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Effects of Carrier Materials and Storage Temperatures on the Viability and Stability of Three Biofertilizer Inoculants Obtained from Potato (*Solanum tuberosum* L.) Rhizosphere

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Abstract: Biofertilizer technology continues to be derailed by the short shelf life of inoculants. The present study investigated the suitability of wheat-bran (WB), rice-husks (RH), farmyard-manure (FYM), bagasse (BG), and sawdust (SD) in the formulation of potato-derived *Klebsiella grimontii* (MPUS7), *Serratia marcescens* (NGAS9), and *Citrobacter freundii* (LUTT5) under refrigerated (8 °C) and room (25 ± 2 °C) storage. The physicochemical properties of the materials were assessed before sterilization and introduction of the inoculants and assessment of their viability for 8 months. Most of the physicochemical properties of the materials varied significantly ($p < 0.05$). Bagasse supported the maximum growth of MPUS7 (5.331 log CFU g^{-1}) under refrigeration and LUTT5 (4.094 log CFU g^{-1}) under both conditions. Under room storage, the maximum growth of MPUS7 (3.721 log CFU g^{-1}) occurred in WB. Formulations that remained viable under room storage can easily be integrated into existing agricultural distribution systems that lack refrigeration.

Keywords: rhizobacteria; carrier materials; biofertilizer; bioformulations; shelf-life

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1. Introduction

Conventional agricultural practices are a major contributor to environmental pollution, global warming, and climate change [1], especially from the use of artificial fertilizers, which are widely associated with greenhouse gas emissions [2]. As such, attempts to find suitable alternative crop fertilization mechanisms and promote agricultural sustainability are quickly gathering momentum worldwide [3]. It is propounded that soil microbiota are key to the development of sustainable cropping systems [4], and plant rhizospheres have been the center of focus for researchers for decades worldwide.

Various plant-root inhabiting bacteria (rhizobacteria) can promote plant growth through various biochemical processes [5], like the production of phytohormones and siderophores, solubilization of phosphates, and biological nitrogen fixation [6,7]. Such beneficial rhizobacteria can be optimized and formulated into biofertilizers for sustainable crop production using different carrier materials [8].

The concept of biofertilizers is widely researched and there exist several practical applications globally, the utilization of this technology [9]. The use of agricultural wastes as carriers for biofertilizer formulations is one commonly explored option because of their ready availability and cost-effectiveness [10]. However, the survivability and efficiency of rhizobacterial inoculants in biofertilizer formulations are greatly dependent on the choice of carrier materials and storage temperatures [11,12]. As such, designing effective biofertilizers with long shelf lives is the greatest bottleneck for biofertilizer technology [10]. Although

various formulations have been evaluated to date, the shelf-life data of most organic formulations are still grossly inadequate. Research should be intensified to develop stable, functional, and reliable biofertilizer inoculants as tools for sustainable agriculture [13]. The present study aimed to investigate the effects of different agricultural wastes as carrier materials for the formulation of selected potato rhizobacterial inoculants and to evaluate their survivability and stability at the end of a storage period under two different temperature conditions. This will ultimately inform us on the suitable carriers and storage conditions for the studied rhizobacterial inoculants and others for sustainable agricultural systems.

2. Materials and Methods

2.1. Rhizobacterial Strains

Potato tubers and rhizosphere soils were sampled from different parts of Tanzania in June 2018. External rhizobacterial strains were isolated from the soil samples by serial dilution (up to 10^{-3}) using sterile saline water ($\frac{1}{4}$ strength Ringer's solution), plating 1 mL aliquots of the 10^{-3} dilution on Tryptic Soy Agar (TSA), and incubating at 28 ± 2 °C for 48 h [14]. For endophytic isolates, the potato tubers were washed with running tap water and sterilized with 70% (*v*/*v*) ethanol and 2% (*w*/*v*) sodium hypochlorite for 30 s and 10 min, respectively, followed by rinsing with sterile distilled water [14] and isolation as described by Aravind et al. [15]. Three rhizobacterial strains, MPUS7, NGAS9, and LUTT5, and identified as *Klebsiella grimontii*, *Serratia marcescens*, and *Citrobacter freundii*, respectively, were selected from among the obtained isolates based on preliminary analyses of their in vitro plant growth-promoting abilities and effects on growth of potted potato plants and later characterized [16] for the formulation of biofertilizers. The GenBank accession numbers of these isolates are CP047604, CP047605, and CP047606, respectively.

