Journal of Root Crops
Indian Society for Root Crops
ISSN 0378-2409, ISSN 2454-9053 (online)
Journal homepage: https://journal.isrc.in

Protocol optimization: A comparative study on phylloplane microbial recovery

S.A. Pavithra[1,2], S.S. Veena[1*], Aundy Kumar[3], K. Krishnaveni[1,4], S. Divya[1,3],
B. Lohitha[1,4], Beegam Nazrin[1,2], Tom Cyriac[1,2], S.U. Shilpa[1,2],
S. Karthikeyan[1], M.L. Jeeva[1], R. Arutselvan[1]and T. Makeshkumar

[1]ICAR-CentralTuber Crops Research Institute, Sreekariyam, Thiruvananthapuram 695017, Kerala, India
[2]Department of Biotechnology, University of Kerala, Kariyavattom, Thiruvananthapuram 695581, Kerala, India
[3]ICAR-Indian Agricultural Research Institute, Pusa, New Delhi, 110 012
[4]ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, 560088

Abstract

Elephant foot yam (*Amorphophallus paeoniifolius*), a herbaceous perennial crop utilizing the C3 photosynthetic pathway and belonging to the family Araceae, is cultivated extensively across eight Indian states. Despite its numerous agronomic and economic benefits, the crop is vulnerable to several diseases. In recent years, symptoms such as yellowing of foliage, the appearance of various types of spots, and eventual partial or complete wilting have been increasingly observed. This disease, provisionally termed 'leaf and pseudostem rot', has been associated with the presence of more than three different pathogens. Investigating the crop's microbiome, particularly the phylloplane, offers potential for use in biological disease management and may provide deeper insights into the complex etiology of the disorder.

In the present study, two protocols were evaluated for isolating phylloplane-associated microbes to determine which method could capture greater microbial diversity. The first protocol involved a single wash of leaf samples, followed by serial dilution and spread plating. The second protocol employed five successive washes of the leaf samples, after which the wash solutions were centrifuged to collect distinct pellets for bacterial and fungal isolation. These pellets were then serially diluted and cultured using dilutions up to 10^{-10}. Results indicated that the second protocol was more effective, yielding a greater number and diversity of microorganisms compared to the direct aliquot method. The fungal and bacterial colony-forming units (CFUs) obtained using the first method were 2.0×10^4 and 9.7×10^5, respectively. In contrast, the five-wash protocol yielded 1.0×10^5 fungal CFUs and 1.0×10^{10} bacterial CFUs. These findings suggest that the sequential washing and centrifugation approach is more efficient for capturing the microbial diversity present on the leaf surface.

Keywords: Elephant foot yam, Phylloplane, Microbiome, Leaf yellowing, Leaf rot

* Corresponding author
E-mail: veena.ss@icar.org.in; Ph: +91 9497536500

Received: 12 September 2024 Revised: 30 November 2024 Accepted: 04 December 2024

Introduction

Tuber crops are among the major agricultural groups that contribute significantly to sustainability and serve as important sources of carbohydrates. In addition to their nutritional value, they play a crucial role in ensuring food security (Rout et al. 2025). Major tuber crops include *Manihot esculenta* (cassava), *Dioscorea* spp. (yam), *Xanthosoma sagittifolium* (tania), *Ipomoea batatas* (sweet potato), *Colocasia esculenta* (taro), and *Amorphophallus paeoniifolius* (elephant foot yam) (Veena et al. 2021). Among these, elephant foot yam (*Amorphophallus paeoniifolius*) is widely regarded as the 'King of Tubers' due to its diverse uses (Srivastava et al. 2022; Ilakiya et al. 2023). A member of the Araceae family, EFY is an underutilized crop and thrives on marginal soils, can withstand low annual rainfall, and performs well under stress conditions such as drought. Additionally, it yields well with minimal chemical inputs when organic waste is used as a supplement (Barua et al. 2022; Kumar et al. 2023; Ilakiya et al. 2023; Remya and Suja, 2024). EFY is also valued for its medicinal properties. It exhibits anti-helminthic and anti-piles activity and is used in Ayurveda to treat haemorrhoids, rheumatism, gastrointestinal disorders, dysentery and diarrhoea. Elephant foot yam (EFY) is recognized for its hepatoprotective, antioxidant, and uterus-stimulating properties (Barua et al. 2022; Srivastava et al. 2022; Ilakiya et al. 2023; Remya and Suja, 2024; Veena et al. 2025). Nutritionally, EFY is rich in carbohydrates and also contains fibre, protein, minerals, sterols, steroids, galactose, glucose, rhamnose, flavonoids, phenols, and tannins (Remya and Suja, 2024; Veena et al. 2025).

