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# A Bio-Based Solution: Repurposing Rice Straw to Control Bacterial Leaf Blight with *Pseudomonas fluorescens*

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Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), remains one of the most destructive diseases affecting rice cultivation, particularly under flooded field conditions. As there are demand for sustainable and eco-friendly alternatives to agrochemicals, beneficial microbes such as *Pseudomonas fluorescens* have the ability to suppress plant pathogens through the production of secondary metabolites, 2,4-diacetylphloroglucinol (2,4-DAPG) which act as a biocontrol to inhibit the growth of *Xoo*. In this study, *P.fluorescens* was immobilized on rice straw, an abundant agricultural byproduct, to create a stable and biodegradable delivery system suitable for field application. Rice straw serves not only as a low-cost carrier but also provides a microenvironment that helps maintain viability of *P.fluorescens* and promotes gradual colonization of the rhizosphere. The immobilization attributed to enhanced production and sustained release of 2,4-DAPG. This approach offers a promising strategy for integrated disease management in paddy plantation, turning crop waste into a bioactive input while reducing reliance on chemical pesticides.

## 1. Introduction

Rice is a staple crop for more than half of the global population, its sustainable production is essential to ensure food security, especially in rice-producing countries (Fukagawa and Ziska, 2019). However, rice cultivation faces significant challenges from plant diseases, particularly bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Teja et al., 2025). Under flooded conditions, common in paddy fields, BLB can rapidly spread and result in severe yield losses. Conventional management strategies rely heavily on chemical pesticides, which raise concerns about environmental degradation, pathogen resistance and risks to farmer health (Asghar et al., 2022). These challenges highlight the urgent need for eco-friendly, resilient agricultural practices that align with the Sustainable Development Goals (SDGs), particularly SDG 2 (Zero Hunger), SDG 12 (Responsible Consumption and Production) and SDG 13 (Climate Action).
Biological control using plant-beneficial microbes offers a sustainable alternative. In this study, *Pseudomonas fluorescens*, a well-known plant growth-promoting rhizobacterium (PGPR), can suppress phytopathogens including *Xoo* by producing 2,4-diacetylphloroglucinol (2,4-DAPG) as a potent antibiotic compound (Megha Mankoti et al., 2024). Several studies have demonstrated the effectiveness of *P.fluorescens* in suppressing pathogenic bacteria in both laboratory and field trials (Srujani Behera et al., 2024). From Srinivas et al. (2024), they have shown that *P.fluorescens* able to supress the growth of *Xoo* by using dual culture method, a clear inhibition zone of about 17.20 mm was observed in their study. However, for field application, microbial survival

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is a major challenge due to environmental stresses such as temperature (Aloo et al., 2022), desiccation and competition (Allouzi et al., 2022). One promising solution is microbial immobilization, a relatively new approach in agriculture, it provides a protective shelter to microbes and extends their viability and activity under field conditions.

In this context, rice straw, a major paddy industry by-product often treated as waste and frequently burned in open fields (Pham et al., 2022), was selected for its potential to be upcycled into a valuable microbial carrier. Despite its high availability, rice straw is rarely used for microbial immobilization. Due to its high lignocellulosic composition (Chen et al., 2020), it offers a suitable surface for bacterial attachment and serves as an additional carbon source (Biswas et al., 2018) able to support microbial growth. While rice straw has been widely studied in composting or as a substrate for biochar, there is currently no study that evaluates its direct use as an immobilization matrix for biofertilizers. In this study, the gap of using rice straw as microbial carrier will be addressed.

This study is among the first to explore the use of different mesh sizes of rice straw to evaluate their impact on *P.fluorescens* immobilization efficiency. It integrates structural analysis (FESEM), microbial load quantification (dry weight) and chemical characterization (CHNS) to assess the compatibility of rice straw as a microbial carrier. This combination of techniques provides new insights into optimizing agro-waste materials for biofertilizer delivery systems, contributing to the circular bioeconomy.

In this study, we aim to analyze the chemical composition of rice straw and evaluate its compatibility for bacterial immobilization. Specifically, we will observe the attachment of *P.fluorescens* on rice straw using field emission scanning electron microscopy (FESEM) by visualizing the colonization at the microstructural level. Additionally, immobilization efficiency of rice straw with different mesh sizes will be determined by assessing the microbial density, thereby identifying the most suitable physical form for effective bacterial loading.

