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Effect of Storage Medium Composition on the Viability of Antagonistic Bacteria Against Stem Rot Disease

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Abstract

Stem rot is a major constraint in sweet corn and maize production, especially under humid conditions that favor pathogen development. Although antagonistic bacteria offer a sustainable alternative to chemical control, their field use is often limited by viability loss during room-temperature storage, making carrier formulation critical for shelf-life and product reliability. This study evaluated the effect of biochar–peat carrier composition on the storage viability of five antagonistic bacterial isolates for stem rot management in sweet corn. A two-factor factorial experiment (4 carrier compositions × 5 isolates) with three replications (60 experimental units) was conducted under laboratory conditions. Carriers were prepared as biochar: peat mixtures (v/v) of 3:1, 1:1, 1:3, and 100% peat. Each carrier unit (10 g) was inoculated with 1 mL bacterial suspension (≈10^8 cfu mL^{-1}), sealed, and stored at room temperature (28–29 °C). Viability was quantified at 30, 60, and 90 days after inoculation (DAI) using serial dilution and plate counts on TSA, expressed as cfu g^{-1}. Data at 60 DAI were analyzed by factorial ANOVA including block (Group), medium (M), isolate (P), and M×P effects, followed by LSD (5%) for mean separation using SPSS 25. At 30 DAI, bacterial densities were high and did not differ among carriers, indicating comparable short-term support across media. At 60 DAI, carrier composition significantly affected bacterial density, whereas isolate and M×P interaction effects were not significant, indicating a general carrier-driven response across isolates. The biochar-rich carrier (3:1) maintained the highest mean population (8.844 × 10^7 cfu g^{-1}). By 90 DAI, all treatments declined, yet the 3:1 carrier retained the highest density (1.089 × 10^7 cfu g^{-1}). Overall, biochar-enriched carriers, particularly the 3:1 biochar: peat mixture, better preserved antagonistic bacterial viability under non-refrigerated storage up to 90 days.

Keywords: Antagonistic bacteria, Storage media, Stem rot disease

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INTRODUCTION

Stem rot disease is a major constraint in maize (*Zea mays* L.) production worldwide, including sweet corn cultivation in tropical and subtropical regions. The disease is commonly associated with soil- and residue-borne pathogens such as *Fusarium graminearum*, *Fusarium verticillioides*, and *Dickeya zeae*, which infect plant tissues through roots or stem wounds and subsequently cause stalk decay, lodging, and premature plant death. In agroecosystems characterized by high soil moisture, abundant organic matter, and limited drainage conditions typical of peatlands and lowland agricultural systems the incidence and severity of stem rot disease are often exacerbated. Yield losses attributable to stem rot can be substantial, not only due to reduced biomass and grain filling but also because lodging interferes with mechanized harvesting and increases postharvest losses (Zhang et al., 2022; Suriani et al., 2023). In addition, infection by *Fusarium* species raises serious food and feed safety concerns because

of the production of mycotoxins such as deoxynivalenol, which pose risks to animal and human health and impose further economic burdens related to quality control and regulatory compliance (Zhang et al., 2022).

Conventional management of stem rot disease has relied predominantly on chemical fungicides. Although fungicides can provide short-term suppression of pathogens, their long-term effectiveness is increasingly constrained by several factors. Repeated fungicide application contributes to environmental pollution, disrupts non-target soil organisms, and accelerates the development of pathogen resistance, thereby reducing efficacy over time (Zhang et al., 2022). Moreover, the high cost of chemical inputs can be prohibitive for smallholder farmers, particularly in developing regions, leading to suboptimal disease control and reduced farm profitability (Suriani et al., 2023). Concerns regarding pesticide residues in food products have also become more prominent, especially as consumer demand for environmentally friendly and sustainably produced crops continues to rise. These limitations highlight the urgent need for alternative disease management strategies that are effective, affordable, and ecologically sustainable.

Within this context, biological control has emerged as a promising component of integrated pest management (IPM) strategies for stem rot disease. IPM emphasizes the coordinated use of biological, cultural, genetic, and chemical approaches to manage plant diseases while minimizing environmental impacts and preserving agroecosystem resilience (Zhang et al., 2022). Among biological control options, antagonistic bacteria often categorized as plant growth-promoting rhizobacteria (PGPR) have received considerable attention due to their multifunctional roles in suppressing pathogens and enhancing plant health. These bacteria can inhibit soil-borne fungal pathogens through multiple mechanisms, including antibiosis, competition for nutrients and niche space, and the induction of systemic resistance in host plants (Tariq et al., 2020; Hidayati et al., 2022).

Antibiosis involves the production of antimicrobial compounds such as lipopeptides, antibiotics, and volatile organic compounds that directly suppress pathogen growth. Species belonging to the genera *Bacillus* and *Pseudomonas* are well known for producing a wide array of bioactive metabolites effective against *Fusarium* spp. and other soil-borne fungi (Ali et al., 2022; Anckaert et al., 2021). In addition to antibiosis, antagonistic bacteria compete with pathogens for limited resources in the rhizosphere, including carbon substrates, iron, and infection sites. This competitive exclusion can significantly reduce pathogen establishment and proliferation, particularly in soils with well-developed beneficial microbial communities (Thakur et al., 2021). Furthermore, certain bacterial strains are capable of inducing systemic resistance in plants by activating defense-related signaling pathways and enhancing the expression of pathogenesis-related proteins, thereby increasing the plant's inherent capacity to withstand pathogen attack (Meena et al., 2020; Chen et al., 2024).