Although elephant foot yam (*Amorphophallus paeoniifolius*) offers several agronomic advantages, it remains vulnerable to a range of diseases. Commonly reported diseases include collar rot, post-harvest rot, and infections caused by *Dasheen mosaic virus* (Veena et al. 2025). In recent years, a new set of symptoms, characterized by yellowing of the foliage, development of various types of leaf spots or blight, and eventual partial or complete wilting has been increasingly reported from multiple regions across India (ICAR-CTCRI, 2022). This condition is currently referred to as 'leaf and pseudostem rot'. The disease typically starts with small, orangish-brown spots encircled by a yellow halo. These lesions expand and are often accompanied by chlorotic patches, which gradually darken and become necrotic. As the infection progresses, the necrotic tissue may dry out, tear, or fall off, leaving behind characteristic shot holes. In several cases, the merging of lesions results in partial or total drying of the leaves or entire plants. The disease severely compromises the plant's photosynthetic capacity, leading to significant yield loss.

Preliminary investigations at ICAR-CTCRI have revealed that more than three pathogens may be involved in this disease complex (ICAR-CTCRI, 2023). These pathogens are capable of infecting both robust and weakened plants at various growth stages, with infections being particularly aggressive under stress conditions. Therefore, accurate identification of the pathogens associated with the diverse symptoms on leaves and pseudostem is essential. There is an urgent need to develop environmentally sustainable and effective management strategies. Exploring the phylloplane microbiome of elephant foot yam may provide vital insights for understanding etiology of the disease completely and help in formulating targeted biological control methods.

The phylloplane microbiome refers to the community of microorganisms present on the surface of leaves. Structures on the leaf surface, such as veins, stomata, and trichomes (leaf hairs) influence microbial colonization by providing physical niches and facilitating interactions between the leaf and microorganisms (Bisht et al. 2025; Zeng et al. 2025). Both bacteria and fungi are commonly found on the leaf surface, with bacteria being the dominant group, typically ranging from 10^5 to 10^6 cells per cm^2 (Chouhan et al. 2025). The leaf surface is directly exposed to the atmosphere; environmental factors such as UV radiation can significantly affect microbial survival. Additionally, microbes can enter the plant through natural openings like stomata or through wounds (Gamit et al. 2025; Bisht et al. 2025).

Microbiome contributes significantly to plant growth promotion, nutrient cycling, stress tolerance, environmental detoxification and biological control. Understanding the functional diversity of phylloplane-associated microorganisms underscores their immense potential in sustainable disease management, particularly through the recruitment of beneficial microbes as natural biocontrol agents (Saleem, 2021; Chao et al. 2024; Lokhande et al. 2024). Many phylloplane microbes are known to secrete compounds that inhibit pathogen growth, while others produce substances that trigger the plant's immune response. Several studies have reported the biocontrol potential of specific phylloplane bacterial species. *Pantoea*, *Pseudomonas* and *Rhizobium* have exhibited volatile-mediated antifungal activity, while *Acinetobacter* and *Sphingomonas* have shown effectiveness against *Magnaporthe oryzae* in rice (Sahu et al. 2021; Prajapati et al. 2025). Their ability to supress a wide range of pathogens, make them significant hope for the development of eco-friendly biocontrol (Saleem et al. 2021).

There are two primary protocols available for isolating the phylloplane microbiome. The first is the leaf imprinting method (Mounika et al. 2024; Dewada et al. 2024; Chouhan et al. 2025). The second is the saline phosphate buffer (PBS) wash technique (Sahu et al.

