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Fermented Rice Water: A Source of Plant Growth-Promoting Microorganisms

Vrushali Jitendra Patil[1], Savanta V. Raut[1*], Tanvi Sawant[1]
[1]*Department of Microbiology, M.M. College of Arts, N.M. Institute of Science, H.R.J. College of Commerce, Bhavans College, Mumbai, India*

***Abstract*: The increasing demand for sustainable agricultural practices has intensified research on eco-friendly biofertilizers derived from natural resources. Fermented rice water (FRW), a common household by-product generated during rice washing, is rich in soluble nutrients and indigenous microbial populations. The present study investigates fermented rice water as a potential source of plant growth- promoting microorganisms (PGPM) and evaluates its effect on the growth of Coriandrum sativum L. Washed rice water was allowed to undergo natural fermentation for three days under ambient laboratory conditions. A significant reduction in pH from 7.5 to 5.0 indicated active microbial metabolism. Fourteen morphologically distinct isolates were obtained using selective and non-selective media. The isolates were screened for plant growth-promoting traits including phosphate solubilization, cellulase activity, and indole-3-acetic acid (IAA) production. Among them, isolates I1, I8, I11, and I12 exhibited comparatively higher IAA production and were selected for consortium development after compatibility testing. Pot trials conducted under sterile and non-sterile soil conditions demonstrated enhanced germination rate, root length, and shoot length in treated plants compared to control. In non-sterile soil, seed soaking treatment showed maximum root elongation (5.3 cm), while microbial consortium application was more effective under sterile soil (3.5 cm). The findings suggest that fermented rice water serves as an inexpensive and sustainable reservoir of beneficial microorganisms with significant potential as a biofertilizer in sustainable agriculture.**

***Keywords*: Fermented rice water, plant growth-promoting microorganisms, microbial consortium, IAA, coriander, sustainable agriculture**

1. Introduction

Modern agriculture has achieved high productivity through the extensive use of chemical fertilizers. However, prolonged and excessive application of synthetic fertilizers has led to soil nutrient imbalance, loss of beneficial soil microbiota, reduced soil fertility, and environmental contamination. These challenges have prompted the scientific community to explore biological alternatives that are environmentally safe and economically feasible.

Plant growth-promoting microorganisms (PGPM) are naturally occurring soil microbes that enhance plant growth through multiple direct and indirect mechanisms. These include nitrogen fixation, phosphate solubilization, production of phytohormones, siderophore production, and suppression of plant pathogens. Among phytohormones, indole-3-acetic acid (IAA) plays a vital role in stimulating root elongation and lateral root formation, thereby improving nutrient absorption.

Washed rice water (WRW) is routinely discarded in households despite being rich in soluble starch, amino acids, vitamins, and minerals such as nitrogen, phosphorus, potassium, and calcium. Rice grains naturally harbor diverse microbial communities on their surface. When WRW is stored under ambient conditions, spontaneous fermentation occurs, resulting in microbial proliferation and biochemical transformation of nutrients. This fermentation process not only enhances nutrient availability but also enriches beneficial microbial populations.

Fermented rice water has traditionally been used in some agricultural practices; however, its scientific evaluation as a source of plant growth-promoting microorganisms remains limited. Therefore, this study aims to isolate and characterize beneficial microorganisms from fermented rice water and assess their impact on the growth of Coriandrum sativum L., a fast-growing and economically important crop widely cultivated for its leaves and seeds.

2. Materials & Methods

A. Fermentation of Washed Rice Water (WRW)

One hundred grams of rice were washed with 300 mL of sterile distilled water in a sterile beaker for 2 minutes with gentle agitation to facilitate the release of surface nutrients and associated microorganisms. The resulting wash water was aseptically decanted into a sterile BOD bottle. The bottle was loosely covered to allow adequate aeration and subjected to spontaneous fermentation under ambient laboratory conditions (25– 30 °C) for a period of three days.

Fermentation progress was monitored daily by observing changes in pH and odor characteristics. The initial pH of the freshly prepared washed rice water prior to fermentation was recorded as 7.5. Gradual changes in pH and development of a characteristic fermented odor were considered indicative of microbial activity and fermentation progression.

