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RESEARCH ARTICLE

PRELIMINARY PERFORMANCE OF *RHIZOBIUM TROPICI* AND *BACILLUS SAFENSIS* ON AGRO-WASTE SUBSTRATES: VIABILITY AND ECONOMIC FEASIBILITY AS ALTERNATIVES TO SOIL-CHARCOAL CARRIER

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ABSTRACT

Carrier materials are critical to the viability and functional delivery of microbial biofertilizers, directly influencing their efficacy in sustainable agriculture. Conventional carriers, such as soil mixed with wood charcoal, are becoming increasingly costly and scarce, prompting the need for alternative low-cost substrates. This study investigated whether locally abundant agro-wastes could serve as effective carriers for biofertilizer formulations. To assess the microbial viability of *Rhizobium tropici* and *Bacillus safensis* co-inoculated on agro-waste substrates—rice hull, coconut coir dust, coconut coir fiber, and corn cobs—and to compare their economic feasibility with traditional soil-charcoal carriers. Sterilized formulations of agro-waste carriers were prepared in three ratios and inoculated with standardized cultures of *R. tropici* and *B. safensis*. Microbial populations were quantified as colony forming units per gram (CFU/g) immediately and at 48 hours post-inoculation using serial dilution and selective agar plating. A direct cost analysis evaluated raw material expenses relative to conventional carriers. Rice hull in a ratio of 120g soil + 40g hull achieved the highest microbial population (3.1×10^9 CFU/g), significantly surpassing the viability threshold of 10^6 CFU/g. Coconut coir dust also supported substantial populations up to 5.3×10^8 CFU/g. In contrast, coconut coir fiber and corn cobs consistently failed to maintain viable counts. Economic analysis indicated that using agro-waste carriers reduced raw material costs by approximately ₱294 per 250 packets compared to soil-charcoal mixtures. Rice hull and coconut coir dust are effective, economical carrier substrates for biofertilizer formulations containing *R. tropici* and *B. safensis*. These findings support the integration of agro-waste carriers into localized biofertilizer production systems, promoting sustainable and cost-efficient agricultural practices.

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INTRODUCTION

Soil fertility management is crucial for sustainable agriculture and food security. Conventional farming's reliance on synthetic fertilizers has led to ecological issues, prompting a shift towards more sustainable practices[1]. Organic amendments and bio-fertilizers offer alternatives that enhance soil structure, nutrient availability, and microbial activity while reducing environmental impacts[1,2]. Ecological intensification, which integrates ecosystem services into crop production, can help meet rising agricultural demands while minimizing negative environmental effects[3]. Organic fertilizers and cover crops improve soil health by enhancing physical, chemical, and biological properties, promoting beneficial microorganisms, and contributing to nutrient cycling and soil stability[4]. This integrated approach to soil fertility management not only supports sustainable food production but also contributes to climate change mitigation through increased carbon sequestration and reduced reliance on chemical inputs[1,4]. Biofertilizers are formulations containing living microorganisms that promote plant growth by increasing nutrient availability when applied to seeds, plants, or soil[5,6]. They enhance soil fertility through processes like nitrogen fixation, phosphorus solubilization, and growth-promoting substance synthesis[5]. Unlike chemical fertilizers, biofertilizers are eco-friendly, non-toxic, and improve soil physicochemical and biological properties[5,6]. They play a crucial role in sustainable agriculture by enhancing soil microbial diversity, improving soil structure, and contributing to carbon sequestration[7]. Biofertilizers also help plants tolerate abiotic and biotic stresses[6]. Despite their benefits, challenges such as limited awareness, inconsistent performance, and inadequate regulatory frameworks hinder widespread adoption[7].Future research should focus on improving formulation techniques and conducting long-term impact studies on soil health[7].

Role and promise of microbial biofertilizers

Microbial biofertilizers offer a sustainable alternative to chemical fertilizers, enhancing soil fertility and crop productivity while minimizing environmental impacts[7,8]. These formulations contain beneficial microorganisms like nitrogen-fixing bacteria, phosphate-solubilizing bacteria, and mycorrhizal fungi, which improve nutrient availability through various mechanisms[9]. Biofertilizers contribute to long-term soil health by fostering microbial diversity, improving soil structure, and increasing organic matter content[7]. They also promote plant growth by synthesizing growth-regulating substances and suppressing soil-borne diseases[8,9]. Despite their advantages, challenges such as limited awareness, inconsistent performance, and inadequate regulatory frameworks hinder widespread adoption[7]. Recent advancements in biofertilizer technology, including multi-functional formulations and microbial consortia, show promise in improving their efficacy[7,10].