2021; Madhusudhan et al. 2024; Kansara, 2023). The leaf imprinting method is particularly useful for comparing the microbial communities on the adaxial (upper) and abaxial (lower) surfaces of the leaf. The commonly used PBS wash technique involves collecting leaf samples, washing them with PBS, and plating the aliquots after serial dilution. An alternative and more refined protocol involving five successive washes followed by centrifugation to separate bacterial and fungal fractions and then serial dilution and plating, has generally been employed for metagenomic studies. In this study, two PBS wash protocols for phylloplane microbial recovery were compared to identify the method yielding greater microbial diversity.

Materials and Methods

The variety of elephant foot yam selected for the study was 'Gajendra'. The plants were cultivated in the experimental fields of ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram, Kerala, India following the recommended agronomic practices.

Sampling and isolation for direct aliquot plating method

Leaf sample of elephant foot yam was aseptically collected using sterile forceps to avoid any external contamination. From the collection, 5g of the sample was weighed with utmost care to maintain maximum aseptic condition. The sample was immediately transferred into a sterile 250 mL Erlenmeyer flask containing 50 mL of phosphate-buffered saline (PBS) solution, which was supplemented with 0.1% Tween 20 to aid in the efficient removal of phylloplane microorganisms. The flask was placed on an orbital shaker and agitated at a speed of 200 revolutions per minute (rpm) for 30 minutes to ensure thorough dislodging of microbial cells from the leaf surface.

Following agitation, the wash solution (aliquot) was subjected to a ten-fold serial dilution up to 10^{-10} using sterile distilled water. From each dilution, 100 μL of the sample was spread onto solid media plates to isolate and enumerate microbial populations. For microbial isolation, Potato Dextrose Agar (PDA) was used to culture fungi, and Nutrient Agar (NA) was selected for bacterial growth. The prepared media were sterilized, melted, and poured into sterile 90 mm Petri dishes, with each plate receiving approximately 15–20 mL of medium under aseptic conditions. Each dilution of the microbiome suspension was plated in triplicate to ensure consistency and statistical reliability. The inoculated plates were incubated at 28 ± 2°C in a BOD incubator to promote the development of colony-forming units (CFUs), which were subsequently used for both quantitative enumeration and qualitative assessment of the phylloplane microbial community (Sahu et al. 2021).

Sampling and isolation for five times wash technique

As described earlier, 5g leaf sample was aseptically transferred into a sterile flask containing 50 mL PBS with 0.1% Tween 20 and shaken at 200 rpm for 30 minutes to dislodge phylloplane microorganisms. Following the initial wash, the aliquot (50 mL) was carefully collected and stored. The leaf sample was then washed again with a fresh 50 mL volume of sterile PBST, and the same shaking procedure was repeated. This washing and collection process was carried out five times in total, yielding a pooled 250 mL PBST wash solution containing microorganisms detached from the leaf surface. The combined wash was mixed thoroughly to ensure uniformity. The pooled microbial suspension was subjected to differential centrifugation to isolate fungal and bacterial fractions. For fungal isolation, the sample was centrifuged at 6000 rpm for 15 minutes, while for bacterial isolation; centrifugation was carried out at 12,000 rpm for 30 minutes. The resulting pellets containing fungal spores and bacterial cells were collected separately. Each pellet was resuspended in sterile distilled water and subjected to ten-fold serial dilutions up to 10^{-10}. As detailed in first method, from each dilution, 100 μL was spread on PDA (for fungi) and NA (for bacteria). Media (15–20 mL) were poured into 90 mm Petri dishes under aseptic conditions. Triplicates were maintained and plates incubated at 28 ± 2°C for CFU analysis.

Results and Discussion

One of the major challenges in the cultivation of elephant foot yam (EFY) is the prevalence of diseases. The crop is predominantly affected by three major diseases: collar rot, *Dasheen mosaic virus*, and post-harvest rot (Veena et al. 2025). More recently, the emergence of a new disease, leaf and pseudostem rot, has posed a serious threat to the survival of EFY plants. As an initial step toward developing a biological management strategy for the disease utilizing the emerging field of agricultural microbiome research, this study compared two widely used protocols for isolating the phylloplane microbiome (Alivisatos et al. 2015).