B. Isolation of Microorganisms from Fermented Rice Water

On the third day of fermentation, fermented rice water samples were aseptically streaked onto Nutrient Agar (NA), de Man Rogosa and Sharpe (MRS) agar, Sabouraud Dextrose Agar

*Corresponding author: svrmicro@yahoo.co.in

(SDA), and Ashby's nitrogen-free agar plates (approximately 50 mL medium per batch). The plates were incubated under suitable conditions for bacterial and fungal growth. After incubation, morphologically distinct colonies differing in size, shape, color, elevation, and margin were selected and purified by two successive streakings to obtain pure cultures. The purified isolates were maintained on agar slants at 4 °C for short-term storage and preserved in 20% glycerol stocks at −20 °C for long-term storage.

Preliminary characterization of bacterial isolates included Gram staining to determine cell wall characteristics and catalase testing using 3% hydrogen peroxide. Immediate bubble formation was recorded as a positive catalase reaction.

C. Characterization of Isolates for Plant Growth Promoting Microorganisms

1) Phosphate Solubilization Activity on Pikovskaya's Agar Medium

Pikovskaya's (PVK) agar medium was prepared according to standard protocol, sterilized by autoclaving, and aseptically poured into sterile Petri plates. After solidification, selected bacterial isolates were spot-inoculated onto the agar surface under aseptic conditions. The plates were incubated at 28–30 °C for up to seven days.

The plates were observed daily for the formation of clear halo zones surrounding bacterial colonies. The appearance of a transparent zone indicated solubilization of insoluble phosphate present in the medium. Isolates exhibiting larger, distinct, and well- defined halo zones were considered efficient phosphate solubilizers and selected for further analysis.

2) Cellulase Activity (Carboxymethyl cellulose) Agar

Qualitative screening for cellulase activity was carried out using Carboxymethyl Cellulose (CMC) agar plates. The medium was prepared, sterilized, and poured into sterile Petri plates. Selected isolates were inoculated onto the agar surface and incubated at 28–30 °C for 24–72 hours.

Following incubation, the plates were flooded with 0.1% Congo red solution and allowed to stain for 15 minutes. Excess stain was carefully decanted, and the plates were destained using 1 M sodium chloride (NaCl) solution for 15 minutes. The presence of yellowish or clear zones around the colonies against a red background indicated cellulose hydrolysis due to cellulase enzyme productions.

3) Detection of IAA (Indole-3-Acetic Acid) production using (Salkowski reagent)

Indole-3-acetic acid (IAA) production was quantified using the Salkowski colorimetric assay. Pure bacterial isolates were inoculated into 10 mL sterile Luria– Bertani (LB) broth supplemented with 0.1% (w/v) L- tryptophan and incubated at 28 °C for 72 hours under shaking conditions (120 rpm). Following incubation, cultures were centrifuged at 3000 rpm for 15 minutes, and 2 mL of the cell-free supernatant was mixed with 2 mL of freshly prepared Salkowski's reagent (0.5 M $FeCl_3$ in 35% $HClO_4$). The reaction mixture was incubated at room temperature for 25 minutes, and development of pink coloration indicated IAA production. Absorbance was measured at 530 nm using a spectrophotometer against a reagent blank. IAA concentration was determined using a standard curve prepared from known concentrations of pure IAA.

D. Compatibility Test of Selected Isolates

Compatibility among the selected bacterial isolates was assessed using the cross-streaking method on sterile Nutrient Agar plates. In this method, one isolate was streaked centrally on the agar surface and the second isolate was streaked perpendicular to the first streak without touching it directly. The plates were further incubated at 37 °C for 24 hours. After incubation, the plates were examined for the presence or absence of inhibition zones at the intersection of the streaks. The absence of a clear inhibition zone indicated compatibility between the isolates, whereas the formation of a distinct inhibition zone suggested antagonistic interaction. Only compatible isolates were selected for consortium development.