Beyond their role as bioprotectants, many antagonistic bacteria also function as biofertilizers and biostimulants. They contribute to nutrient solubilization, phytohormone production, and improved root development, leading to enhanced plant growth and stress tolerance. In sweet corn, indigenous PGPR isolated from peat soils have been successfully applied as seed biopriming agents, promoting early plant growth while simultaneously suppressing *Fusarium*-induced stem rot under controlled conditions (Hidayati et al., 2022). These multifunctional attributes make antagonistic bacteria particularly attractive for sustainable crop production systems, where inputs are expected to deliver multiple agronomic and environmental benefits.

Despite their demonstrated potential, the large-scale adoption of antagonistic bacteria in agricultural practice remains limited. One of the most critical constraints is the difficulty of maintaining bacterial viability and functional stability during storage prior to field application. For microbial biocontrol agents to be effective, they must retain sufficiently high viable cell populations and metabolic activity from the point of production to the time of use. However,

many bacterial formulations exhibit a rapid decline in viable cell numbers during storage, especially under room-temperature conditions commonly encountered at the farm level (Sunarno et al., 2021; Compant et al., 2021). Reduced viability not only diminishes biocontrol efficacy but also increases the amount of inoculum required for effective disease suppression, thereby raising production costs and reducing economic feasibility.

Shelf-life and storage stability are therefore pivotal determinants of the commercial success of microbial biocontrol agents. Storage at room temperature poses particular challenges because fluctuations in temperature and humidity can accelerate metabolic activity, leading to nutrient depletion, accumulation of toxic metabolites, desiccation stress, and eventual cell death (Teixidó et al., 2022; Kumar et al., 2023). Inadequate storage conditions also increase the risk of contamination by opportunistic microorganisms that may outcompete or inhibit the target antagonistic bacteria (Riseh et al., 2021). These challenges are compounded when formulations involve multiple bacterial species with differing physiological traits, nutrient requirements, and stress tolerances.

The suitability of the carrier medium plays a central role in determining the shelf-life and viability of antagonistic bacteria. Carrier materials provide a physical matrix that protects bacterial cells, supplies or retains nutrients, regulates moisture, and buffers environmental stress during storage. Various carriers have been explored, including liquid suspensions, talc-based powders, sterile soils, and organic substrates. However, many conventional carriers fail to provide long-term stability, particularly under non-refrigerated conditions (Lehmann & Joseph, 2015; Lee et al., 2023). Consequently, the identification of carrier systems that can sustain high bacterial viability under practical storage conditions remains a critical research priority.

Biochar and peat have attracted increasing attention as potential carrier materials for microbial inoculants. Biochar, a carbon-rich material produced through the pyrolysis of biomass residues, is characterized by high porosity, large specific surface area, and substantial cation exchange capacity. These properties enable biochar to create protected microhabitats for microorganisms, enhance aeration, and retain nutrients and moisture, thereby supporting microbial survival and persistence (Lehmann et al., 2011; Zhu et al., 2017). Biochar derived from agricultural residues such as rice husks is particularly attractive in tropical regions because it is locally available, cost-effective, and environmentally beneficial through waste valorization (Yuzairi et al., 2022).

Peat, on the other hand, has long been used as a carrier for microbial inoculants due to its high organic matter content, strong water-holding capacity, and ability to support microbial growth. Peat-based carriers can provide readily available carbon sources and maintain moisture levels favorable for bacterial survival (Maftu'ah & Nursyamsi, 2019). However, peat is typically acidic and can become anaerobic under high moisture conditions, factors that may limit long-term bacterial viability for certain aerobic species (Wang et al., 2019). Moreover, peat decomposition over time can generate organic acids and phenolic compounds that may inhibit microbial activity (Hodgson et al., 2018).

Combining biochar and peat into a composite carrier system offers a potentially synergistic approach that leverages the complementary properties of both materials. Biochar can improve aeration, structural stability, and nutrient retention, while peat can supply organic matter and maintain moisture. Recent studies have demonstrated that biochar organic matter amendments can enhance microbial biomass, diversity, and functional stability in soils (Li et al., 2022; Rijk et al., 2024). However, despite these promising findings, information remains limited regarding the use of biochar–peat mixtures specifically as storage media for antagonistic bacteria. In particular, the effects of different biochar–peat ratios on the long-term viability of multiple bacterial species under room-temperature storage conditions have not been systematically evaluated.

This knowledge gap is especially pronounced for biochar produced from agricultural residues and peat materials commonly found in tropical regions, where storage infrastructure such as refrigeration is often unavailable. Furthermore, most existing studies focus on single microbial strains, whereas practical biocontrol applications increasingly involve multiple antagonistic species or consortia. Differences in physiological traits among bacterial species such as spore-forming capacity in *Bacillus* spp. or stress sensitivity in non-spore-forming bacteria may lead to differential survival responses to carrier media during storage. Understanding whether carrier effects are consistent across bacterial species or whether specific interactions occur is therefore essential for rational formulation design.