The colony-forming units (CFUs) of bacteria and fungi observed at various dilutions of phylloplane microbiome suspensions were compared using two different isolation methods.

Isolation of microbes by direct aliquot plating method

Bacterial colonies began to appear within 24 hours of incubation, with colony counts recorded at 24 and 48 hours. Similarly, fungal colonies became visible after 72 hours, and their counts were recorded at 96 hours (Table 1; Fig. 1 and 2).

Table 1. Population of bacterial and fungal colonies appeared on NA and PDA by using the direct aliquot plating method

Dilution	No. of fungal colonies	CFU ($100\ \mu l^{-1}$)	No. of bacterial colonies	CFU ($100\ \mu l^{-1}$)
10^{-1}	Too many, could not count		Too many, could not count	
10^{-2}	165	1.65×10^{4}	Too many, could not count	
10^{-3}	21	2.1×10^{4}	Too many, could not count properly	
10^{-4}	2.0	2.0×10^{4}	97	9.7×10^{5}
10^{-5}	0.0	0.0	9.0	9.0×10^{5}
10^{-6}	0.0	0.0	0.0	0.0

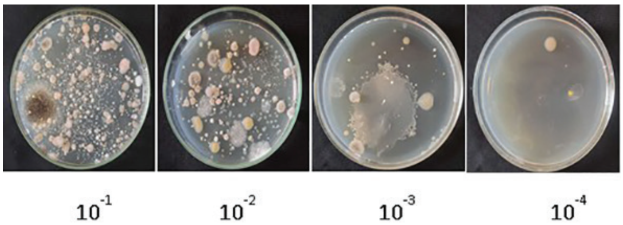

Fig. 1. Fungal colonies on PDA by adopting the protocol of one-time wash and direct aliquot plating after serial dilution

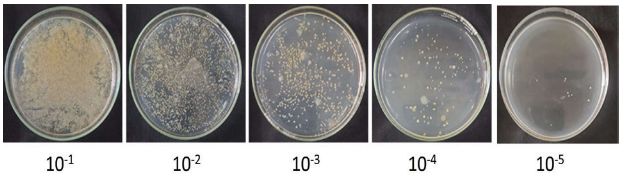

Fig. 2. Bacterial colonies on NA by adopting the protocol of one-time wash and direct aliquot plating after serial dilution

Isolation of microbes by adopting five times washes technique

Bacterial growth was observed within 24 hours, with colony counts noted at 24 and 48 hours. Fungal colonies emerged by 72 hours, and counts were taken at 96 hours (Table 2; Fig. 3 and 4).

The comparison of the two protocols for isolating the phylloplane microbiome revealed that the second protocol, which involved five successive washes, was more effective than the first protocol, which used a single wash. In the direct aliquot plating method, bacterial

Table 2. Population of fungal and bacterial colonies by adopting five times washes technique

Dilution	No. of fungal colonies	CFU ($100\ \mu l^{-1}$)	No. of bacterial colonies	CFU ($100\ \mu l^{-1}$)
10^{-1}	Too many, could not count		Too many, could not count	
10^{-2}	285.0	2.85×10^{4}	Too many, could not count	
10^{-3}	89.0	8.9×10^{4}	Too many, could not count	
10^{-4}	15.0	1.5×10^{5}	Too many and colonies merged	
10^{-5}	2.0	2.0×10^{5}	Too many and colonies merged	
10^{-6}	1.0	1.0×10^{5}	Too many and colonies merged	
10^{-7}	Nil		123.0	12.3×10^{8}
10^{-8}	Nil		19.0	19.0×10^{8}
10^{-9}	Nil		3.0	3.0×10^{9}
10^{-10}	Nil		1.0	1.0×10^{10}