E. Microbial Consortium Development

Compatible isolates were individually cultured in 20 mL sterile Nutrient Broth under shaking conditions until an optical density (OD_{600}) of 0.8–1.0 ($\approx 10^8$ CFU mL^{-1}) was attained to ensure uniform cell density. Equal volumes (5 mL each) of the selected cultures were aseptically combined and homogenized to formulate the microbial consortium. To enhance stability and maintain viability, 1% sterile molasses was incorporated as a nutritive stabilizer. The consortium was stored at 4 °C until use, and its functional efficiency was evaluated through phosphate solubilization, cellulase activity, and IAA production assays.

F. Assessment of Plant Growth Promoting Potential of Microbial Consortium in Coriander (Coriandrum sativum L.)

Coriander (Coriandrum sativum) seeds were surface sterilized to eliminate external contaminants. The seeds were sequentially immersed in 70% ethanol for 1 minute, followed by 1% sodium hypochlorite solution for 2 minutes. Subsequently, the seeds were rinsed five times with sterile distilled water to remove residual disinfectant. Sterilized seeds were air-dried under aseptic conditions for 30–60 minutes prior to sowing. Plastic pots (15 cm diameter) were filled with 2 kg of sterilized and non-sterilized potting mixture to evaluate microbial performance under both controlled and natural soil conditions. Ten seeds were sown uniformly in each pot. The experiment was arranged in a completely randomized design (CRD) with two replicates per treatment. The treatments included:

i. Sterile water application (placebo control),
ii. Seeds soaked in microbial consortium prior to sowing, and
iii. Direct soil application of 20 mL microbial consortium per pot.

Consortium applications were performed weekly for 15 days. Growth parameters such as shoot length, root length, and number of leaves were recorded at weekly intervals. At the time of harvest, plants were carefully uprooted to avoid root damage. The recorded parameters were used to evaluate the growth-promoting efficiency of the developed microbial consortium on coriander.

G. Data Analysis

Data were expressed as mean ± SD and analyzed using ANOVA ($p < 0.05$).

3. Results

A. Isolation of Microorganisms from Fermented Rice Water on Nutrient Agar, Sabouraud Dextrose (SAB) Agar, De Man, Rogosa and Sharpe (MRS) Agar and ASHBY'S Mannitol Agar Medium

A total of microbial isolates were obtained from fermented rice water using different selective and general-purpose culture media. On MRS agar (De Man, Rogosa and Sharpe agar), 4 isolates were recovered, indicating the presence of lactic acid bacteria. Sabouraud Dextrose Agar (SAB agar) yielded 4 isolates, suggesting the presence of fungi or yeasts. Nutrient Agar (NA), a general-purpose medium supporting the growth of non-fastidious bacteria, also produced 4 isolates. Ashby's Mannitol Agar, selective for nitrogen-fixing bacteria such as Azotobacter species and using mannitol as a carbon source, yielded 2 isolates. These results demonstrate that different microbial populations were successfully recovered using specific and non-specific media, highlighting the microbial diversity present in fermented rice water.

Table 1
Isolates obtained on MRS agar

Isolates	Size	Shape	Elevation	Opacity	Colour	Margin	Consistency	Gram Nature	Morphology
1	>1mm	Irregular	Flat	Opaque	White	Irregular	Smooth	Gram positive	Cocci
2	>1mm	Circular	Flat	Opaque	White	Entire	Smooth	Gram positive	Rod
3	1mm	Irregular	Convex	Opaque	White	Irregular	Smooth	Gram positive	Rod
4	1mm	Circular	Flat	Opaque	White	Entire	Smooth	Gram positive	Cocci

Table 2
Isolates obtained on SAB agar

Isolates	Size	Shape	Elevation	Opacity	Color	Margin	Consistency	Gram Nature	Morphology
5	3mm	Circular	Convex	Opaque	Cream	Entire	Smooth	Gram positive	Oval
6	2mm	Circular	Convex	Opaque	White	Entire	Smooth	Gram positive	Oval
7	>1mm	Circular	Flat	Opaque	Off White	Entire	Smooth	Gram positive	Rod
8	4mm	Irregular	Convex	Opaque	Off White	Irregular	Smooth	Gram positive	Rod