2.2. Characterization of Carrier Materials and Formulation of Biofertilizers

Approximately 3.5 kg (dry weight) of 5 types of agricultural wastes (bagasse (BG), farmyard manure (FYM), rice husks (RH), sawdust (SD), and wheat bran (WB)) were sourced from locally available agro-industries in Arusha for use as carrier materials. The pH of the materials was determined as described by Arora et al. [17]. The carrier materials were sterilized in the oven for 24 h at 105 °C and the sterilization efficiency was confirmed by plating out 1 g aliquots of samples on TSA and observing no growth after incubating at 28 ± 2 °C for another 24 h. The final (%) moisture contents (MC) were determined on a wet and dry mass basis as expressed in Equation (1).

$$(\%)\ \text{Moisture content} = \frac{Mass\ before\ drying\ (g) - Mass\ of\ dry\ sample\ (g)}{Mass\ of\ sample\ before\ drying\ (g)} \times 100\% \quad (1)$$

The water holding capacity (WHC) of each material was assessed using the Keen–Raczkowski cup method following the procedures described by Joshi and Setty [18] and Equation (2).

$$(\%)\ \text{WHC} = \frac{Mass\ of\ saturated\ and\ drained\ sample\ (g) - Mass\ of\ dry\ sample\ (g)}{Mass\ of\ dry\ sample\ (g)} \times 100\% \quad (2)$$

The electrical conductivity (EC) of carriers was determined using the saturated paste method (Chi and Wang, 2010) while the organic carbon (OC) and organic matter (OM) contents were evaluated using the potassium dichromate wet digestion method [19]. The N contents were quantified using the micro-Kjeldahl method [20] while the Mehlich III extraction method [21] was used to extract exchangeable Zn and P from materials. The ammonium acetate extraction method [22] and the 1, 10-phenanthroline complex method [23] were used to extract exchangeable K and Fe from the carriers, respectively. To estimate the quantities of P, K, Zn, and Fe in the respective carrier extracts, their optical densities were obtained spectrophotometrically at A690, A799, A399, and A510, respectively, using a multi-mode reader (Synergy HTX-Biotek). The quantities of P, K, and Zn in mg kg^{-1}

were calculated from standard curves prepared from standard solutions of KH_2PO_4, KCl, $ZnSO_4$ and $Fe(NH_4)_2$ $(SO_4)_2.6H_2O$, respectively.

The rhizobacterial strains were multiplied in TSB medium by inoculating and incubating at 28 ± 2 °C in a shaking incubator (180 rpm) for 24 h for them to attain log phase growth with a cell load of about l.8 × 10^6 CFU mL^{-1} [24,25]. The carriers were used to formulate biofertilizers for the 3 rhizobacterial inoculants (*K. grimontii* MPUS7, *S. marcescens* NGAS9, and *C. freundii* LUTT5) following the procedure described by Abd El-Fattah [24]. For each of the 3 inoculants, two sets of each sterilized carrier material (~20 g) were packed in sterile high-density clear plastic containers (40 × 30 × 15 cm) in triplicates for storage at 8 °C and 25 ± 2 °C, respectively, for the monthly evaluations of inoculant viability. A summary of the design used to formulate these biofertilizers is shown in Table 1.

Table 1. A summary of the design used to formulate biofertilizers out of the rhizobacterial inoculants.