In this context, antagonistic bacteria such as *Bacillus cereus*, *Burkholderia cepacia*, *Pseudomonas luteola*, *Bacillus subtilis*, and *Brevibacillus laterosporus* are of particular interest. These species have previously been identified as potential stem rot antagonists and plant growth-promoting agents, yet they differ markedly in physiological characteristics, metabolic capabilities, and stress tolerance (Hidayati et al., 2022). Evaluating their survival in different carrier media can provide valuable insights into the general applicability and robustness of biochar–peat formulations for biocontrol purposes.

Therefore, this study aimed to evaluate the effect of different biochar–peat storage medium compositions on the viability of five antagonistic bacterial isolates with potential for controlling stem rot disease in sweet corn. Specifically, bacterial population dynamics were assessed under room-temperature storage conditions (approximately 28–29 °C) over storage periods of 30, 60, and 90 days, using viable cell density (cfu g^{-1}) as the primary response variable. This research was designed as a laboratory-based viability study and did not include greenhouse or field efficacy evaluations. By focusing on storage performance, the study seeks to address a critical bottleneck in the practical deployment of antagonistic bacteria and to provide empirical evidence for the development of effective, locally adaptable carrier formulations.

The findings of this study are expected to contribute to the optimization of microbial biocontrol formulations by identifying biochar–peat ratios that best maintain bacterial viability during storage. Such information is essential for bridging the gap between laboratory-scale screening of antagonistic bacteria and their successful application in sustainable stem rot disease management within integrated pest management frameworks.

METHOD

Study Design and Treatments

This study evaluated the storage viability of antagonistic bacteria using a two-factor factorial experiment arranged in a Completely Randomized Design (CRD) under laboratory conditions. The first factor was storage medium composition (M), consisting of four levels based on the volume/volume (v/v) ratio of biochar and peat: m1 (biochar:peat = 3:1), m2 (1:1), m3 (1:3), and m4 (100% peat). The second factor was bacterial isolate type (P), consisting of five antagonistic bacterial isolates: Bacillus cereus (p1), Burkholderia cepacia (p2), Pseudomonas luteola (p3), Bacillus subtilis (p4), and Brevibacillus laterosporus (p5) (Hidayati et al., 2022). Each treatment combination was replicated three times, resulting in a total of 60 experimental units (4 storage media × 5 isolates × 3 replications). The experiment was conducted over a six-month period from November 2023 to April 2024. The following table presents treatment structure used in the factorial experiment.

Bacterial viability (cfu g^{-1}) was quantified at 30, 60, and 90 days after inoculation (DAI). The storage workflow and sampling scheme follow the configuration shown in Figure 1 (Storage of antagonistic bacteria), while summary outcomes and statistical outputs are presented in Table 2 (ANOVA at 60 DAI), Table 3 (treatment means at 60 DAI), and Table 4 (viability at 30/60/90 DAI) in the Findings section.

Table 1. Treatment structure used in the factorial experiment

Factor	Code	Description
Storage medium (M)	m1	3 parts biochar : 1 part peat (v/v)
	m2	1:1 biochar : peat (v/v)
	m3	1:3 biochar : peat (v/v)
	m4	100% peat (v/v)
Bacterial isolate (P)	p1	*Bacillus cereus*
	p2	*Burkholderia cepacia*
	p3	*Pseudomonas luteola*
	p4	*Bacillus subtilis*
	p5	*Brevibacillus laterosporus*

Rationale for Carrier Selection

Biochar and peat were selected as carrier components because their physical and chemical properties are widely recognized as relevant to microbial survival during storage. Biochar provides a porous structure and large surface area that can support microbial attachment and protected microhabitats, while improved cation exchange capacity (CEC) may enhance nutrient retention and microbial persistence (Zhu et al., 2017; Lehmann et al., 2011). Studies have demonstrated that higher-temperature biochars may increase surface area and adsorption capacity, which can support beneficial bacterial survival (Zhang et al., 2021), and biochar additions can improve soil pore structure conducive to microbial habitats (Akhtar & Kulhary, 2025). In addition, biochar can enhance nutrient retention linked to increased CEC (Qodarrohman et al., 2024) and may strengthen ecological interactions when used with PGPR (Ren et al., 2020).

Peat provides organic carbon and strong moisture retention that can support microbial survival (Maftu'ah & Nursyamsi, 2019), though peat pH and moisture status may influence viability excess acidity or excessive moisture can reduce performance of some beneficial microbes (Hafif et al., 2022; Chen et al., 2024). These considerations motivated testing different biochar–peat ratios under room-temperature storage.

Bacterial Isolates and Culture Maintenance

The antagonistic bacterial isolates used in this study were previously identified as potential biological control agents against stem rot disease caused by Fusarium spp. (Hidayati et al., 2022). Pure cultures of each isolate were maintained on Nutrient Agar (NA) media and stored at 4 °C prior to use. Prior to inoculation, cultures were refreshed on NA to ensure active growth. All microbiological manipulations were performed aseptically under a laminar airflow cabinet to prevent contamination.