Critical importance of carriers in biofertilizer formulations

Carrier materials play a crucial role in biofertilizer formulations, affecting microbial viability and effectiveness during storage and application. Ideal carriers should support high microbial populations, be cost-effective, and environmentally friendly[11]. Various materials have been explored as carriers, including clay soil, fly-ash, press mud, and lignite, with some formulations maintaining microbial populations up to six months[12]. Vermicast has shown promise as a carrier, increasing bacterial survival compared to charcoal[13]. Lantana charcoal, derived from Lantana camara biomass, has emerged as a novel carrier material for *Azotobacter chroococcum*, demonstrating high water-holding capacity, neutral pH, and superior microbial support during storage[14]. These studies highlight the ongoing research to develop effective, sustainable carrier materials for biofertilizers, addressing challenges such as maintaining microbial viability and reducing environmental impacts associated with traditional carriers like wood charcoal and garden soil.

Agro-waste valorization as a sustainable alternative

Agricultural waste valorization presents a sustainable solution to manage the vast quantities of residues generated globally. These wastes, including rice husks, coconut fiber, and crop residues, can be transformed into valuable products, aligning with circular economy principles[15,16]. Agro-wastes serve as effective carriers for microbial inoculants, supporting beneficial bacterial populations and enhancing soil fertility[17]. Various conversion techniques can produce activated carbons for environmental remediation, energy storage, and crop productivity improvement[16]. Additionally, agricultural wastes can be utilized to create biofuels, biofertilizers, bio-plastics, and other value-added products[18]. This approach not only addresses waste management challenges but also contributes to sustainable agriculture, mitigates climate change, and supports the bioeconomy[15,18]. Implementing these strategies can significantly reduce environmental pollution and fossil fuel dependence while promoting agricultural sustainability

Focus of this study

This research explores the potential of microbial biofertilizers to enhance crop productivity while reducing chemical fertilizer use. *Bacillus safensis* and *Bacillus siamensis* strains demonstrated phosphorus solubilization, nitrogen fixation, and phytohormone production capabilities, promoting plant growth when used in combination[19]. Various nitrogen-fixing and potassium-solubilizing bacteria have been identified as potential biofertilizers[20]. Bioengineering approaches can improve the efficiency of nitrogen fixation, phosphate solubilization, and potassium mobilization in microbial communities[21]. In a study on common beans, inoculation with *Rhizobium tropici*, especially when combined with other beneficial microorganisms like *Trichoderma harzianum*, enhanced nodule formation and improved agronomic parameters. Co-inoculation with *Azospirillum brasilense* significantly increased grain yield, particularly in clayey soil with reduced NPK fertilization, suggesting the potential for sustainable agriculture with decreased reliance on mineral fertilizers[22].

Objectives and broader relevance

This study aimed to evaluate the viability of co-inoculated *R. tropici* and *B. safensis* on four locally abundant agro-waste substrates—rice hull, coconut coir dust, coconut coir fiber, and corn cobs—each tested in three formulation ratios. Additionally, an economic analysis compared the direct material costs of these agro-waste carriers with conventional soil-charcoal systems. By identifying effective, low-cost alternatives, the study seeks to provide practical solutions that can be adopted by local farmers, cooperatives, and community-based biofertilizer production units, thereby contributing to more resilient, ecologically sound farming systems.

MATERIALS AND METHODS

Study design and rationale

The experiment was structured to comprehensively assess both the biological and economic viability of using agro-waste carriers. A factorial approach was employed, testing four carrier types across three formulation ratios, allowing a robust comparison of how carrier composition influenced microbial survival.

Carrier preparation

The agro-waste materials—rice hull, coconut coir dust, coconut coir fiber, and chopped corn cobs—were collected from local farms and agro-industrial processing sites. Each material was sun-dried to reduce moisture content and then ground to achieve reasonably uniform particle sizes, improving homogenization with soil and inoculum. Three carrier formulations were prepared for each material:

- 80g sterilized garden soil + 80g agro-waste (Ratio 1)
- 120g sterilized garden soil + 40g agro-waste (Ratio 2)
- 160g agro-waste alone, no soil (Ratio 3)

All mixtures were sterilized using a conventional autoclave at 121°C for 30 minutes to eliminate background microbial populations, ensuring that subsequent counts reflected only the introduced inoculants.

Inoculum preparation and application: Pure cultures of ***R. tropici*** were grown in Yeast Mannitol Broth (YMB) for nitrogen-fixers, while *B. safensis* cultures were maintained in parallel in Pikovskaya's broth for phosphate-solubilization and *Aleksandrow's* broth for potassium mobilization. After reaching microbial growth phase, 20 mL of *R. tropici* and 20 mL of combined *B. safensis* cultures were mixed to create a 40 mL inoculum. This was uniformly incorporated into each 160g carrier formulation under aseptic conditions.

