

# Case study: Mangrove phyllosphere fungal populations in agro-waste management

## Abstract

**Introduction:** The article highlights the critical role of sustainable solid waste management (SWM) in agro ecosystems and emphasizes the need for effective microbial strains to improve waste management processes. It specifically focuses on isolating fungal strains from the phyllosphere of mangrove leaves in the Sundarbans region of West Bengal, India, during three different seasons.

**Materials & methods:** After isolation, the fungal strains are preserved in laboratory conditions, and pure cultures are established to assess their abilities in decomposing various types of waste substances. Additionally, the study includes an enzyme assay to evaluate the enzymatic activities of the isolated fungal strains, specifically targeting amylase, catalase, and polyphenol oxidase enzymes.

**Result:** The findings reveal that each isolated fungal strain exhibits unique enzyme production capabilities, with notable levels of amylase, catalase, and polyphenol oxidase enzymes. Moreover, the study suggests that employing multiple fungal strains together could prove effective for agricultural solid waste management and sustainable bioremediation technologies for future generations.

**Conclusion:** Key findings from the study reveal that each isolated fungal strain exhibits distinct enzyme production capabilities, with significant levels of amylase, catalase, and polyphenol oxidase enzymes. The research suggests that utilizing a combination of multiple fungal strains could be highly effective for agricultural solid waste management and sustainable bioremediation technologies for future generations. This study significantly contributes to the development and improvement of sustainable technologies for solid waste management. It underscores the potential of fungal strains in waste decomposition and bioremediation processes, highlighting the importance of microbial biodiversity. The research encourages further exploration of novel fungal strains to discover more efficient waste management solutions.

**Keywords:** solid waste management, phyllosphere, fungi, agriculture, bioremediation

## Introduction

Mangrove ecosystems are rich in biodiversity, hosting numerous microbial communities on their aerial parts, known as the phyllosphere. Among these, fungi play a crucial role in nutrient cycling and ecosystem stability. This case study explores the diversity of mangrove phyllo sphere fungi and their potential application in managing agricultural waste, a growing environmental concern. Bioremediation and agricultural waste management are crucial areas in environmental science and sustainable agriculture. Here's an overview of each:

## Bioremediation

Bioremediation is a process that uses microorganisms, plants, or microbial enzymes to detoxify or remove pollutants from the environment. It is often used to clean up contaminated soil and groundwater. There are several methods of bioremediation:

- In situ bioremediation:** Treatment of the contaminated material in its original place.

**Bioventing:** Supplying air and nutrients to the contaminated soil to stimulate the activity of indigenous bacteria.

**Biosparging:** Injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants.

**Phytoremediation:** Use of plants to absorb contaminants from soil and water.

- Ex situ bioremediation:** Removal of the contaminated material to be treated elsewhere.

**Biopiles:** Excavated soils are piled and treated with aeration and nutrient/water supply.

- Bioreactors:** Contaminated material is placed in a bioreactor where conditions are optimized for microbial degradation.

**Land farming:** Contaminated soils are spread over a prepared bed and periodically tilled to aerate the soil.

## Agricultural waste management

Agricultural waste management involves the handling and disposal of waste generated from farming activities. Effective waste management practices are essential to reduce environmental impact, enhance sustainability, and improve soil health. Key methods include:

- Composting:** Biological decomposition of organic materials under controlled aerobic conditions to produce a stable product (compost) that can be used as a soil amendment.

- Anaerobic digestion:** Biological process that breaks down organic material in the absence of oxygen, producing biogas (methane) and digestate, which can be used as a fertilizer.

**3. Manure management:** Proper handling, storage, and application of animal manure to reduce pollution and enhance soil fertility. Methods include:

**Manure spreading:** Applying manure to fields as fertilizer.

**Manure storage:** Using lagoons, tanks, or pits to store manure until it can be applied or treated.

**Manure processing:** Technologies such as drying, pelletizing, or composting manure to make it easier to handle and apply.

**4. Crop residue management:** Managing leftover plant material after harvest to improve soil health and reduce waste. Practices include:

**Mulching:** Using crop residues to cover soil, reducing erosion and moisture loss.

**Incorporation:** Plowing crop residues into the soil to enhance organic matter content.

**Grazing:** Allowing livestock to graze on crop residues.

These fungi are not only crucial for the health of mangrove ecosystems but also hold potential for biotechnological applications, such as agro-waste management. This case study investigates the diversity and functionality of mangrove phyllosphere fungi in degrading agricultural waste.