Preparation of Biochar–Peat Storage Media

Biochar was produced from rice husks using a pyrolysis method, while peat soil of fibric maturity was collected from Kalampangan Village, Central Kalimantan. Storage media were prepared according to the designated biochar–peat ratios (v/v). Each medium was sterilized by autoclaving at 121 °C for 15 minutes. After cooling to room temperature under sterile conditions, 10 g of each sterilized storage medium was placed into sterile plastic containers and sealed with cling wrap until bacterial inoculation.

Inoculum Preparation and Bacterial Inoculation

Bacterial inoculation and storage procedures were adapted from (Sudrajat et al.,2014) with minor modifications. Bacterial cultures grown on Nutrient Agar (NA) media were transferred into Nutrient Broth (NB) and incubated at 30 °C for 24 hours. Following incubation, the bacterial suspensions were centrifuged at 5,000 rpm for 10 minutes, and the resulting pellets were resuspended in sterile 0.85% NaCl solution. Cell concentrations were adjusted to approximately 1×10^8 cfu mL^{-1} using a spectrophotometer and confirmed by plate count

methods. Each storage medium (10 g) was aseptically inoculated with 1 mL of the bacterial suspension, resulting in an initial bacterial population of approximately 1×10^7 cfu g^{-1} of carrier material.

Immediately after inoculation, each container was sealed to reduce moisture loss and exposure to contaminants. The physical storage layout and container sealing approach correspond to the workflow depicted in Figure 1 (Findings section).

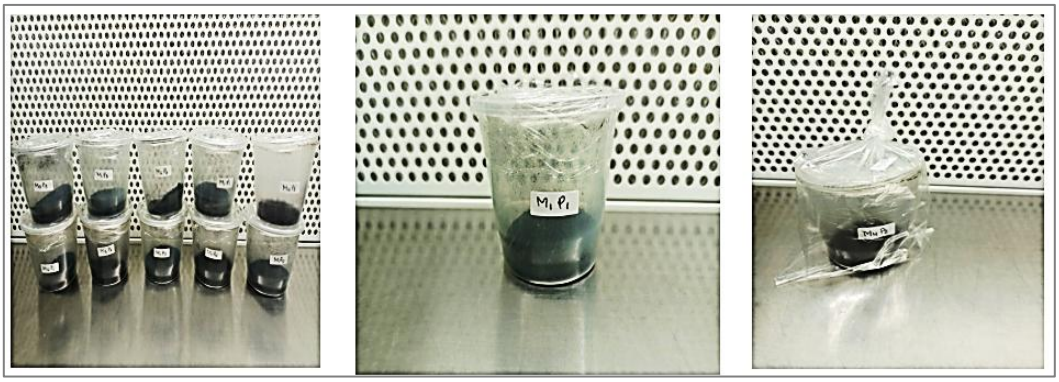

Figure 1. Storage of antagonistic bacteria

Storage Conditions and Viability Assessment

Inoculated biochar–peat carrier media were sealed and stored at room temperature (approximately 28–29 °C) for up to 90 days, reflecting practical non-refrigerated conditions commonly encountered in on-farm handling (Figure 1). Bacterial viability was evaluated at 30, 60, and 90 days after inoculation (DAI) using a standardized plate count procedure. At each sampling time, 1 g of inoculated carrier material was aseptically transferred into 9 mL of sterile 0.85% NaCl to obtain the first dilution (10^{-1}), followed by serial tenfold dilutions up to 10^{-7}. From an appropriate dilution, 0.1 mL was spread onto Tryptic Soy Agar (TSA) plates and incubated at 27 °C for 24 h, after which colonies were counted and expressed as cfu g^{-1} of carrier material. CFU enumeration followed standard dilution plating principles, where colony counts represent viable population estimates and reliability improves when plate counts fall within practical counting ranges (Bankole et al., 2020; Islam et al., 2022; Odo et al., 2021; Pedroso et al., 2022; Waris & A.S., 2025). The resulting viability trends are summarized in Table 4 (30, 60, and 90 DAI), while treatment means at 60 DAI are shown in Table 4.

Across storage durations, viability outcomes exhibited clear temporal and carrier-dependent patterns. At 30 DAI, bacterial densities did not differ significantly among storage media (Table 4), with mean populations ranging from approximately 1.25×10^8 to 2.35×10^8 cfu g^{-1}. This short-term stability suggests that, during early storage, moisture retention and available organic resources in both biochar- and peat-based matrices were sufficient to support survival across media compositions. Similar early-stage stability across substrate types has been observed where carrier moisture and organic carbon initially remain adequate to sustain viable populations (Ismail et al., 2023; Luera & Gabler, 2022; Aytar & Kömpe, 2023).