Microbial enumeration

Microbial viability was assessed immediately after inoculation and again at 48 hours. Samples were serially diluted and plated on Nutrient Agar to calculate ***R. tropici*** and ***B. safensis***. Colony forming units per gram (CFU/g) were calculated using standard plate count techniques, with results averaged over three replicate plates per sample.

Economic analysis

To determine the feasibility of scaling these formulations, a cost analysis compared the direct raw material expenses for producing 250 biofertilizer packets using agro-waste carriers versus the traditional garden soil and wood charcoal blend. Local market rates in Ilagan City, Isabela were used to compute total expenses, enabling a straightforward cost comparison.

RESULTS

Microbial viability across carrier formulations

Results revealed significant variability in microbial survival depending on carrier type and ratio.

- Rice hull consistently exceeded the critical viability threshold of 10^6 CFU/g across all ratios, corroborating previous findings[23]. Notably, Ratio 2 (120 g soil + 40 g rice hull) yielded the highest microbial counts, reaching 3.1×10^9 CFU/g. Ratio 3 (pure rice hull) also performed well at 4.8×10^8 CFU/g, underscoring rice hull's intrinsic suitability as a microbial carrier due to its high porosity and moisture retention capacity.
- Coconut coir dust demonstrated moderate effectiveness, with Ratio 3 (pure coir dust) achieving 5.3×10^8 CFU/g and Ratio 2 at 3.4×10^8 CFU/g, which is consistent with previous observations[24]. However, Ratio 1 did not sustain sufficient populations, possibly due to dilution effects from higher soil content or uneven moisture distribution that may have compromised microbial survival.
- In contrast, coconut coir fiber and corn cobs consistently failed to support viable microbial populations across all tested ratios, with counts falling below detectable limits. This outcome likely reflects the presence of inhibitory phenolic compounds in coir fiber as previously documented[25], and the dense, compact structure of corn cobs which restricts aeration and microbial colonization.

Initial short-term viability

Within 48 hours post-inoculation, rice hull and coconut coir dust maintained robust populations, indicating minimal immediate die-off. These carriers effectively stabilized the introduced microbes, preserving populations well above the threshold required for biofertilizer quality standards.

Economic assessment

The cost comparison revealed dramatic differences. Producing 250 packets of biofertilizer carrier using the soil-charcoal mixture required approximately ₱318.75, primarily due to the high price of wood charcoal. In contrast, formulations based on agro-wastes cost only ₱25.00, representing a savings of ₱293.75. This 92% reduction in carrier material costs highlights a compelling economic incentive for adopting agro-waste substrates.

DISCUSSION

This study underscores the potential of agricultural waste products as alternative carriers for microbial biofertilizers. Rice husk exhibited particularly promising characteristics, including low moisture content, high porosity, and strong water absorption capacity, which collectively supported microbial growth and plant development[23]. Coconut coir dust also proved moderately effective, especially in pure formulations, consistent with earlier findings that demonstrated its ability to enhance microbial survival in crop systems[24]. However, the inconsistent performance observed across different ratios highlights the need for optimizing parameters such as moisture content and implementing pre-treatment strategies to mitigate inhibitory phenolic compounds[25]. Conversely, coconut coir fiber and corn cobs were unsuitable under the conditions tested. The likely causes include phenolic toxicity and poor structural compatibility for inoculant adherence, consistent with findings in bioremediation studies where certain lignocellulosic substrates inhibited microbial colonization[26].

CONCLUSION

This study demonstrated that rice hull, especially in a formulation of 120g soil + 40g hull, serves as an optimal carrier for co-inoculated *R. tropici* and *B. safensis*, achieving CFU counts surpassing 3.1×10^9. Coconut coir dust also emerged as a viable alternative under specific ratios. These agro-waste carriers not only ensured superior microbial viability but also reduced carrier costs by over 90% compared to traditional soil-charcoal mixtures. By integrating agro-waste substrates into local biofertilizer production, this approach promotes environmentally sustainable, economically accessible, and community-driven nutrient management systems. Future studies should explore long-term shelf-life under ambient storage and validate field performance across multiple crop systems, paving the way for widespread adoption of this low-cost, eco-friendly technology. Economically, the shift from soil-charcoal to agro-waste carriers presents a transformative opportunity for resource-constrained farming communities. Beyond reducing input costs, this strategy valorizes local residues that otherwise contribute to environmental burdens, effectively closing nutrient loops and supporting circular bioeconomies.

Glossary of Abbreviations

CFU – Colony Forming Unit
DA – Department of Agriculture
LGU – Local Government Unit
RFO – Regional Field Office

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