## Objectives

**Identification and characterization:** To identify and characterize fungal populations present in the phyllosphere of mangrove trees. Aims to identify and utilize efficient microbial strains that can enhance the decomposition of agricultural waste.

**Degradation potential:** To assess the ability of these fungi to degrade various types of agricultural waste.

**Field application feasibility:** To evaluate the practical application of these fungi in managing agricultural waste in field conditions.

So, Bioremediation is indeed a promising solution for solid waste management. Researchers worldwide are continuously striving to discover better remedial measures. This study aims to explore novel technologies for sustainable solid waste degradation.<sup>1</sup> The mangrove forests of West Bengal were chosen as the study area due to minimal anthropogenic interference, which increases the likelihood of discovering new microbial strains. Emphasis is placed on the phyllosphere microbiota (fungi) because, compared to the rhizosphere, it has been less explored by researchers. The microbial communities inhabiting leaves are diverse and include various species of bacteria, yeasts, filamentous fungi, and, less commonly, protozoa and nematodes. Among these, filamentous fungi are considered predominant transient inhabitants of leaf surfaces.<sup>2</sup>

## Significance of study

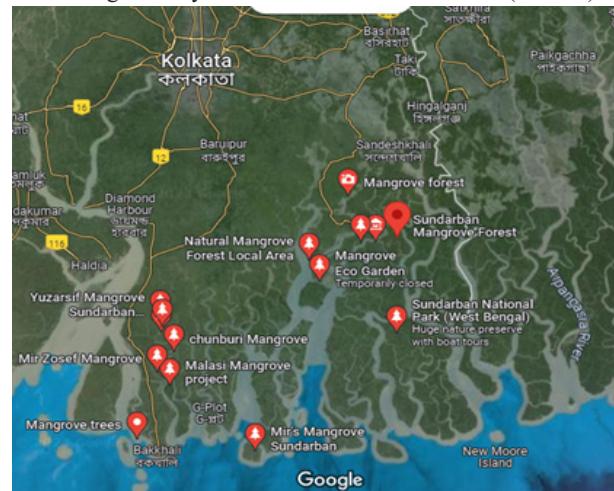
- Emphasizes the critical role of effective SWM in maintaining healthy agro ecosystems.
- Highlights the need for innovative approaches to handle agricultural waste sustainably.

## Literature review

### Material & methods

**Study site:** The study site is situated in the coastal zone of West Bengal, within the lower 'Bengal Basin'. It mainly encompasses the

districts of Parganas, both south and north, and partly Medinapole. This area constitutes approximately 73% of the total land area within West Bengal and is located between longitude 86°E and 89°10'E, and latitude 21°30'N to 22°30'N. The entire mangrove habitat within this region is classified as CRZ-1 (Coastal Regulation Zone-1), indicating its crucial importance for coastal ecology and conservation. Those areas were selected, where mangrove forest is natural. These regions have been chosen to capture the diverse ecological characteristics of the coastal ecosystem in the study area, providing a comprehensive understanding of its dynamics and conservation needs (MAP-1).



Map I Sample site collection area.

### Collection Procedure

#### Sample collection:

- Samples were collected from mangrove species including *Avicennia officinalis*, *Excoecaria agallocha*, *Heritiera globosa*, *Acanthus ilicifolius*, *Bruguiera gymnorhiza*, *Xylocarpus obovatus*, *Pneumatophorus decandrus*, and *Sonneratia apetala*.
- Collection involved traveling by boat to the required locations and then walking through the intertidal virgin forest region to gather mangrove leaves. Appropriate footwear was essential to prevent foot injuries.
- Sampling was conducted three times a year (in different seasons) to assess seasonal microbiological diversity. This approach helps capture the dynamic changes in fungal populations throughout the year, accounting for variations due to environmental factors such as temperature, humidity, and precipitation.

#### Fungal isolation and identification:

- Imprints were taken from both the dorsal and ventral surfaces of the leaves and cultured in Potato Dextrose Agar (PDA) medium.
- Fungal colonies were isolated and purified for further study.
- Identification of fungal species was carried out using morphological characteristics.

#### Screening for agro-waste degradation:

- Isolated fungi were tested for their ability to degrade agricultural wastes such as rice straw, corn husks, and sugarcane bagasse.
- Degradation assays involved incubating fungi with these substrates and monitoring changes in weight and biochemical composition over time.