At 60 DAI, viability differentiation among media became evident. The factorial analysis (Table 2) indicated that storage medium composition significantly influenced bacterial density, whereas the interaction between storage medium and bacterial type was not significant. Consistent with this, the biochar-dominant medium m1 (biochar:peat = 3:1) produced the highest mean population at 8.844×10^7 cfu g^{-1}, exceeding the peat-only medium m4 (2.988×10^7 cfu g^{-1}) and remaining statistically comparable to m2 (1:1) (Table 3). This pattern supports the functional role of carrier properties particularly porosity, surface area, and nutrient retention—in sustaining microbial persistence during storage and incubation (Lehmann et al., 2011; Zhu et al., 2017; Qodarrohman et al., 2024; Ren et al., 2020; Gulyás et al., 2022). Evidence that biochar can enhance microbial microhabitats and resource retention further aligns with the improved persistence observed in biochar-enriched carriers (Zhang et al., 2021;

Akhtar & Kulhary, 2025). Moreover, biochar is increasingly evaluated as a component of peat-reduced substrates, supporting its relevance for peat–biochar carrier development (Chen et al., 2024).

By 90 DAI, bacterial densities declined in all treatments (Table 4), consistent with progressive nutrient depletion and metabolite accumulation under sealed storage. Nonetheless, biochar-based carriers continued to maintain higher populations than peat alone, with m1 retaining the highest density (1.089×10^7 cfu g^{-1}) compared with m4 (5.333×10^6 cfu g^{-1}). Reduced persistence in peat-dominant media may reflect shifts in moisture and aeration status, where excessive moisture can limit oxygen availability and accelerate viability loss in aerobic formulations (Richardson & Sun, 2023; Chen et al., 2024; Ismail et al., 2023). The absence of significant interaction effects suggests that carrier composition remained the dominant driver of viability across isolates, consistent with formulation literature emphasizing the importance of matrix properties and immobilization environments under storage constraints (Mukiri et al., 2021; Gulyás et al., 2022; Eigenbrode & Adhikari, 2023).

Data Analysis

Bacterial population density data (cfu g^{-1}) were analyzed using factorial two-way ANOVA at a 5% significance level ($\alpha = 0.05$). Because the experiment was arranged as a factorial Randomized Block Design (RBD) with three blocks (Group), the statistical model included storage medium (M), bacterial isolate (P), their interaction (M×P), and block (Group) effects:

$$\boldsymbol{Y_{ijk} = \mu + M_i + P_j + (MP)_{ij} + G_k + \varepsilon_{ijk}}$$

where Y_{ijk} is the observed bacterial density for the i-th storage medium, j-th bacterial isolate, and k-th block; μ is the overall mean; M_i is the storage medium effect; P_j is the bacterial isolate effect; $(MP)_{ij}$ is the interaction effect; G_k is the block (Group) effect; and ε_{ijk} is the residual error. When ANOVA detected significant effects, treatment means were separated using the Least Significant Difference (LSD) test at 5%, consistent with the BNJ 5% notation reported in the Findings tables. All analyses were performed using SPSS version 25.

The ANOVA output is presented in Table 2 (Findings), while mean separation results are shown in Table 3. The temporal viability trends across storage duration (30, 60, and 90 DAI) are summarized descriptively in Table 4, supporting interpretation of storage-time patterns alongside the factorial results.

RESULTS AND DISCUSSION

Effect of storage media composition on bacterial density

The analysis of variance at 60 days after inoculation (DAI) indicated that storage medium composition (M) significantly affected bacterial population density, whereas bacterial isolate (P) and the M×P interaction were not significant at this observation time. This pattern confirms that, under the tested room-temperature storage conditions, the dominant driver of population differences was the carrier matrix rather than isolate identity, and that media effects were broadly consistent across isolates as presented in Table 2.

Table 2. Results of the analysis of bacterial density variance at 60 days after planting (DAI)

Diversity	df	Sum^2	Mean^2	F Count	F-Tabel 5%	F-Tabel 1%
Group	2	7.580×10^{15}	3.790×10^{15}	102.85**	3.24	5.21
Media (M)	3	1.012×10^{15}	3.373×10^{14}	9.15**	2.85	4.34
PGPR (P)	4	2.197×10^{13}	5.496×10^{12}	0.15 tn	2.62	3.86
MxP	12	1.430×10^{14}	1.191×10^{13}	0.32 tn	2.02	2.69
Galat	38	1.400×10^{15}	3.685×10^{13}			
Total	**59**	**1.016×10^{16}**				

Description: ** means significantly different, tn means not significantly different

Table 2 shows that the storage medium (M) had a highly significant effect on bacterial population density at 60 days after inoculation (DAI), as indicated by the F value exceeding the F-table at both 5% and 1% significance levels. The Group factor also had a highly significant effect on bacterial density. In contrast, the type of bacteria (PGPR, P) and the interaction between storage medium and bacterial type (M×P) did not show significant effects on bacterial density at 60 DAI. These results indicate that variations in bacterial density at this observation time were primarily influenced by the composition of the storage medium rather than by bacterial type or their interaction.

In line with this statistical result, mean comparisons at 60 DAI showed that the biochar-rich carrier m1 (biochar:peat = 3:1) sustained the highest bacterial density (8.844×10^7 cfu g^{-1}), and it was statistically comparable to m2 (1:1) but significantly higher than peat-dominant media particularly m4 (100% peat), which produced the lowest density (2.988×10^7 cfu g^{-1}). These results demonstrate that carriers containing biochar maintained higher viable populations than peat-only or peat-dominant carriers during intermediate storage.