Beyond plant disease control, this biofertilizer strategy supports broader environmental goals. Incorporating microbial inputs aligns with low-carbon agricultural policies and national climate commitments such as Malaysia’s Climate Change Act (2025) and its Nationally Determined Contributions (NDCs). Transforming rice straw into a carbon-storing bioinput reduces emissions from open burning and promotes a circular bioeconomy in agriculture. This work highlights an integrative approach to by-product valorisation, climate-smart agriculture, and sustainable biocontrol — showcasing how microbial biotechnology can meet both environmental and food security demands.

## 2. Methodology

According to Figure 1, this immobilization study included multiple steps to evaluate the potential of rice straw as a microbial carrier for *P.fluorescens*. Rice straw of varying particle sizes was prepared and added into a nutrient medium to facilitate microbial attachment. Following cultivation, bacterial immobilization efficacy was assessed through dry weight measurements. For further investigation, chemical composition and morphology of rice straw samples were analyzed by CHNS analysis and FESEM imaging, respectively.

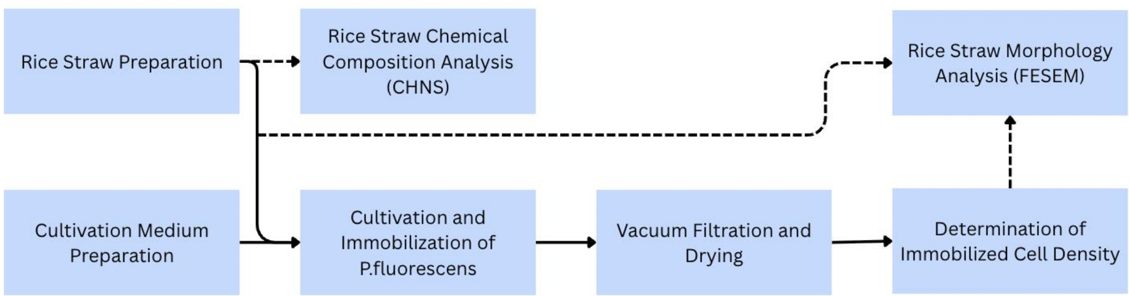


*Figure 1: Flow chart outlining the methodology for evaluating rice straw as a microbial carrier for P. fluorescens immobilization*

The rice straw was obtained from a local paddy field, where they are the by-product after the harvesting of rice. The rice straw was ground and sieved into three particle sizes, the 20-40 mesh, 40-60 mesh, 60-80 mesh and 80-100 mesh. To remove residual fine carbon and other impurities, the sieved rice straw was washed with deionized water until the rinse water was clear. The pretreated rice straw was then dried and stored for further use.

For cultivation medium, the Tryptic Soy Broth (TSB; Merck, Germany) was used, preparation was according to the manufacturer's instructions and used as the basal medium for microbial cultivation. To evaluate the effect of rice straw particle size on bacterial immobilization, pre-treated rice straw was added to the TSB at a concentration of 1 % (w/v). The complete medium, including rice straw, was autoclaved at 121 °C for 15 min to ensure sterility before inoculation.

Afterwards, *P.fluorescens* was inoculated into the sterile rice straw-tryptic soy broth (TSB) medium to facilitate bacterial growth and immobilization. The culture was incubated at 37 °C with shaking at 120 rpm for 24 h. During incubation, the bacteria adhered to the rice straw surfaces to form immobilized biomass.

After 24 hours incubation, the immobilized bacterial cell density was determined using dry cell weight (DCW) measurements. The liquid medium was removed via vacuum filtration, leaving rice straw and attached cells on filter paper. The filter paper and immobilized rice straw were dried at 50 °C until a constant weight was achieved. The increase in dry weight was used to estimate immobilized cell density.

The rice straw samples were sent for CHNS analysis to determine its chemical composition. Rice straw samples are oven-dried at 60 °C for 24 h ground to a fine powder and sieved through a ≤0.5 mm mesh to ensure homogeneity. Approximately 2–3 mg of each powdered sample was weighed and encapsulated in tin capsules. Elemental analysis for carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) was performed using a Thermo Fisher FlashSmart CHNS/O analyzer. Samples were combusted at 950 – 1,000 °C in the presence of pure oxygen, and the resulting gases were carried by helium through reduction and separation columns before detection by a thermal conductivity detector (TCD). Calibration was done using a certified reference material, and all measurements were conducted in triplicate, with results reported as the average percentage of each element on a dry weight basis.