**Enzyme activity assays:** Fungi that demonstrated effective degradation were further analyzed for enzyme production, focusing on cellulase, ligninase, and pectinase activities. Standard assays were used to quantify enzyme activity levels.

## Result & discussion

**Fungal diversity:** The study identified 14 different fungal populations over three different seasons, including *Aspergillus versicolor*, *A. niger*, *A. nidulans*, *A. flavus*, *A. ochraceus*, *Pyricularia*, *Penicillium expansum*, *Penicillium funiculosum*, *Chaetomium globosum*, *Mucor racemosus*, *Fusarium* sp., and *Helminthosporium expansum*.

*Acanthus ilicifolius* had the highest biological spectrum at 7.54%, while *Aspergillus versicolor* and *Aspergillus niger* had the lowest microbial population at 1.749%.

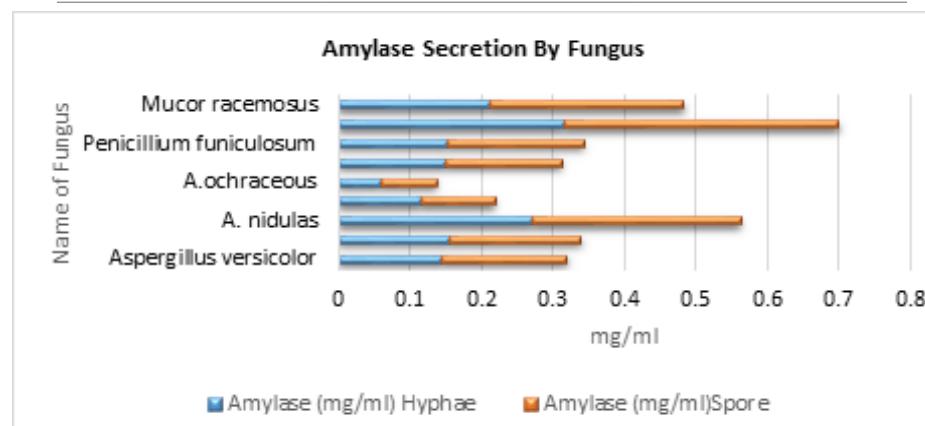
Pathogenic fungi identified included *Alternaria alternata*, *Fusarium* sp., *Pyricularia*, and *Helminthosporium expansum*.