Table 3. Effect of media and PGPR treatments on cfu gr^{-1} population density at 60 DAI

Treatment	p_1	p_2	p_3	p_4	p_5	Average
m_1	$9.062x10^7$	$8.025\ x10^7$	$8.308\ x10^7$	$8.797\ x10^7$	$8.389\ x10^7$	$8.844x10^{7\ c}$
m_2	$6.724x10^7$	$6.758\ x10^7$	$6.472\ x10^7$	$6.518\ x10^7$	$6.300\ x10^7$	$6.554x10^{7\ b}$
m_3	$5.855x10^7$	$5.980\ x10^7$	$5.761\ x10^7$	$5.205\ x10^7$	$7.044\ x10^7$	$6.555\ x10^{7\ b}$
m_4	$3.131x10^7$	$2.960\ x10^7$	$2.975\ x10^7$	$2.941\ x10^7$	$2.934\ x10^7$	$2.988\ x10^{7\ a}$
BNJ 5%			**M= $1.328\ x10^7$**			

Description: Numbers in a column or row followed by the same letter mean there is no difference according to the LSD test at the 5% level.

The observed advantage of biochar-based and biochar–peat carriers is consistent with biochar's dual role as a physical shelter and a chemical buffer. Biochar typically provides high porosity and large surface area that create protected microsites for microbial colonization and reduce exposure to environmental stressors; it can also adsorb nutrients and maintain their availability in the carrier matrix (Lehmann et al., 2011; Lehmann & Joseph, 2015; Santi et al., 2012; Rijk et al., 2024). In nutrient-poor substrates such as peat, biochar addition commonly increases pH and improves nutrient availability, conditions that can enhance microbial activity and persistence (Ullah et al., 2020; Iacomino et al., 2023; Wu et al., 2020). Laboratory syntheses also describe how biochar can act as a microbial "refugia," strengthening biomass and community stability in mixed substrates (Rodriguez et al., 2022; Frene et al., 2021; Pot et al., 2022).

Conversely, peat can support microbial survival in the short term because it supplies organic matter and maintains moisture availability, but its acidic pH and decomposition chemistry may become limiting during longer storage. Peat acidity has been widely reported in Indonesian peat systems and can constrain microbial performance depending on isolate tolerance (Noor, 2001). In addition, peat decomposition may generate phenolic compounds and organic acids that inhibit microbial metabolism under certain conditions (Hodgson et al., 2018). This inhibition is consistent with broader peat literature showing that soluble phenolics can suppress microbial processes and enzyme activity, particularly under waterlogged or oxygen-limited conditions (Cory et al., 2021; Kim et al., 2021; Teickner et al., 2022). These properties provide a plausible explanation for why m4 (peat only) exhibited the lowest density at 60 DAI.

Viability dynamics over storage duration (30, 60, and 90 DAI)

Viability patterns over time indicate that populations tended to decline from 30 to 90 DAI, but biochar-rich media maintained more stable populations than peat-dominated media. Editorially, a summary of these temporal dynamics is presented in a time-course table so readers can immediately see the population change trend across observation periods as shown in Table 4.

Table 4. Response of rhizobacterial viability/population density (cfu g^{-1}) to storage media treatment at 30, 60, and 90 DAI observations

Treatment	30 DAI	60 DAI	90 DAI
m_1 = 3 Biochar: 1 Peat moss	2.349 x 10^8 a	8.844x10^7 c	1.089 x 10^7 b
m_2 = 1 Biochar: 1 Peat moss	2.057 x 10^8 a	6.554x10^7 b	7.333 x 10^6 a
m_3 = 1 Biochar: 3 Peat moss	1.283 x 10^8 a	6.555x10^7 b	1.111 x 10^6 a
m_4 = Gambut	1.255 x 10^8 a	2.988x10^7 a	5.333 x 10^6 a

Note: Numbers followed by the same letter in the same column are not significantly different based on the 5% LSD test. DAI = days after inoculation

The results showed that the biochar and peat media compositions had different effects on microbial population dynamics at 30, 60, and 90 days after isolation (DAI). At 30 DAI, all media supported similarly high populations, indicating that early storage conditions still allowed sufficient access to nutrients and moisture for survival. This uniformity in early incubation is consistent with the view that readily decomposable carbon and accessible resources can mask substrate differences during initial periods (Ma et al., 2020).

By 60 DAI, differences among media became evident, with biochar-rich media maintaining higher densities than peat-dominant carriers. This divergence suggests that biochar contributed to longer-term stability of the microenvironment, likely through maintenance of aeration pathways, creation of protective microhabitats, and retention of nutrients and moisture in forms accessible to bacteria (Lehmann et al., 2011; Lehmann & Joseph, 2015; Rijk et al., 2024). Biochar-related improvements in microbial properties under constrained conditions have also been reported in laboratory studies where pH buffering and habitat effects were linked to higher microbial activity and resilience (Iacomino et al., 2023; Frene et al., 2021; Pot et al., 2022).