Table 3
Isolates obtained on NA agar

Isolates	Size	Shape	Elevation	Opacity	Color	Margin	Consistency	Gram Nature	Morphology
9	1mm	Circular	Convex	Opaque	White	Entire	Smooth	Gram positive	Rod
10	>1mm	Circular	Convex	Opaque	White	Entire	Smooth	Gram positive	Rod
11	1mm	Circular	Flat	Opaque	Cream	Entire	Smooth	Gram positive	Rod
12	>1mm	Irregular	Flat	Opaque	White	Irregular	Smooth	Gram positive	Rod

Table 4
Isolates obtained on ASHBY'S Mannitol agar

Isolates	Size	Shape	Elevation	Opacity	Color	Margin	Consistency	Gram Nature	Morphology
13	1mm	Circular	Flat	Opaque	White	Entire	Smooth	Gram positive	Rod
14	>1mm	Irregular	Flat	Opaque	White	Irregular	Smooth	Gram positive	Cocci

B. Characterization of Isolates for Plant Growth Promoting Microorganisms

1) Phosphate Solubilization Activity on Pikovskaya's Agar medium

All the obtained isolates were spot-inoculated onto Pikovskaya's agar medium, and slight halo zones were observed around the colonies, indicating phosphate solubilization activity.

2) Cellulase Activity (Carboxymethyl cellulose)

After incubation of bacterial isolates on CMC agar plates at 37 °C for 24–48 hours, visible colony growth was observed. Following Congo red staining and subsequent destaining with 1 M NaCl, slight clear zones developed around several colonies. The presence of light halo zones indicates cellulase enzyme production by the respective isolates. However, the comparatively small diameter of the clearance zones suggests low to moderate cellulolytic activity. Mild cellulase activity was specifically observed in isolates I1, I6, I7, I8, I11, and I12, as evidenced by the formation of slight zones of hydrolysis around the spotted colonies.

3) Detection of IAA (Indole-3-Acetic Acid) Production Using (Salkowski reagent)

Table 5
Standard table for IAA

Concentration (ppm)	Stock (ml)	Diluent (ml)	Total volume	Reagent (ml)	Incubation	Absorbance at 540 nm
5	0.015	2.985	3	2	For 25 min at Room temperature	0.04
10	0.030	2.970	3	2		0.09
20	0.060	2.940	3	2		0.13
50	0.150	2.850	3	2		0.37
100	0.300	2.700	3	2		0.63
Blank	-	3.000	3	2		0.00

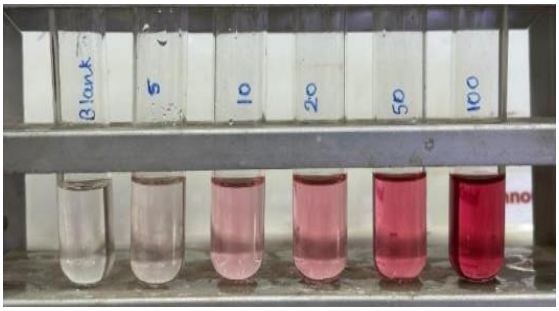

Fig. 1.

Table 6
Quantitative Estimation of (IAA) production by obtained isolates

Isolates No.	Absorbance at 540nm	Concentration of IAA
I1	**0.23**	**33. 96**
I2	0.11	15.16
I4	0.11	15.16
I8	**0.24**	**35.53**
I9	0.16	22.99
I10	0.20	29.26
I11	**0.26**	**38.66**
I12	**0.30**	**44.93**
I13	0.21	30.83
I14	0.16	22.99