**Table 1** Quantitative enzyme assay result

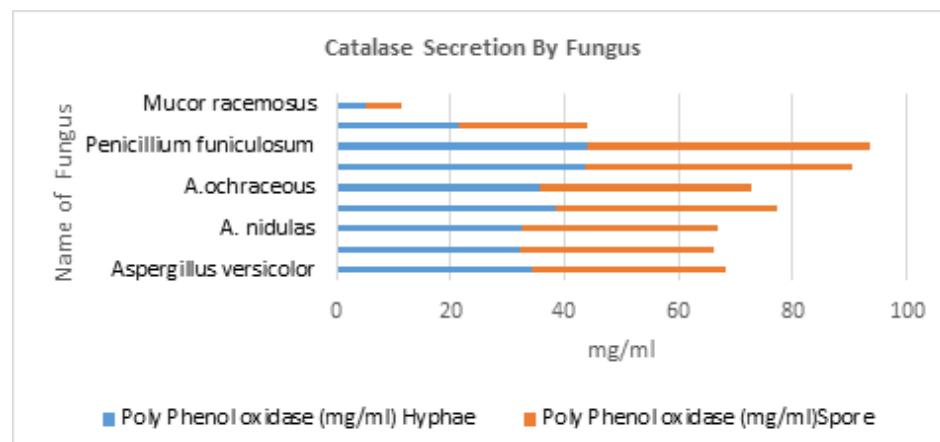
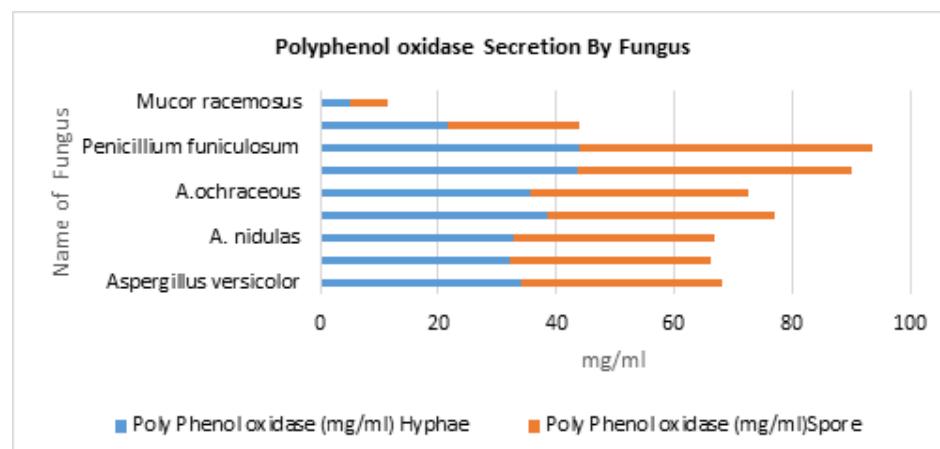
Name of Fungus	Name of Enzymes					
	Amylase (mg/ml)		Catalase (mg/ml)		Polyphenol oxidase (mg/ml)	
	Hyphae	Spore	Hyphae	Spore	Hyphae	Spore
<i>Aspergillus versicolor</i>	0.145	0.175	34.13	34.16	0.268	0.267
<i>A. niger</i>	0.155	0.185	32.13	34.12	0.238	0.258
<i>A. nidulans</i>	0.272	0.292	32.70	34.2	0.192	0.205
<i>A. flavus</i>	0.115	0.105	38.42	38.72	0.212	0.272
<i>A. ochraceous</i>	0.058	0.079	35.70	36.92	0.272	0.301
<i>Penicillium expansum</i>	0.151	0.162	43.56	46.71	0.104	0.152
<i>Penicillium funiculosum</i>	0.152	0.192	43.86	49.71	0.108	0.172
<i>Chacotonium globosum</i>	0.315	0.385	21.59	22.34	0.183	0.201
<i>Mucor racemosus</i>	0.211	0.272	05.10	06.20	0.137	0.262

**Table 2** Fungal enzyme production capability

Fungus	Enzyme Produced	Capability (Units/ml)
<i>Aspergillus versicolor</i>	Polyphenol oxidase (PPO)	10.5
<i>Aspergillus niger</i>	Cellulase	8.2
<i>Fusarium</i> sp	Lipase	6.7
<i>Penicillium expansum</i>	Amylase	7.9
<i>Pyricularia</i>	Protease	9.1
<i>Alternaria alternate</i>	Lactase	5.4



**Figure 1** Fungal amylase enzyme secretion.

**Figure 2** Fungal catalase enzyme secretion.**Figure 3** Fungal polyphenol oxidase enzyme secretion.

The study reveals that spores are more active and efficient in enzyme production than vegetative bodies or hyphae. Specifically, the polyphenol oxidase (PPO) enzyme, which catalyzes the oxidation of phenolic compounds into highly reactive quinones, is found to be produced by *Aspergillus versicolor* at a capability of 10.5 Units/ml.

Plant food wastes and by-products may contain a variety of enzymes capable of transforming bio-organic molecules, making them potentially useful in bioremediation procedures. Vegetable peels, being abundant plant food waste, may be considered a promising means of bioremediation as they contain the oxidative enzyme polyphenol oxidase (PPO), which can degrade or oxidize a range of pollutants.

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The polyphenol oxidase (PPO) enzyme catalyzes the phenolic compounds' oxidation into highly reactive quinones. Plant food wastes and by-products might have a range of enzymes that can transform bio-organic molecules, and thus they may have possible uses in bioremediation procedures. Vegetable peels are plentiful plant food waste and might be considered a hopeful means of bioremediation since they comprise the oxidative enzyme polyphenol oxidase (PPO), which can degrade or oxidize a range of pollutants.<sup>6</sup> Polyphenol oxidase (PPO) is not only crucial for its role in catalyzing the oxidation of phenolic compounds but also supports preventing wilt in tomato plants. Tomato plants possess PPO in their tissues,

which helps in preventing wilt by catalyzing the oxidation of phenolic compounds produced in response to stress, such as pathogen attack or environmental factors. The oxidation of these phenolic compounds results in the formation of compounds such as quinones, which have antimicrobial properties and help in strengthening the plant's defense mechanisms against pathogens.<sup>7</sup>