At 90 DAI, all treatments showed reduced populations, reflecting the cumulative impact of resource depletion and the accumulation of metabolic by-products in sealed carrier systems. Nonetheless, the biochar-rich medium m1 maintained the highest density at this time point (1.089×10^7 cfu g^{-1}), demonstrating superior long-term stability relative to peat-only or peat-dominant carriers. This finding is consistent with formulation studies reporting that biochar-based matrices can maintain measurable CFU over extended storage periods, largely due to the protective structure and buffering environment created by the carrier (Ajeng et al., 2023; Sawatphanit et al., 2022; Puteri et al., 2021). The sensitivity of viability to environmental conditions such as moisture and nutrient availability during storage is also emphasized in studies assessing bacterial survival in different carriers and storage environments (Ali et al., 2023; Tamašauskaitė et al., 2024).

The overall decline from 30 to 90 DAI is not unexpected and aligns with general observations in microbial storage systems, where reductions are commonly associated with diminishing readily utilizable substrates, intensified competition, and progressive shifts in microenvironmental conditions (Cheng et al., 2017; Widdig et al., 2020). In peat-rich carriers, additional constraints may arise from acidity and inhibitory decomposition products, and potentially from micro-anaerobic zones when water retention is high (Wang et al., 2019; Cory et al., 2021; Kim et al., 2021). Collectively, these mechanisms provide a coherent explanation for why biochar-rich carriers better preserved viability at later storage times.

The type of bacteria stored (P) also showed a significant effect ($p < 0.05$) on bacterial density during storage (Table 3). In general, *B. cereus* (p1) showed a relatively higher density of 8.844 x 10^7 cfu g-1 compared to other bacterial species at both observation times. Studies by (Chung et al., 2007; Lim & Kim, 2010) also demonstrated the ability of *B. subtilis* to survive under various storage conditions. Differences in survival between bacterial species (Hidayati et al., 2022) may be due to physiological differences and the adaptability of each species to storage media conditions. Some bacteria may be better able to utilize the resources available in the media or more resistant to environmental stress during storage.

Effect of bacterial isolate and interaction with storage media

In this study, the factorial ANOVA at 60 DAI indicates that the bacterial isolate factor (P) was not significant, and the media × isolate interaction (M×P) was also not significant (Table 2). Accordingly, any isolate-related interpretations should be presented as descriptive trends rather than inferential conclusions, unless additional analyses (e.g., isolate means across 30, 60, and 90 DAI) are provided. In practical terms, the non-significant P and M×P effects imply that differences in viability at 60 DAI were driven predominantly by carrier composition, and that the relative ranking of carrier performance was broadly similar across the five tested antagonists.

Even without a significant isolate effect, Table 3 shows that isolates maintained comparable orders of magnitude ($\approx 10^7$ cfu g^{-1}) when stored in biochar-enriched media, particularly in m1 (3:1) and m2 (1:1). This pattern supports the interpretation that biochar-rich matrices create a generally supportive storage environment across taxa. Such a "carrier-dominant" outcome is consistent with formulation principles emphasizing that matrix physicochemical properties including porosity, surface area, adsorption, aeration, and nutrient retention often exert stronger control on survival than isolate identity under a shared storage regime (Lehmann et al., 2011; Lehmann & Joseph, 2015; Santi et al., 2012; Rijk et al., 2024). Recent storage-focused studies also highlight biochar's role as a protective carrier that can sustain viable bacterial populations over extended periods, reinforcing its suitability as a broadly compatible formulation platform (Ajeng et al., 2023).

The absence of a significant M×P interaction is particularly informative. Biologically, it suggests that the stabilizing advantage of biochar-rich carriers is not restricted to a single isolate but reflects a generalizable carrier effect. This is advantageous for product development because it implies that a single biochar–peat formulation may support multiple antagonistic bacteria without requiring isolate-specific carrier customization, thereby simplifying standardization and scaling (Rijk et al., 2024; Ajeng et al., 2023). Mechanistically, the porous structure of biochar can provide protected microhabitats and maintain aeration, while its adsorption and nutrient retention properties can buffer cells against nutrient limitation and other stresses typical of sealed, non-supplemented storage (Lehmann et al., 2011; Lehmann & Joseph, 2015; Santi et al., 2012). By contrast, peat contributes moisture and organic matter but may impose constraints during longer storage due to acidity and decomposition-derived inhibitory chemistry (Noor, 2001). The net result is a matrix environment where biochar inclusion tends to stabilize survival outcomes across isolates rather than generating divergent isolate-specific responses.

Although isolate effects were not statistically significant at 60 DAI, the descriptive tendency of *Bacillus cereus* (p1) showing relatively higher average density can be discussed cautiously as a plausible reflection of physiological resilience. *Bacillus* spp. are capable of forming endospores, a trait often linked to enhanced tolerance to desiccation, nutrient limitation, and prolonged storage, and Bacillus-based formulations frequently show improved persistence relative to non-spore-forming bacteria under comparable storage constraints (Chung et al., 2007; Lim & Kim, 2010). However, because P was non-significant in the ANOVA at 60 DAI (Table 2), this explanation should be framed as contextual rather than definitive for this dataset. To strengthen isolate-level interpretation in future work, an isolate-by-time summary (means at 30, 60, and 90 DAI averaged across media, or within the best carrier) would directly address the reviewer request for evidence beyond a single time point. Biosafety and regulatory limitation. Reviewer concerns regarding biosafety are valid and should be explicitly acknowledged. Both *B. cereus* and *B. cepacia* can raise biosafety or regulatory issues in some jurisdictions, meaning that any progression toward field deployment should include strain-level safety screening (e.g., enterotoxin/virulence profiling where relevant) and alignment with applicable regulations.