Morphology of non-immobilized and immobilized rice straw were observed with field emission scanning electron microscopy (FESEM) (MERLIN Compact, ZESS), it provided morphological observations of rice straw surfaces and bacterial attachment.

## 3. Result and Discussion

### 3.1 Chemical Profile of Rice Straw

The chemical composition of rice straw was analyzed to determine its compatibility as an immobilizer for *P.fluorescens*. From Table 1, it indicates that rice straw showed a high carbon content of 37.034 %, which is typical for lignocellulosic materials like rice straw due to the abundance of cellulose, hemicellulose and lignin (Sharma et al., 2023). This high carbon content makes rice straw a good carbon source to support the growth of beneficial microbes, including *P.fluorescens*, both in cultivation and when applied to soil (Saharan et al., 2024). In microbial formulations, such a carbon-rich matrix can help sustain microbial viability and activity, contributing to improved colonization and gradual release of biocontrol compounds in the rhizosphere.

In contrast, the nitrogen (0.029 %), hydrogen (4.899 %) and sulphur (0 %) contents were relatively low, which is expected for untreated plant residues. Low nitrogen and sulphur indicate limited protein and amino acid content, meaning rice straw alone cannot supply essential nutrients for microbial metabolism. However, in combination with a nutrient-rich medium like TSB or when applied to soils with sufficient fertility, the hydrogen and nitrogen levels are sufficient to support microbial activity while minimizing unwanted nutrient leaching or competition. These values confirm that rice straw serves primarily as a carbon scaffold, not a complete nutrient source, making it ideal for microbial immobilization and slow-release applications.

*Table 1: The nitrogen, carbon, hydrogen and sulphur composition (%) of rice straw*

| Sample Name | Nitrogen | Carbon | Hydrogen | Sulphur |
|---|---|---|---|---|
| Rice Straw | 0.029 | 37.034 | 4.899 | 0 |

### 3.2 Surface Morphology Study of *P.fluorescens* Immobilized Rice Straw

The surface morphology of the rice straw is being observed under FESEM to better understand its morphological structure and observe the *P.fluorescens* attachment on rice straw. According to Figure 2(a) and 2(b), the non-immobilized rice straw has fibrous, uneven, and rough surface, with ridge-like grooves structures are being observed at lower magnifications (100x), this likely associated with vascular bundles or broken silica cells (Kaur et al., 2018). These surface features create a naturally porous and micro-structured architecture, offering large surface area for microbial attachment. The interconnected fibrils and crevices may serve as shelters to protect bacteria from environmental stress, making rice straw a suitable microbial immobilization carrier.

In contrast, the immobilized rice straw shown in Figure 3(a) and 3(b) shows significant structural change, including the presence of bacterial, which indicating the colonization of *P.fluorescens*. Under 1,000x and 2,000x magnification, bacterial cells are well-distributed within surface pores and grooves, this demonstrating high efficacy in bacterial immobilization. The bacterial adhesion likely being enhanced through biofilm formation from *P.fluorescens* and chemical bonds of rice straw itself. As extracellular polymeric substances (EPS) produced by microbes is playing a crucial role in microbial aggregation and attachment (Wang et al., 2024). This interaction not only stabilizes the cells but also creates a microenvironment that may retain moisture and nutrients, thus promoting microbial survival and sustained release (Saharan et al., 2024). These observations confirm that the physical structure of rice straw plays a vital role in enhancing immobilization efficacy, making it an excellent low-cost and biodegradable carrier for field applications in biofertilizer and biocontrol formulations.