## Conclusion

Indeed, catalase is a vital enzyme known for catalyzing the breakdown of hydrogen peroxide into oxygen and water. It holds one of the highest turnovers among all enzymes, capable of decomposing more than one million molecules of hydrogen peroxide per molecule of enzyme. This enzyme's remarkable efficiency and ability to rapidly convert hydrogen peroxide into harmless oxygen and water make it invaluable in various biotechnological applications. Catalase is extensively used in bioremediation and waste management processes due to its capability to degrade toxic substances and pollutants effectively. In bioremediation, catalase can be employed to detoxify environments contaminated with hydrogen peroxide or other pollutants by accelerating their breakdown into harmless by-products. It is also utilized in waste management to treat wastewater or industrial effluents containing hydrogen peroxide, ensuring safe disposal and minimizing environmental impact. Overall, catalase's contributions to environmental sustainability and pollution control are significant, making it a key component in the arsenal of biotechnological tools aimed at preserving and protecting our planet.<sup>8,9</sup>

Certainly, amylase enzyme finds wide applications in agricultural waste management. Here are some ways in which it is utilized: Bioconversion of agricultural residues: Agricultural waste, such as crop residues, straw, husks, and pomace, contains complex polysaccharides like starch, cellulose, and hemicellulose. Amylase enzymes are used to break down starch into simpler sugars like glucose, which can then be fermented into biofuels such as ethanol. This bioconversion process helps in the efficient utilization of agricultural waste for energy production, reducing waste accumulation and dependence on fossil fuels. Composting: Amylase enzymes can accelerate the decomposition of organic matter in agricultural waste during composting. By breaking down starch and other complex carbohydrates into simpler compounds, amylase enhances the composting process, resulting in faster production of nutrient-rich compost. This compost can then be used to improve soil fertility and enhance crop yields, closing the loop on agricultural waste management. Livestock feed: Agricultural by-products containing starch, such as cereal grains, may not be fully digestible by livestock due to their complex structure. Adding amylase enzymes to animal feed helps to break down starch into more digestible sugars, improving the nutritional value of the feed and promoting better digestion in animals. This not only reduces feed wastage but also enhances animal health and productivity. Soil remediation: In certain cases, agricultural practices can lead to soil contamination with pesticides or other chemicals. Amylase enzymes, along with other enzymes, can be used in bioremediation processes to degrade these contaminants, making the soil safer for agricultural use.

Overall, the use of amylase enzyme in agricultural waste management helps to maximize resource utilization, reduce environmental pollution, and improve agricultural productivity.<sup>10-13</sup> The studied fungal population demonstrates the ability to synthesize amylase, catalase, and polyphenol oxidase enzymes simultaneously. These enzymes are all capable of breaking down waste, particularly organic waste. Agricultural solid wastes, rich in organic material, can benefit from a combination of these fungal strains, which would support the degradation of organic waste more efficiently. Through bioremediation, this combination of enzymes can accelerate the decomposition of agricultural solid wastes, partially converting them into bio-fertilizer. This process not only reduces waste accumulation but also enhances soil fertility. The bio-fertilizer produced can provide essential nutrients for plant growth, promoting healthier crops and improving agricultural productivity. Furthermore, the bioremediation process can also help prevent pathogenic attacks. By breaking down organic waste more rapidly, the growth of pathogens is inhibited, reducing the risk of disease outbreaks in plants. Additionally, the improved soil fertility resulting from the bioremediation process can strengthen plants' natural defense mechanisms, making them more resilient to pathogenic attacks.<sup>14-20</sup>

In summary, the simultaneous synthesis of amylase, catalase, and polyphenol oxidase enzymes by the studied fungal population enables efficient bioremediation of agricultural solid wastes. This process not only converts waste into valuable bio-fertilizer but also enhances soil fertility and provides preventive measures against pathogenic attacks, ultimately benefiting agricultural sustainability and productivity.<sup>21-23</sup>

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## Recommendations

Based on the findings that spores are more active and efficient in enzyme production than vegetative bodies or hyphae, and considering the potential of polyphenol oxidase (PPO) in bioremediation, the following recommendations are proposed:

**Optimize cultivation conditions:** Research and develop optimal growth conditions specifically for spore production in the studied fungal populations. This can include adjusting factors such as temperature, humidity, nutrient availability, and light exposure.