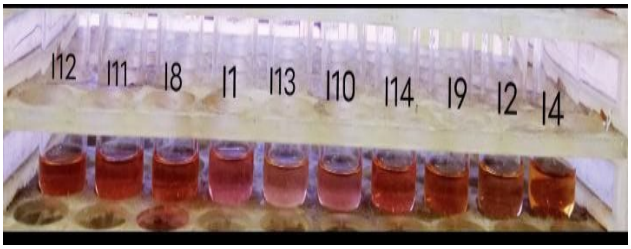

Fig. 2. IAA detection in cell free supernatant of isolates using Salkowski Assay

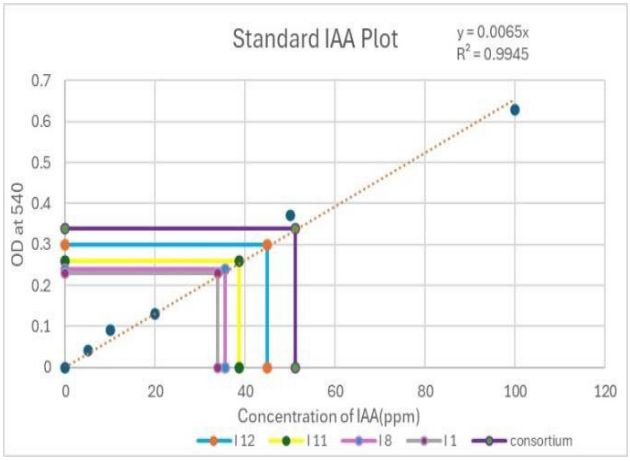

Fig. 3. Standard curve of Indole-3-Acetic Acid (IAA) using Salkowski Assay

Based on the screening results, isolates I1, I8, I11, and I12 were chosen for further consortium formulation due to their comparatively higher indole-3-acetic acid (IAA) production and positive phosphate solubilization and cellulase activity on Pikovskaya's and CMC agar plates.

C. Compatibility Test of Selected Isolates

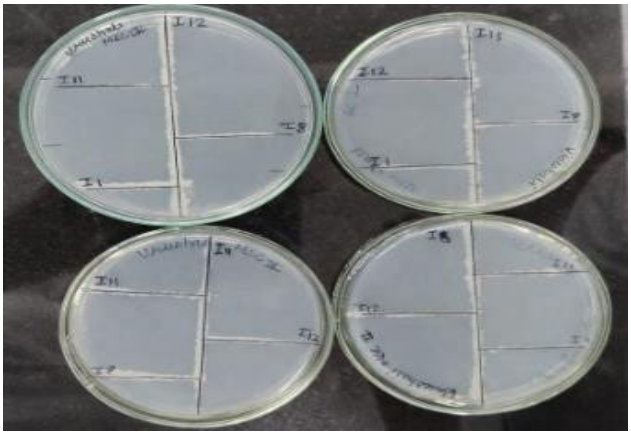

Fig. 4.

Compatibility test of isolates I1, I8, I11, and I12 demonstrating compatible growth without inhibitory interaction.

D. Microbial Consortium Development

The four compatible isolates were cultured separately in sterile Nutrient Broth (NB) and incubated under appropriate conditions until sufficient growth was obtained. The optical density (OD) of each culture was adjusted to 0.1 to standardize the cell concentration. Subsequently, 5 mL from each standardized culture was aseptically combined to formulate the microbial consortium.

E. Plant Growth Promoting Activity of Microbial Consortium

The developed microbial consortium was evaluated for plant growth-promoting traits including phosphate solubilization, cellulase activity, and indole-3-acetic acid (IAA) production. Upon spot inoculation on Pikovskaya's agar and CMC agar, the consortium exhibited slightly clear halo zones, indicating positive phosphate solubilization and cellulase activity. IAA production was confirmed by the development of pink coloration in the colorimetric assay, with an absorbance value of 0.34 at 540 nm. These findings demonstrate that the formulated microbial consortium possesses multiple plant growth-promoting characteristics and has potential to enhance nutrient availability and plant growth.

F. Assessment of Plant Growth Promoting Potential of Microbial Consortium in Coriandrum sativum L.

After 15 days of growth, enhanced germination and plant development were observed in consortium- treated seeds under both sterile and non-sterile soil conditions. In sterile soil, seeds coated with the microbial consortium exhibited a higher germination rate and increased plant height compared to the control, indicating effective growth promotion in the absence of native microflora. Similarly, under non-sterile soil conditions, although all treatments including the control showed germination and normal growth, the consortium-treated seeds demonstrated comparatively higher germination percentage and greater plant height. These observations confirm the growth-promoting potential of the developed microbial consortium under both controlled and natural soil environments.

