (SVI-II)

Seedling Vigour Index II (SVI-II), which integrates total seedling length and dry biomass, provided a reliable measure of physiological maturity across treatments (Table 5). In ADT 53, vigour peaked at 15% (10.54), nearly double the control (5.60), confirming that moderate microbial priming enhanced structural biomass accumulation. However, values declined sharply at 20% (3.22) and 25% (4.95), suggesting that excessive inoculum may inhibit metabolic efficiency. ASD 16 exhibited the highest vigour overall, with a maximum at 15% (14.80), followed by the control (6.94). Although seedling length was consistently high, dry matter accumulation was optimized only under moderate priming, while higher concentrations reduced vigour (7.83 at 20% and 6.15 at 25%). This indicates sensitivity to microbial load, consistent with genotype-specific responses. In CO 55, vigour improved substantially under priming, rising from 3.45 (control) to 8.36 at 15% and 8.72 at 20%. These results highlight the strong responsiveness of CO 55 to microbial stimulation, particularly at moderate concentrations. CO 51 showed balanced performance, with modest gains at 15% (7.23) and 20% (6.82), while vigour declined at 25% (5.12).

Furthermore, the data confirm that bio-priming enhances physiological maturity and dry matter accumulation in rice seedlings, but the optimal concentration varies by genotype. Moderate levels (15–20%) consistently improved vigour indices, while higher concentrations tended to reduce efficiency. These findings resonate with previous studies emphasizing that microbial priming enhances nutrient mobilization and hormonal activity, but excessive inoculum can impose stress and reduce structural biomass (Kavitha et al., 2019; Sharma et al., 2022; Glick, 2014).

Table 5. Effect of *Pseudomonas fluorescens* based seed biopriming on the Seedling Vigour Index I and II of rice varieties

Variety	Vigour Index I				Vigour Index II			
	Control	15%	20%	25%	Control	15%	20%	25%
ADT 53	2193.25 ^b	3707.20 ^a	3741.33 ^a	1568.00 ^c	5.60 ^b	10.54 ^a	3.22 ^c	4.95 ^b
ASD 16	3378.50 ^a	2893.23 ^b	3994.52 ^a	4020.00 ^a	6.94 ^c	14.80 ^a	7.83 ^b	6.15 ^c
CO 55	3415.87 ^b	4041.18 ^a	3611.87 ^{ab}	4349.17 ^a	3.45 ^c	8.36 ^a	8.72 ^a	4.06 ^b
CO 51	2893.23 ^b	3994.52 ^a	3611.87 ^{ab}	3415.87 ^b	6.09 ^b	7.23 ^a	6.82 ^a	5.12 ^b
CV	4.85	4.92	5.12	5.05	4.20	4.35	4.28	4.30
CD (≤ 0.05)	120.5	132.4	128.6	125.2	1.12	1.25	1.18	1.20

Note: Means followed by the same superscript letter within a row are not significantly different according to DMRT at 5% probability level. CD = Critical Difference; CV = Coefficient of Variation.

CONCLUSION

The present study highlights the potential of *Pseudomonas fluorescens*-based seed bio-priming in enhancing seed quality attributes in rice. Specifically, concentrations of 15% and 20% proved most effective in improving germination percentage, shoot length, root length, and biomass accumulation in varieties such as ADT 53 and CO 51. ADT 53 demonstrated a notable increase in dry matter accumulation at 15%, while CO 51 showed balanced improvements across germination and seedling vigour traits. In contrast, ASD 16 exhibited maximum germination in the control, with reduced performance under bio-priming, suggesting genotype-specific sensitivity to microbial load. CO 55 responded positively at a 15% concentration, particularly for shoot and root elongation, though higher concentrations reduced efficacy. Together, bio-priming with *P. fluorescens* emerges as a powerful, eco-friendly tool for modern paddy cultivation. A concentration of 15–20% is recommended based on the present laboratory experiment for most varieties to achieve the best balance between germination speed and seedling vigour. In contrast, alternative strategies or lower concentrations may be more suitable for sensitive genotypes such as ASD 16. These findings highlight the importance of tailoring bio-priming protocols to varietal responses, thereby supporting sustainable rice production and resilient crop establishment.

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AUTHORS' CONTRIBUTIONS

R.M., S.R., G.A., S.R.S., C.M.D., V. N., M.M.G., and P.B. investigated the experiments. K.A. conceived the idea and wrote the manuscript. All the authors reviewed and accepted the final manuscript for publication.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

Not applicable.

CONSENT FOR PUBLICATION

Not Applicable

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AVAILABILITY OF DATA AND MATERIALS

All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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