Carrier Material		BG [1]	FYM [2]	SD [3]	WB [4]	RH [5]	Purpose
Klebsiella grimontii MPUS7	Set 1	20 g	20 g	20 g	20 g	20 g	8 °C storage
	Set 2	20 g	20 g	20 g	20 g	20 g	25 °C storage
Serratia marcescens NGAS9	Set 1	20 g	20 g	20 g	20 g	20 g	8 °C storage
	Set 2	20 g	20 g	20 g	20 g	20 g	25 °C storage
Citrobacter freundii LUTT5	Set 1	20 g	20 g	20 g	20 g	20 g	8 °C storage
	Set 2	20 g	20 g	20 g	20 g	20 g	25 °C storage

[1] Bagasse, [2] Farmyard manure, [3] Sawdust, [4] Wheat bran, [5] Rice husks.

The preparations were thoroughly mixed in sterile Petri-dishes (90 mm) using sterile glass rods to ensure uniformity in distribution and absorption of the liquid cultures into the carriers and left to cure under sterile conditions in the laminar flow hood for 24 h, repackaged in the sterile plastic containers and sealed aseptically. The 1st sets of each bio-formulation were stored at 8 °C and the 2nd sets at room temperature (25 ± 2 °C) for the purpose of determining the conducive storage temperatures. The total number of formulations was thus, 30. During the experiment, aliquots of non-inoculated carrier materials were maintained under the same incubation conditions to check the maintenance of axenic conditions. The biofertilizers packaged for storage are displayed in Figure 1.

Figure 1. Formulated biofertilizers packaged in different carrier materials for storage and monthly evaluations.

2.3. Laboratory Evaluation of the Viability of the Biofertilizer Formulations

Periodical samples were taken aseptically from each of the 30 formulations for 8 consecutive months for evaluation of the stability and viability of the rhizobacterial inoculants. Sampling was done by scooping about 1 g of each formulation using a sterile spatula and suspending in 9 mL of sterile distilled water, followed by vigorous mixing and filtering in Whatman (No. filter papers. The number of viable cells g^{-1} of each formulation was determined using the plate count technique (28 °C; 24 h) using the prepared filtrates on TSA plates [24,25].

2.4. Statistical Analyses

All statistical analyses were performed using the XLSTAT (Version 2.3, Adinsoft) at a 95% level of confidence. The Shapiro–Wilk test was used to test for normality of

data and multiple comparisons of variances were performed using Multivariate Analysis of Variance (MANOVA). Variables with significantly different means were subjected to posthoc analysis using Tukey's Honest Significant Difference (HSD) test. A t-test for paired samples was used to evaluate the viability of the biofertilizer formulations in the different carrier materials under room and refrigerated storage.

3. Results and Discussions

3.1. The Physicochemical Characteristics of the Carrier Materials

The physicochemical properties of the carrier materials that were used in the formulation of biofertilizers are portrayed in Table 2. According to Gade et al. [26], these properties can largely affect inoculant survival and viability. The results showed that the materials were moderately acidic (pH 4.73 ± 0.13 to 5.4 ± 0.21) except for FYM which was slightly alkaline (pH 8.38 ± 0.24). Such pH, which is generally near-neutral, is known to support large inoculant populations and maintain their viability [27–29] and maintain their viability.

Table 2. Characteristics of the carrier materials used in the formulation of biofertilizers for in vitro and field evaluations.