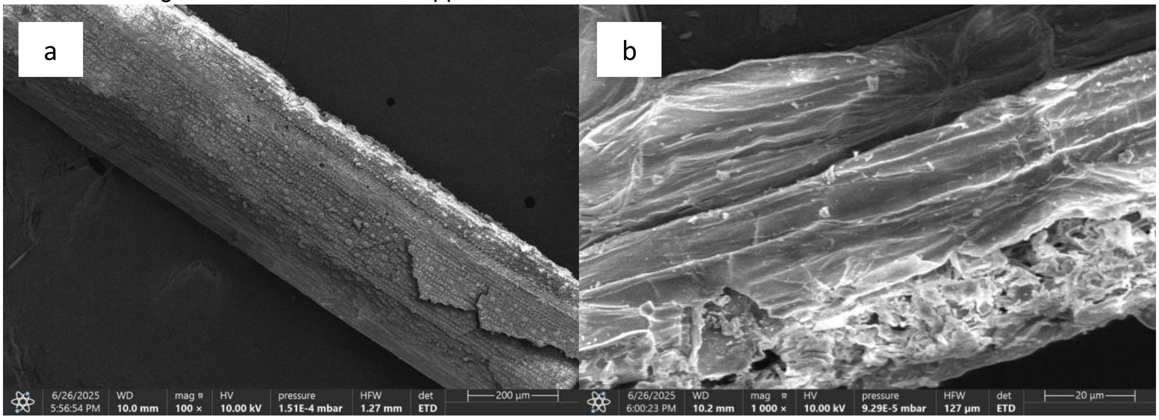


*Figure 2: The FESEM image of untreated rice straw's surface structure (a) with 100x magnification; (b) with 500x magnification*

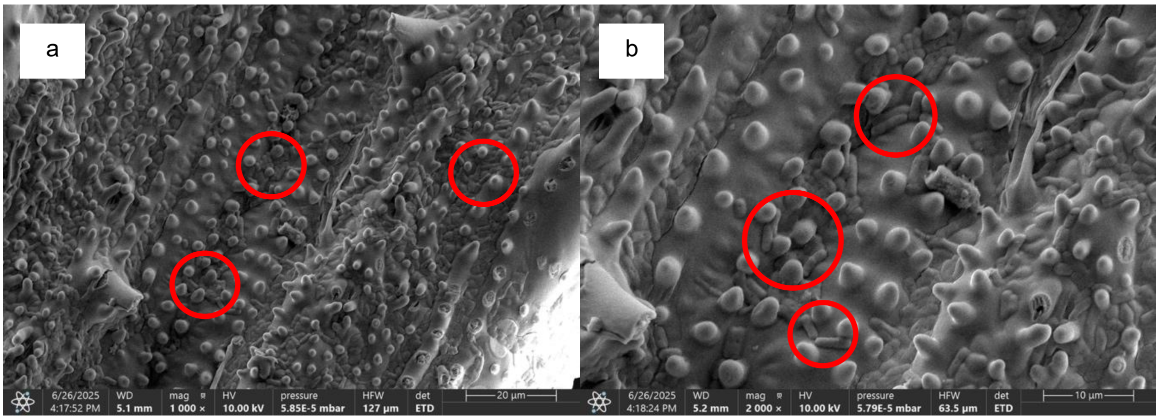


*Figure 3: The FESEM image for P.fluorescens immobilized rice straw, red circles indicating microbial immobilization occurred; (a) Surface structure with 1,000x magnification; (b) Surface structure with 2,000x magnification*

### 3.3 Microbial Density of *P.fluorescens* Immobilized on Rice Straw

The immobilized microbial density was analyzed with the dry weight to compare immobilization efficiency of different mesh sizes rice straw based on microbial density. The relationship between rice straw particle size and *P.fluorescens* immobilization efficiency is clearly demonstrated by the dry cell weight results shown in Figure 4. Among all tested mesh sizes, 60 - 80 mesh and 80 - 100 mesh rice straw showed the highest levels of bacterial immobilization, with dry cell weights of approximately 4.80 g/L and 4.93 g/L, respectively. These results suggest that finer particle sizes offer a more optimal physical structure for bacterial attachment and retention (Wu et al., 2019). The loosely packed, moderately porous nature of these mesh sizes allows better penetration of the bacterial suspension, while also providing sufficient surface area and microenvironments for cells to attach and multiply. The structural observations from FESEM images support this as Figure 3 is showing dense colonization and visible bacterial clusters on the rice straw surfaces.

In contrast, the larger particle sizes, 20-40 mesh and 40–60 mesh resulted in significantly lower dry cell weight, approximately 1.33 g/L and 2.52 g/L, respectively. These coarser particles have reduced effective surface contact with the bacterial culture due to their lesser available surface area, leading to less microbial attachment during cultivation. From the findings, it proved that particle size of rice straw is an important factor in affecting immobilization efficacy, as it plays a key role in influencing bacterial adhesion, colonization behavior and spatial availability on the carrier matrix (Fan et al., 2025). Therefore, by optimizing mesh size is critical to maximize microbial attachment, particularly when formulating biofertilizers for field application where consistent and high microbial density is essential for biocontrol efficacy.