Table 7
Effect of microbial treatments on Shoot Length (cm) of Coriandrum sativum under sterile and non-sterile soil conditions (Mean ± Sd)

Soil Type	Treatment	No. of seed Germinated (n)	Shoot Length (cm) Mean ± Sd
Non-Sterile	Control	2	5.3 ± 0.42
	Microbial Consortium	2	7.2 ± 0.28
	Seed Soaked	3	6.0 ± 0.20
Sterile	Control	2	4.0 ± 0.28
	Microbial Consortium	2	9.5 ± 0.28
	Seed Soaked	5	6.5 ± 0.16

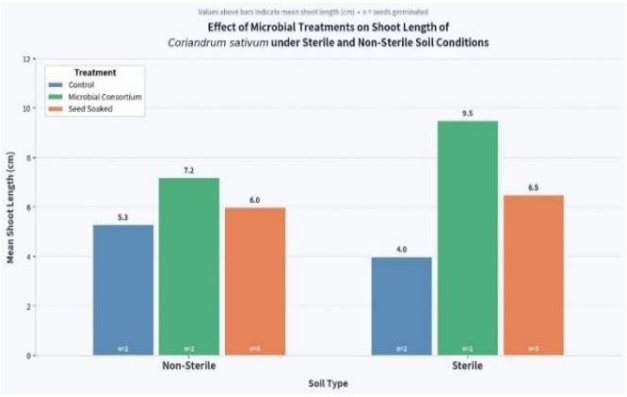

Fig. 5. Impact of microbial consortium on shoot growth of coriander

Table 8
Effect of microbial treatments on root length (cm) of Coriandrum sativum under sterile and non-sterile soil conditions (Mean ± Sd)

Soil Type	Treatment	No. of Seed germinated (n)	Root Length (cm) Mean ± Sd
Non-Sterile	Control	2	2.2 ± 0.28
	Microbial Consortium	2	3.5 ± 0.28
	Seed Soaked	3	5.3 ± 0.20
Sterile	Control	2	2.7 ± 0.28
	Microbial Consortium	2	3.5 ± 0.28
	Seed Soaked	3	3.3 ± 0.10

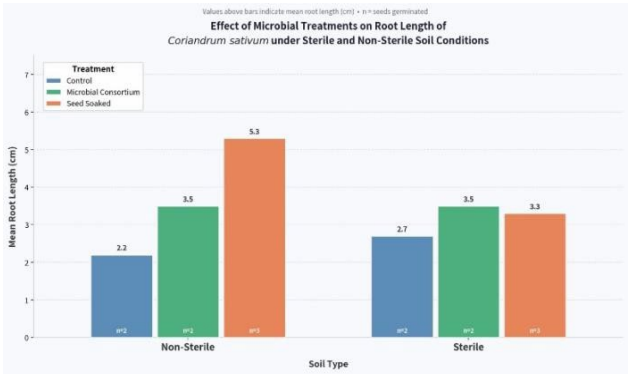

Fig. 6. Impact of Microbial Consortium on root growth of coriander

G. Data analysis

Two-way ANOVA revealed a significant effect of microbial treatment on both shoot and root length ($p < 0.001$), confirming that consortium application significantly enhanced plant growth compared to the control. Soil type also had a significant influence ($p < 0.05$), and a significant interaction between soil type and treatment ($p < 0.05$) indicated that the growth-promoting effect varied under sterile and non-sterile conditions. Maximum growth was observed in consortium-treated plants under sterile soil conditions.

4. Discussion

The findings of the present study clearly show that washed rice water (WRW), which is generally discarded as household waste, can serve as a valuable source of plant growth-promoting microorganisms. The reduction in pH during the three-day fermentation period, along with the development of a characteristic sour odor, confirmed active microbial metabolism. Such acidification is typically associated with organic acid production by fermentative bacteria, creating conditions that favor the growth of beneficial microbial populations. This indicates that spontaneous fermentation not only increases microbial load but also enhances the biological quality of the solution. Isolation on different selective and non-selective media resulted in fourteen morphologically distinct colonies, demonstrating the microbial diversity present in fermented WRW. Growth on MRS agar and Ashby's nitrogen-free agar suggests the presence of lactic acid bacteria and potential nitrogen-fixing organisms. The predominance of Gram-positive isolates further indicates that acid-tolerant bacteria may have adapted more efficiently to the fermentation environment. The presence of diverse functional groups is particularly important for consortium development, as multifunctional microbial communities generally perform better than single-strain inoculants.