	Sawdust	Wheat Bran	FYM	Bagasse	Rice Husks	Average	*p* Value
pH	4.73 ± 0.13 ^{b}	4.63 ± 1.07 ^{b}	8.38 ± 0.24 ^{a}	4.35 ± 0.19 ^{b}	5.37 ± 0.21 ^{b}	5.49 ± 1.59	0.000 *
EC (uS cm^{-1})	0.116 ± 0.027 ^{c}	1.174 ± 0.634 ^{ab}	1.985 ± 0.349 ^{a}	0.194 ± 0.062 ^{c}	0.89 ± 0.026 ^{bc}	0.993 ± 0.853	0.000 *
WHC (%)	388 ± 22 ^{b}	155 ± 28 ^{c}	101 ± 7 ^{c}	568 ± 90 ^{a}	62 ± 46 ^{c}	255 ± 203	0.000 *
MC (%)	17.1 ± 0.6 ^{a}	10.3 ± 0.5 ^{b}	15.8 ± 1.4 ^{a}	8.6 ± 1.5 ^{b}	8.2 ± 0.3 ^{b}	11.9 ± 3.9	0.000 *
N (%)	0.100 ± 0.03	0.060 ± 0.04	0.130 ± 0.01	0.090 ± 0.08	0.160 ± 0.01	0.110 ± 0.05	0.154
OC (%)	1.84 ± 0.17	0.70 ± 0.51	1.45 ± 0.14	1.07 ± 0.92	1.31 ± 0.37	1.27 ± 0.58	0.154
OM (%)	3.17 ± 0.28	1.20 ± 0.88	2.50 ± 0.24	1.85 ± 1.58	2.25 ± 0.63	2.20 ± 1.00	0.152
P (mg kg^{-1})	248.7 ± 103.7 ^{b}	323.6 ± 109.2 ^{b}	1354.6 ± 68.9 ^{a}	183.3 ± 29.9 ^{b}	270.8 ± 64.4 ^{b}	476.2 ± 462.0	0.000 *
K (mg kg^{-1})	9.26 ± 5.16	9.06 ± 1.44	11.70 ± 0.96	6.24 ± 0.52	7.49 ± 0.96	8.75 ± 2.99	0.225
Zn (mg kg^{-1})	221.3 ± 136.5 ^{ab}	203.8 ± 69.33 ^{ab}	442.6 ± 30.99 ^{a}	87.4 ± 33.22 ^{b}	266.1 ± 192.4 ^{ab}	244.2 ± 152.3	0.034 *
Fe (mg kg^{-1})	0.99 ± 0.29 ^{b}	1.24 ± 0.49 ^{b}	3.68 ± 1.62 ^{a}	0.72 ± 0.14 ^{b}	0.84 ± 0.33 ^{b}	1.49 ± 1.32	0.005 *

Values are means of three replicates ± standard deviation. Means with similar letter superscripts within the same row are not significantly different (Tukey's HSD; $p > 0.05$). * Significantly different at $p < 0.05$.

The average EC of carrier materials ranged from ~0.116 to 1.985 dS cm^{-1}. The average EC of RH and BG were 0.89 ± 0.23 and 0.19 ± 0.06 dS m^{-1}, respectively. However, in a study by Khavazi et al. [30] these two carriers portrayed a relatively higher average EC of ~4.20 and 1.62 dS m^{-1}, respectively. Similar findings in the range of 3.89 to 4.03 dS m^{-1} have also been reported by Abdel Nabi et al., (2016). The EC of carrier materials depicts the concentration of soluble salts which can definitely influence the activities and survivability of inoculants.

The WHC of the carrier materials ranged from about 62 to 387%. The average WHC of WB, FYM and RH were all <150% while BG portrayed the greatest average WHC of 568 ± 90% followed distantly by SD at 388 ± 22%. High WHC (>50%) is a desirable feature in carrier materials to support proper bacterial growth and multiplication [28,29,31]. In the present study, the WHC of the carrier materials ranged from 62 to 387%, suggesting their potential as carriers for biofertilizer production. This is because high WHC favors the enzymatic processes involved in the degradation of the organic materials that provide important nutrients for bacteria [32]. These present findings are also comparable to previous reports [26,27,30]. Sawdust had the 2nd highest average WHC (388 ± 22%) after BG (568 ± 90%), agreeing with previous reports about its suitability for biofertilizer formulation [17,25].

The MC of the carrier materials ranged from 8.2 to 17.1%. Additionally, FYM and SD had significantly higher MC averages of 15.8 ± 1.4% and 17.1 ± 0.6%, respectively, than WB, SD, and BG. Arora et al. [27], while studying different organic carrier materials,

similarly reported an average MC of 16.8% but BG had a lower average MC of 6.8%. The MC of carrier materials can exert a great effect on inoculant survival and longevity [11,33]. Generally, dry formulations with low MC can extend microbial survival for longer periods and at higher temperatures, subsequently reducing marketing and maintenance costs since refrigeration is not required [34]. This is probably because the inoculants can remain inactive and resistant to environmental stresses and insensitive to contamination in carriers with low MC [33].