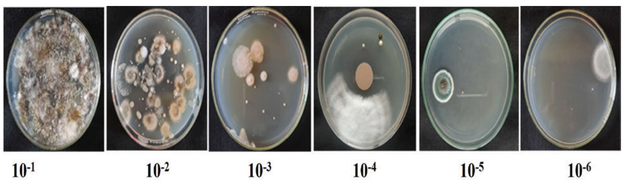

Fig. 3. Fungal colonies on PDA by adopting the protocol of five times wash followed by centrifugation

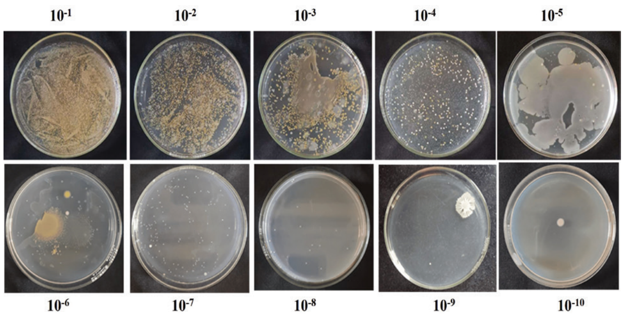

Fig. 4. Bacterial colonies on NA by adopting the protocol of five times wash followed by centrifugation

colonies were observed only up to a dilution of 10^{-5}. In contrast, the second protocol yielded bacterial colonies up to a 10^{-10} dilution, with distinct colony growth evident at 10^{-7} and 10^{-8} dilutions. This indicates a significantly higher bacterial load in the five times wash method, as confirmed by standard serial dilution and plate-counting techniques (Yousef and Carlstrom, 2003). Phylloplane bacterial populations are generally reported to range from 10^6 to 10^7 cells per cm^2 (Chouhan et al. 2025). Based on colony morphology (Madigan et al. 2018), 15 morphologically distinct bacterial colonies were identified using the five-times wash protocol, while only 7 distinct colonies were observed using the direct aliquot method.

A similar trend was observed for fungal populations. Fungal colonies were detectable up to 10^4 dilution in the first protocol, whereas they were observed up to 10^{-6} dilution using the five-times wash method. Furthermore, seven distinct fungal colonies were recorded in the first protocol, compared to fourteen in the second.

Overall, the five-time wash technique significantly improved microbial dislodgement from the leaf surface compared to the single-wash method (Compant et al. 2010; Berg et al. 2014). Additionally, the inclusion of Tween 20 enhanced microbial recovery by reducing surface tension and facilitating the detachment of epiphytic microbes from the leaf surface (Silva et al. 2024). Centrifugation also aided in enriching low-abundance microbes by concentrating them into a pellet (O'Leary et al. 2014). Overall, the phylloplane tends to harbour a higher density of bacterial communities compared to fungal communities.

Conclusion

This study successfully demonstrated that the refined protocol involving five successive washes followed by centrifugation was highly effective in dislodging microbial populations from the leaf surface of elephant foot yam plants. It also proved efficient in concentrating sparsely populated microorganisms. This method yielded higher colony-forming unit (CFU) counts and greater microbial diversity for both bacteria and fungi.

References

Alivisatos, A.P., Blaser, M.J., Brodie, E.L., Chun, M., Dangl, J.L., Donohue, T.J. and Taha, S., 2015. A unified initiative to harness Earth's microbiomes. *Sci*, **350** (6260): 507–508. https://doi.org/10.1126/science.aac8480

Barua, S., Hanewald, A., Bächle, M., Mezger, M., Srivastav, P.P. and Vilgis, T.A. 2022. Insights into the structural, thermal, crystalline and rheological behavior of various hydrothermally modified elephant foot yam (*Amorphophallus paeoniifolius*) starch. *Food Hydrocoll.,* **129** (2): DOI: 10.1016/j.foodhyd.2022.107672

Berg, G., Grube, M., Schloter, M. and Smalla, K. 2014. Unraveling the plant microbiome: looking back and future perspectives. *Front. Microbiol*. **5**:148. doi: 10.3389/fmicb.2014.00148

Bisht, N., Singh, T., Ansari, M.M. and Chauhan, P.S. 2025. The hidden language of plant-beneficial microbes: chemo-signaling dynamics in plant microenvironments. *World J. Microbiol. Biotechnol*., **41**(2): 1-16.