Implications for formulation and storage recommendations

From a formulation standpoint, the results indicate that biochar-enriched carriers—especially m1 (3:1)—are most effective for maintaining viable populations during room-temperature storage. This matters because viability losses can reduce the capacity of antagonistic bacteria to establish in the rhizosphere, compete for resources, and express antagonistic functions, ultimately lowering disease suppression potential (Yadav et al., 2020; Compant et al., 2021). Declining viability also increases the inoculum required to reach an effective dose, raising production costs and limiting practical adoption (Mulawarman et al., 2021). In this context, the consistently higher densities maintained in biochar-rich carriers provide a strong rationale for prioritizing biochar-based matrices as storage carriers in biocontrol-oriented formulations.

At the same time, a strict recommendation to cap storage at "two months" should be stated cautiously unless linked to an efficacy threshold. While a clear decline is evident by 90 DAI, densities in the best carrier remain on the order of 10^7 cfu g^{-1}, which may still be biologically meaningful depending on the minimum effective dose for colonization and disease suppression. Therefore, the most defensible interpretation is that storage beyond 60 days can substantially reduce viable populations, and the practical consequences for disease control performance should be verified through greenhouse or field trials that assess antagonism and disease outcomes after storage. This phrasing retains the applied relevance of the findings while avoiding an unsupported efficacy cutoff.

Limitations and future directions

Several limitations should be explicitly stated to satisfy transparency requirements and strengthen the discussion. First, the study is laboratory-based, measuring viability (CFU) rather than post-storage functional performance such as antagonism against the target pathogen or colonization in planta. Second, isolate-level inference is constrained because the factorial ANOVA emphasizes 60 DAI, and isolate-by-time summaries are not provided; adding a time-course by isolate would substantively improve interpretation of species persistence and address reviewer requests. Third, biochar and peat characteristics can vary by feedstock, pyrolysis conditions, and source, meaning that reporting carrier physicochemical properties would improve reproducibility and cross-study comparability. Finally, potential biosafety and regulatory constraints for *B. cereus* and *B. cepacia* require explicit follow-up testing and compliance assessment prior to any recommendation for wide-scale field application.

CONCLUSION

This laboratory-based study demonstrates that carrier composition is a primary determinant of antagonistic bacterial viability during room-temperature storage (28–29 °C). Across storage periods of 30, 60, and 90 DAI, bacterial populations declined over time, but the decline was consistently slower in biochar-enriched media than in peat-dominant media. The biochar:peat mixture at 3:1 (m1) maintained the most stable populations and retained the highest viable density at extended storage, indicating that biochar-based matrices can provide a protective microenvironment that supports survival under non-refrigerated conditions. In contrast, peat-only media (m4) generally supported lower densities during storage, which is plausibly linked to constraints associated with acidity, aeration, and decomposition-derived inhibitory compounds. Importantly, the media × isolate interaction was not significant at 60 DAI, suggesting that the benefits of biochar-rich carriers are broadly applicable across the tested isolates rather than being isolate-specific. Overall, these findings help address the limited empirical evidence on biochar–peat composites as storage carriers and support their potential utility as locally adaptable carrier materials for microbial biocontrol formulations targeting stem rot disease in sweet corn.

RECOMMENDATION

Future work should extend these storage-viability findings toward practical disease management outcomes. First, the best-performing carrier (biochar:peat = 3:1) should be evaluated in greenhouse and field trials to confirm whether post-storage inoculants retain functional traits relevant to stem rot suppression, including rhizosphere establishment, antagonistic activity, and plant protection under realistic environmental conditions. Second, storage studies should be expanded to include durations beyond 90 days and a wider range of ambient conditions (including temperature and humidity variability) to better represent farm-level handling constraints and to identify formulation stability limits under non-refrigerated storage. Third, because carrier physicochemical attributes strongly influence survival, future studies should measure and report key properties of biochar and peat (e.g., pH, moisture content, surface area, cation exchange capacity, and organic carbon) and relate these parameters to viability outcomes to improve reproducibility and cross-study comparison. Finally, because some isolates (e.g., Bacillus cereus and Burkholderia cepacia) may raise biosafety or regulatory concerns in certain contexts, subsequent research should incorporate strain-level safety screening and risk assessment prior to large-scale deployment, while also exploring multi-species or consortium formulations using biochar-based carriers to enhance robustness and scalability of biocontrol products.

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AUTHOR CONTRIBUTIONS STATEMENT

This study applies the Contributor Roles Taxonomy (CRediT) to describe the contributions of each author as follows:

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Nurul Hidayati	✓	✓	✓	✓	✓	✓		✓	✓	✓			✓	
S. Salamiah	✓	✓		✓		✓		✓	✓	✓		✓		
Raihani Wahdah			✓	✓		✓	✓	✓	✓		✓		✓	
Fahrur Razie							✓		✓			✓	✓	
Fahruddin Arfianto			✓	✓		✓	✓	✓	✓		✓		✓	

C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

CONFLICT OF INTEREST STATEMENT

Authors state no conflict of interest.

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