Generally, the carrier materials were rich in C, N, K, and micronutrients (Fe and Zn) which makes them suitable for microbial growth because these macro and micro-nutrients are required for carbohydrate metabolism [28], protein synthesis, enzyme activation and different growth processes of microbes [35]. For N, OC and OM, averages of 0.100 ± 0.1%, 1.3 ± 0.6% and 2.2 ± 1% were recorded, respectively. Farmyard manure seemed to contain more macro and micronutrients than the other carrier materials but its Zn content was not significantly higher ($p > 0.05$) than the rest except for BG. For N, OC and OM, averages of 0.100 ± 0.1%, 1.3 ± 0.6% and 2.2 ± 1% were recorded, respectively, in FYM. Previous studies also indicate that FYM contains 0.500 to 1.000% N [36,37], while RH and BG, averagely 0.400% [30]. The SD in this study also contained substantially high OC/OM which makes it a desirable carrier material [17]. Generally, carrier materials with high OM content can increase bacterial survival and enhance the efficacy of bio-formulations [38], by supporting proper inoculant growth and multiplication [31].

3.2. Laboratory Evaluation of the Viability and Stability of the Formulated Biofertilizers

3.2.1. Laboratory Evaluation of the Shelf Life of the Formulated Biofertilizer

The present study also investigated the viability and stability of rhizobacterial biofertilizers that were formulated from the five different carrier materials (WB, SD, FYM, RH, and BG) for 8 months (February–September 2019) under refrigeration (8 °C) and room storage (25 ± 2 °C). The results are displayed in Figure 2a–f. The determination of shelf lives of formulations is a crucial step during the development of biofertilizers [8,39], and the prolonged survival of inoculants during storage is a desirable feature that can enhance the industrial applicability of biofertilizer formulations [8].

The maximum inoculant population (5.331 log CFU g^{-1}) of *K. grimontii* MPUS7 was achieved in BG after 4 months of storage under refrigeration but the FYM formulation also maintained relatively high *K. grimontii* MPUS7 populations for most of the storage period under the same conditions (Figure 2a), a strong indication of its potential suitability as a carrier for this inoculant under refrigeration, probably due to its lower average MC (8.62 ± 1.45%) which may make inoculants to remain relatively inactive and resistant to environmental stresses [33]. The WB formulation did not sufficiently maintain high *K. grimontii* MPUS7 populations under refrigeration and by the 7th month of storage, no viable cells were detected from this formulation. Nevertheless, carriers that can maintain inoculants for at least 2–3 months are still suitable for commercial purposes [39,40]. Interestingly, under room storage, the WB formulation maintained the highest inoculant populations for most of the storage period except from the 7th month onwards where the inoculant population dropped to 1.5 log CFU g^{-1} below levels observed in the other formulations (Figure 2b). Although the storage of biofertilizers is mostly reported to be better under refrigeration than room conditions [26,41–43], the present results show that WB could sufficiently maintain *K. grimontii* MPUS7 under room storage, which may allow for its integration in the existing agricultural distribution systems that lack refrigeration facilities [44].