**Spore-based bioremediation:** Prioritize the use of fungal spores in bioremediation projects to take advantage of their superior enzyme production capabilities. Develop protocols for the efficient application of spores to contaminated sites or agricultural wastes.

**Utilize plant food wastes:** Exploit the abundance of vegetable peels and other plant food wastes that contain PPO for bioremediation processes. Develop methods to extract and concentrate PPO from these wastes for more effective application.

**Pollutant degradation:** Conduct further studies to identify the range of pollutants that can be effectively degraded or oxidized by PPO. Tailor bioremediation strategies to target specific pollutants present in agricultural or industrial waste streams.

**Bio-Fertilizer Production:** Combine the enzymatic breakdown of agricultural solid wastes with the production of bio-fertilizers. Utilize the resultant bio-fertilizers to enhance soil fertility and support sustainable agriculture.

**Pathogen Control:** Implement enzymatic bioremediation as a means to control soil-borne pathogens. By accelerating the decomposition of organic waste, reduce the substrates available for pathogen growth and proliferation.

## Pilot projects and scaling up

**Field trials:** Initiate pilot projects to test the practical application of spore-based and PPO-mediated bioremediation on a larger scale. Monitor and document the effects on soil health, crop yields, and environmental impact.

**Scaling up:** Develop scalable bioremediation systems that can be adopted by agricultural communities and waste management facilities. Ensure that these systems are cost-effective, efficient, and easy to implement.

## Collaborative Research and Development:

**Interdisciplinary collaboration:** Foster collaboration between microbiologists, environmental scientists, agronomists, and biotechnologists to refine and advance bioremediation techniques.

**Knowledge Sharing:** Create platforms for knowledge exchange and dissemination of best practices in bioremediation. Encourage the publication of research findings and case studies to promote wider adoption.

## Policy and regulation support

**Supportive policies:** Advocate for policies and regulations that support the use of bioremediation techniques in waste management and agricultural practices. Seek government incentives and funding for research and implementation.

**Environmental standards:** Establish environmental standards and guidelines for the use of enzymatic bioremediation to ensure safety, efficacy, and minimal ecological disruption.

By implementing these recommendations, the potential of fungal spores and PPO enzymes in bioremediation can be fully realized, contributing to more sustainable waste management practices and improved agricultural productivity.

## Future plan

Given the findings, future research and application strategies should focus on several key areas to maximize the benefits of the identified fungal populations and their enzymatic capabilities:

**Optimizing fungal cultivation:** Develop and refine techniques to enhance the growth and enzyme production efficiency of fungal spores, considering their demonstrated superiority over vegetative bodies or hyphae. This includes exploring optimal environmental conditions and substrates that can support maximal enzyme yield.

**Scaling up bioremediation processes:** Implement pilot projects to scale up the use of fungi and their enzymes, such as amylase, catalase, and PPO, in bioremediation. This could involve treating larger volumes of agricultural waste and monitoring the effects on soil fertility, pathogen reduction, and crop productivity.

**Exploring diverse agricultural wastes:** Extend research to a wider variety of agricultural wastes and by-products to determine the broad applicability of these fungal enzymes. This could help identify specific wastes that are particularly amenable to enzymatic breakdown and subsequent conversion into bio-fertilizers.

**Developing integrated waste management systems:** Create integrated systems that combine fungal bioremediation with other waste management strategies. This holistic approach can ensure comprehensive waste reduction, soil enhancement, and sustainable agricultural practices.

**Enhancing pathogen resistance in crops:** Investigate the potential of PPO and other enzymes in fortifying crops against pathogens. Genetic studies and biotechnological interventions could be pursued to increase the endogenous production of such enzymes in plants, thereby boosting their natural defense mechanisms.

**Environmental impact assessment:** Conduct thorough assessments of the environmental impacts of deploying fungal bioremediation on a large scale. This includes studying the long-term effects on soil health, biodiversity, and ecosystem balance to ensure sustainable and eco-friendly practices.

**Collaboration with agricultural stakeholders:** Engage with farmers, agricultural researchers, and policymakers to promote the adoption of bioremediation techniques. Educational initiatives and practical demonstrations can help in disseminating knowledge and encouraging widespread implementation.

By focusing on these areas, future efforts can harness the full potential of mangrove phyllosphere fungal populations in agro-waste management, contributing to sustainable agriculture and environmental conservation.

## Acknowledgments

None.

## Conflicts of interest

There is no conflict of interest in this research work.

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