Chao, S., Sun, Y., Zhang, Y., Chen, Y., Song, L., Li, P., Tang, X., Liang, J. and Lv, B. 2024. The response of microbiome assembly within different niches across four stages to the cultivation of glyphosate-tolerant and conventional soybean varieties. *Front. Microbiol.*, **15**: 1-14. https://doi.org/10.3389/fmicb.2024.1439735

Chouhan, V., Thalor, S., Charishma, K., Javed, M., Kumar, S., Sharma, J., Munjal, V. and Kumar, A. 2025. Microbiome succession on the pomegranate phylloplane during bacterial blight dysbiosis: Functional implications for blight suppression. *Microbiol. Res.*, **293**: 128050. https://doi.org/10.1016/j.micres.2025.128050

Compant, S., Clément, C. and Sessitsch, A. 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization, *Soil Biol. Biochem.*, **42**(5): 669–678. https://doi.org/10.1016/j.soilbio.2009.11.024

Dewada, R., Khunt, M.D., Tandel, D.H., Suthar, H. and Bhimani, H.D. 2024. Isolation, characterization and in vitro efficacy of banana phyllospheric bacteria. *Emerg. Life Sci. Res.*, **10**: 90-96.

Gamit, H.A., Manoharadas, S. and Amaresan, N. 2025. Inoculation of methylotrophs mitigates heat and UV stress in mung bean (*Vigna radiata* L.) and enhances growth, antioxidant, and functional diversity. *Photochem. Photobiol.,* **101**(1): 1–17. https://doi.org/10.1111/php.13934

ICAR-CTCRI 2022. Annual Report 2021. ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala, India, 180p.

ICAR-CTCRI 2023. Annual Report 2022. ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala, India, 179p.

Ilakiya, T., Swarnapriya, R., Pugalendhi, L., Geethalakshmi, V., Lakshmanan, A., Kumar, M. and Lorenzo, J.M. 2023. Carbon accumulation, soil microbial and enzyme activities in elephant foot yam-based intercropping system. *Agriculture*, **13**(1), 187. https://doi.org/10.3390/agriculture13010187

Kansara, S. 2023. Investigation of Phylloplane microflora of tomato. *Res. Agric. Sci. Technol.,* **4**(1): 1-3.

Kumar, J.S., Sunitha, S., Sreekumar, J., Mamatha, K., Das, B., Sengupta, S., Kamalkumaran, P.R., Thangamani, C., Mitra, S., Tarafdar, J. and Patel, H.B. 2023. Weed management strategies in elephant foot yam (*Amorphophallus paeoniifolius*) under different agro environments in India. *Indian J. Agric. Sci.*, **93**(12): 1314-1319.

Lokhande, K.S., Jagiya, A. A. and Zingare, A. 2024. Dynamics of phylloplane and rhizoplane mycoflora in sustainable crop management of green gram and black gram. *Int. J. Plant Environ.*, **10**(3): 174–178. https://doi.org/10.18811/ijpen.v10i03.22

Madhusudhan, K.N., Tuboi, L., Vinayarani, G., Moorthy, S.M. and Gandhi Doss, S. 2024. Isolation and characterization of antibacterial phylloplane bacteria *Bacillus subtilis* isolated from *Terminalia arjuna* leaves. *Microbiol. Res. Int.*, **12**(1): 1-9.

Madigan, M.T., Stahl, D.A., Sattley, W.M., Buckley, D.H. and Bender, K.S. 2018. *Brock Biology of Microorganisms*. 15th Global Edition, 1064p.