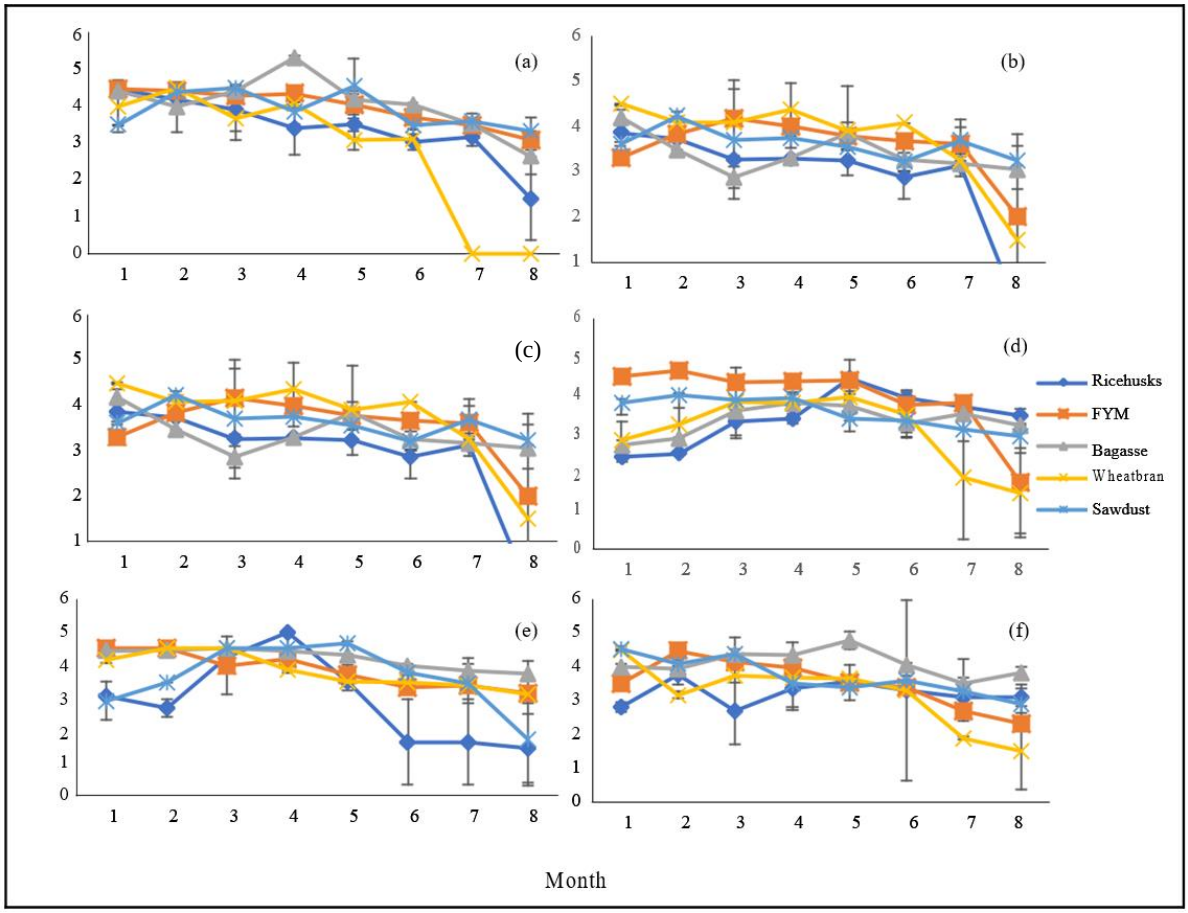

Figure 2. The 8-month viability of the formulated biofertilizers under room and refrigerated conditions in different carrier materials. The graphs represent *K. grimontii* MPUS7 under refrigerated (**a**) and room (**b**) storage, *S. marcescens* NGAS9 under refrigerated (**c**) and room (**d**) storage, and *C. freundii* LUTT5 under refrigerated (**e**) and room (**f**) storage in the different carrier materials. The point values are means of three replicates ± standard deviation. The y-axes represent the Log CFU g^{-1} of the biofertilizer formulations.

Similar observations were made for FYM formulation which also maintained relatively high inoculant populations for most of the storage period and by the 8th month, the population of inoculants was still 2.0 log CFU g^{-1}. Under these conditions, the inoculant populations in RH and WB formulations dropped to 0.0 CFU g^{-1} and 1.5 log CFU g^{-1}, respectively, by the 8th month of storage. Farmyard manure, WB, BG, and SD formulations could all maintain high *S. marcescens* NGAS9 populations throughout the study period under refrigerated conditions (Figure 2c), demonstrating their suitability in supporting the growth and multiplication of this inoculant. Although previous studies have demonstrated RH as a good carrier material for inoculant formulation [25,31,45], such results were not replicated in the present study for *S. marcescens* NGAS9 under refrigeration (Figure 2c) probably due to physiological differences among inoculants [46].