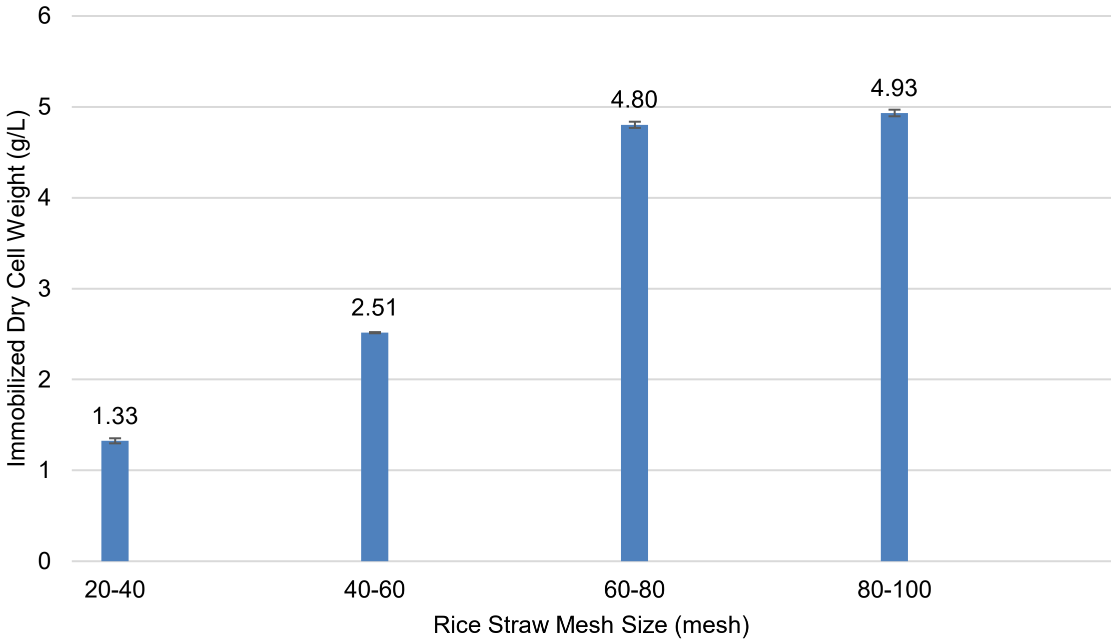


*Figure 4: Relationship Between Dry Cell Density of Immobilized P.fluorescens on Different Mesh Sizes Rice Straw. Error bar according to standard deviation*

## 4. Conclusion

This study demonstrated that rice straw is a suitable and effective carrier for the immobilization of *P.fluorescens*, and mesh size playing a significant role in determining immobilization efficiency. Among the tested sizes, 60–80 and 80–100 mesh rice straw supported the highest bacterial dry cell weights, indicating that finer structure provides optimal conditions for microbial attachment and colonization. In contrast, coarser particles limited bacterial retention as effective surface area is lower, these findings highlighted the importance of optimal particle size for biocontrol development. This research has reinforced the potential of rice straw not only as a low-cost agricultural waste material but also as a sustainable and biodegradable immobilization matrix for beneficial microbes. By optimizing mesh size can contribute to the development of more effective biocontrol-based fertilizer to support sustainable agriculture and align with broader goals in waste valorization and climate-smart farming.

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**References**

Allouzi, M.M.A., Allouzi, S.M.A., Keng, Z.X., Supramaniam, C.V., Singh, A., Chong, S., 2022, Liquid biofertilizers as a sustainable solution for agriculture, Heliyon, 8(12).

Aloo, B.N., Mbega, E.R., Makumba, B.A.,Tumuhairwe, J.B., 2022, Effects of Carrier Materials and Storage Temperatures on the Viability and Stability of Three Biofertilizer Inoculants Obtained from Potato (Solanum tuberosum L.) Rhizosphere, Agriculture, 12(2), 140.

Asghar, M., Hussain, M., Ahmad, I., Hasan, T., Iqbal, M.F., Mahdi, M.M., Ali, M.A., 2022, Evaluation of the chemical control of bacterial leaf blight (Xanthomonas oryzae pv. oryzae) of rice in transplanted rice (Oryza sativa L.) crop, Journal of Pure and Applied Agriculture.