Mounika, K., Madhusudhan., Raju, M. and Paramasiva, I. 2024. Isolation and identification of phylloplane bacteria from rice ecosystem of Andhra Pradesh against *Magnaporthe oryzae*., Andhra Agric. J., **71:** 410-414. 10.61657/aaj.2024.153

O'Leary, B.M., Rico, A., McCraw, S., Fones, H.N. and Preston, G.M. 2014. The infiltration-centrifugation technique for extraction of apoplastic fluid from plant leaves using *Phaseolus vulgaris* as an example. *J. Vis. Exp.,* **19**(94):52113. doi: 10.3791/52113. PMID: 25549068; PMCID: PMC4396939.

Prajapati, M.D., Gayakwad, D.M., Suman, S. and Kansara, S.S. 2025. Phylloplane bacteria and their role in plant disease management, *Int. J. Agric. Food Sci.,* **7**(1): 268-273. DOI: https://doi.org/10.33545/2664844X.2025.v7.i1d.267

Remya, K. and Suja, G. 2024. Crop–weed dynamics, nutrient uptake, and soil microclimate in elephant foot yam under conservation agriculture. *J. Plant Nutr.*, **47**(2): 281-295.

Rout, P., Mohanty, G., Dash, D. and Barik, M. 2025. Harnessing the power of underutilized root and tuber crops for global nutrition. In: *Sustainable Production of Root and Tuber Crops,* Kumar, R., Lal, M.K., Tiwari, R.K., and Brajesh Singh, B. (Eds.), Routledge 4 Park Square, Milton Park, Abingdon, Oxon OX14 4RN, 27-38.

Sahu, K.P., Patel, A., Kumar, M., Sheoran, N., Mehta, S., Reddy, B., Eke, P., Prabhakaran, N. and Kumar, A. 2021. Integrated metabarcoding and culturomic-based microbiome profiling of rice phyllosphere reveal diverse and functional bacterial communities for blast disease suppression. *Front. Microbiol.*, **12**: 1-21. 780458. doi: 10.3389/fmicb.2021.780458

Saleem, B. 2021**.** Phyllosphere microbiome: Plant defense strategies. In: *Microbiomes and the Global Climate Change*, Lone, S. A. and Malik, A. (Eds.), 173–201. https://doi.org/10.1007/978-981-33-4508-9_11

Silva, M.d.G.C., Medeiros, A.O., Converti, A., Almeida, F.C.G. and Sarubbo, L.A. 2024. Biosurfactants: promising biomolecules for agricultural applications. *Sustainability,* **16:** 449. https://doi.org/10.3390/su16010449

Srivastava, S., Pandey, V.K., Singh, P., Bhagya Raj, G.V.S., Dash, K.K. and Singh, R., 2022. Effects of microwave, ultrasound, and various treatments on the reduction of antinutritional factors in elephant foot yam: A review. *eFood*, **3**(6): doi: 10.1002/efd2.40

Veena, S.S., Chandra, C.V., Jeeva, M.L. and Makeshkumar, T. 2021. Postharvest diseases of tropical tuber crops and their management. In: *Postharvest handling and diseases of horticultural produce.* Singh, D., Sharma, R.R., Devappa, V. and Kamil, D (Eds). CRC Press, 6000 Broken Sound Parkway NW, Suite 300, Boca Raton, FL 33487-2742. 397-414 (437pp).

Veena, S.S., Sreekumar, J., Jeeva, M.L., Byju, G., Suja, G., Sengupta, S., Thangamani, C., Thakur, P., Narayan, A., Gudadhe, P.S. and Sunitha, S. 2025. Optimizing management interventions against *Sclerotium rolfsii* Sacc. on elephant foot yam (*Amorphophallus paeoniifolius* (Dennst.) Nicolson) in India. *Crop Prot.*, **188**: 107013. https://doi.org/10.1016/j.cropro.2024.107013

Yousef, A.E. and Carlstrom, C. 2003. *Food Microbiology: A Laboratory Manual,* 227p.

Zeng, Q., Hu, H.W., Ge, A.H., Xiong, C., Zhai, C.C., Duan, G.L., Han, L.L., Huang, S.Y. and Zhang, L.M. 2025. Plant–microbiome interactions and their impacts on plant adaptation to climate change. *J. Integr. Plant Biol.* **67**: 826–844. https://doi.org/10.1111/jipb.13863