Screening for plant growth-promoting traits revealed that several isolates were capable of phosphate solubilization, cellulase production, and indole-3- acetic acid (IAA) synthesis. Halo zone formation on Pikovskaya's agar indicates the ability of isolates to convert insoluble phosphate into plant-available forms. Slight clearance zones observed on CMC agar following Congo red staining confirm cellulolytic activity, suggesting a role in organic matter breakdown and nutrient release. Although the zones were moderate, such activity can become more significant under natural soil conditions where microbial interactions enhance overall efficiency.

Among the isolates, I1, I8, I11, and I12 showed comparatively higher IAA production and were therefore selected for consortium formulation. Since IAA plays a key role in root elongation and lateral root development, its production likely contributed to improved plant growth observed in pot experiments. Compatibility testing confirmed the absence of antagonistic interactions among selected isolates, ensuring stable coexistence. The formulated consortium exhibited improved functional performance compared to individual strains, indicating synergistic interaction among the combined microorganisms.

The pot trial results further validated these laboratory findings. Under sterile soil conditions, consortium- treated seeds showed higher germination rates and increased plant height compared to the control. In non- sterile soil, although all treatments germinated, consortium-treated plants demonstrated comparatively better growth performance. Mean root length and shoot length values were consistently higher in consortium-treated plants than in untreated controls under both soil conditions, confirming the positive influence of microbial inoculation.

Statistical analysis using two-way ANOVA supported these observations. A highly significant effect of microbial treatment on both shoot and root length was observed ($p < 0.001$), confirming that the consortium application significantly enhanced plant growth. Soil type also showed a significant effect ($p < 0.05$), indicating differences between sterile and non-sterile conditions. Furthermore, a significant interaction between treatment and soil type ($p < 0.05$) suggests that the growth-promoting effect of the consortium varied depending on soil environment. The highest mean growth values were recorded in consortium-treated plants, particularly under sterile soil conditions, demonstrating the strong contribution of the introduced microbial inoculum.

The overall improvement in plant growth can be attributed to combined mechanisms such as nutrient solubilization, phytohormone production, and enhanced rhizosphere colonization. Unlike chemical fertilizers that mainly supply nutrients, the fermented WRW-based consortium improves both nutrient availability and microbial diversity in soil.

5. Conclusion

The findings of the present investigation confirm that washed rice water (WRW), commonly discarded as household waste, can be effectively utilized as a source of plant growth-

promoting microorganisms (PGPM). Natural fermentation for three days resulted in microbial enrichment, as evidenced by a decrease in pH and characteristic sour odor, indicating active metabolic activity and favorable conditions for beneficial microbial proliferation.

A total of fourteen morphologically distinct isolates were obtained from fermented WRW, several of which demonstrated key plant growth-promoting traits, including phosphate solubilization, cellulase activity, and indole-3-acetic acid (IAA) production. Based on their comparatively higher functional efficiency, isolates I1, I8, I11, and I12 were selected for consortium development. Compatibility testing confirmed their mutual coexistence, enabling the formulation of a stable and multifunctional microbial consortium.

Application of the consortium in pot experiments significantly enhanced germination rate, shoot length, and root length of coriander plants under both sterile and non-sterile soil conditions. Mean growth values were consistently higher in consortium-treated plants compared to controls. Statistical evaluation using two- way ANOVA revealed a highly significant effect of microbial treatment ($p < 0.001$), a significant effect of soil type ($p < 0.05$), and a significant interaction between treatment and soil conditions. These results validate the growth-promoting potential of the developed consortium.

In conclusion, fermented rice water represents a low- cost, sustainable, and eco-friendly source of beneficial microorganisms with promising application as a biofertilizer. The conversion of a routinely discarded by-product into a biologically active agricultural input supports waste valorization and sustainable crop production. Further large-scale and field-level studies are recommended to establish its long-term efficacy and practical applicability in diverse agro-ecosystems.

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