Biswas, B., Singh, R., Kumar, J., Singh, R., Gupta, P., Krishna, B.B., Bhaskar, T., 2018, Pyrolysis behavior of rice straw under carbon dioxide for production of bio-oil, Renewable Energy, 129, pp.686–694.

Chen, C., Chen, Z., Chen, J., Huang, J., Li, H., Sun, S., Liu, X., Wu, A. and Wang, B., 2020, Profiling of Chemical and Structural Composition of Lignocellulosic Biomasses in Tetraploid Rice Straw, Polymers, 12(2), 340.

Fan, F., Ren, Y., Mao, Z., Li, B., Yu, C., Gao, J., Gu, Y., Ding, J., Li, H., Wu, Q.L., 2025, Particle-size dependent of bacterial diversity associated with Suspended particulate matter continuum in Lake Taihu, FEMS Microbiology Ecology.

Fukagawa, N.K., Ziska, L.H., 2019, Rice: Importance for Global Nutrition, Journal of Nutritional Science and Vitaminology, 65.

He, Z., Cao, H., Qi, C., Hu, Q., Liang, J., Li, Z., 2023, Straw management in paddy fields can reduce greenhouse gas emissions: A global meta-analysis, Field Crops Research, 306, 109218.

Kaur, P., Kocher, G.S. and Taggar, M.S., 2018, Development of fungal consortium for the pretreatment of rice straw under optimized solid state and shake flask conditions, Environmental Progress and Sustainable Energy, 38(2).

Megha Mankoti, Pandit, N.K., Meena, S.S., Mohanty, A., 2024, Investigating the genomic and metabolic abilities of PGPR Pseudomonas fluorescens in promoting plant growth and fire blight management, Molecular Genetics and Genomics, 299(1).

Mordor Intelligence, 2025, Crop Protection Chemicals Market Size and Share Analysis - Industry Research Report - Growth Trends, Mordorintelligence.

Pham C.D., Le T.M., Nguyen C.T.X., Vo N., Do N.H.N., Le K.A., Mai T.P., Le P.T.K., 2022, Review of The Role of Pretreatment Step in Nanocellulose Production from Rice Straw, Chemical Engineering Transactions, 97, 67-72.

Saharan, B.S., Dhanda, D., Mandal, N.K., Kumar, R., Sharma, D., Sadh, P.K., Jabborova, D., Duhan, J.S., 2024, Microbial contributions to sustainable paddy straw utilization for economic gain and environmental conservation, Current Research in Microbial Sciences, 7, 100264.

Sharma, N., Allardyce, B.J., Rajkhowa, R., Agrawal, R., 2023, Rice straw-derived cellulose: a comparative study of various pre-treatment technologies and its conversion to nanofibers, Scientific Reports, 13(1), 16327.

Srinivas, B., Patil, V.A., Shinde, C.U., John, P., Garde, Y.A., Waghunde, R.R., 2024, Effect of Antagonists and Botanicals Against Xanthomonas oryzae pv. oryzae In Vitro, International Journal of Economic Plants, 11(Feb, 1), 7-11.

Srujani Behera, Sunita Behera, Nayak, B.S., 2024, Management of Bacterial leaf blight of rice caused by Xanthomonas oryzae pv. oryzae through Integrated approach in western undulating zones of Odisha, Journal of Cereal Research, 16(1).

Teja, B.S., Jamwal, G., Gupta, V., Verma, M., Sharma, A., Sharma, A., Pandit, V., 2025, Biological control of bacterial leaf blight (BLB) in rice–A sustainable approach, Heliyon, 11(2), 41769.

Wang, D., Chen, H., Han, H., Yang, W., Sun, Q., Cao, C., Ning, K., Huang, Z., Wu, T., 2024, Interaction of biochar with extracellular polymers of resistant bacteria restrains Pb(II) adsorption onto their composite: Macro and micro scale investigations, Bioresource Technology, 414, 131602.

Wei, Y., Dong, B., Wu, X., Zhao, M., Wang, D., Li, N., Zhang, Q., Zhang, L., Zhou, H., 2023, RpoZ regulates 2,4-DAPG production and quorum sensing system in Pseudomonas fluorescens 2P24, Frontiers in Microbiology, 14.

Wu, T., Zhai, C., Zhang, J., Zhu, D., Zhao, K., Chen, Y., 2019, Study on the Attachment of Escherichia coli to Sediment Particles at a Single-Cell Level: The Effect of Particle Size, Water, 11(4), 819.