Under room storage, the formulations could all maintain sufficient *S. marcescens* NGAS9 populations during the entire storage period (Figure 2d), suggesting their suitability for the formulation of this inoculant and storage under room conditions which can allow for its integration into existing agricultural distribution channels that lack refrigeration facilities [44]. The suitability of these materials as carriers for other inoculants under room storage has also been demonstrated for other inoculants [27,47]. By the 8th month of room storage, only FYM and WB formulations had low inoculant populations (<1.5 log CFU g^{-1}).

The BG formulation maintained consistently high *C. freundii* LUTT5 populations throughout the study period under refrigeration (Figure 2e).

The compositional analysis of BG has in earlier studies revealed that it is equipped with monosaccharides, hemicellulose, and amino acids that together make it a nutritionally rich material for microbial growth [48]. In a similar study, when BG formulations were stored at 4 °C for 6 months, the survival of inoculants was high and densities of 10^9 cells g^{-1} were maintained [30]. Under room storage, *C. freundii* LUTT5 populations did not vary widely in the formulations (Figure 2f). Nevertheless, the BG formulation proved to be the best at maintaining high *C. freundii* LUTT5 populations and by the 8th month of storage, this formulation still had an average of 3.8 log CFU g^{-1}. Bagasse has also been reported to maintain high numbers of *P. fluorescens* (9.3 log CFU g^{-1}) and *R. leguminosarum* (8.9 log CFU g^{-1}) after 6 months of room storage (25 ± 2 °C) [27]. The shelf life of inoculants depends on several factors including the production technology, carrier material, storage conditions, and packaging material [49]. In the present study, the shelf lives of the formulated biofertilizers were only established in terms of the storage conditions and carrier materials. The viability of inoculums in formulations for a sufficient period is important for the commercialization and applicability of biofertilizers [27]. However, it should be noted that inoculant survival in carriers is not only vital during storage but also after introduction in the soil where they have to compete with well-established indigenous soil microbes [40].

3.2.2. Comparative Effects of Storage Temperatures on the Viability of Biofertilizer Formations

The present study evaluated the viability of each of the biofertilizer formulations in different carrier materials under room (25 ± 2 °C) and refrigerated (8 °C) conditions using the t-test for paired samples/two-tailed tests. The results are portrayed in Figure 3a–o. Temperature is one of the factors that affect the longevity and survival of inoculants and should be evaluated to optimize storage conditions for long-term inoculant survival [11,33]. Significant differences ($p < 0.05$) were noted between the number of viable cells under room (25 ± 2 °C) and refrigerated (8 °C) conditions for the WB formulations of all 3 biofertilizer inoculants (Figure 3a–c). In a study by Sandikar and Awasthi [42], biofertilizer inoculants also showed a maximum population under refrigeration (8 °C). The prolonged shelf life of inoculants under refrigeration has previously been linked to the reduction of metabolic activities and physiological activities [26,41]. While investigating the shelf lives of *Rhizobium* carrier-based biofertilizers under different storage temperatures, refrigeration (8 °C) was also demonstrated to be more suitable for inoculant viability probably because of lowered MC at higher temperatures [43].

Except for *K. grimontii* MPUS7, the viability of the tested inoculants in the RH formulations although not significant, seemed to be better under refrigeration than room storage (Figure 3j–l). Similar to the observations made for FYM formulations, the viability of inoculants in the BG formulations also seemed to be better under refrigerated conditions (Figure 3k–o). For the SD formulations, the number of viable cells of the three inoculants was also greater under refrigerated than room storage (Figure 3d–f). However, significant differences in 14 these numbers between the two conditions were only evident for *S. marcescens* NGAS9 ($p = 0.001$) (Figure 3e). Similarly, for the FYM formulations, the number of viable cells of all three biofertilizer inoculants was higher under refrigerated conditions but this was only significant for *C. freundii* LUTT5 ($p = 0.020$) (Figure 3g–i). Due to the biological nature of inocula, the survival of formulated cells at room temperature for longer storage is a persistent problem probably due to their growth and metabolic activities which lead to pH changes, death, and loss of viability [50].

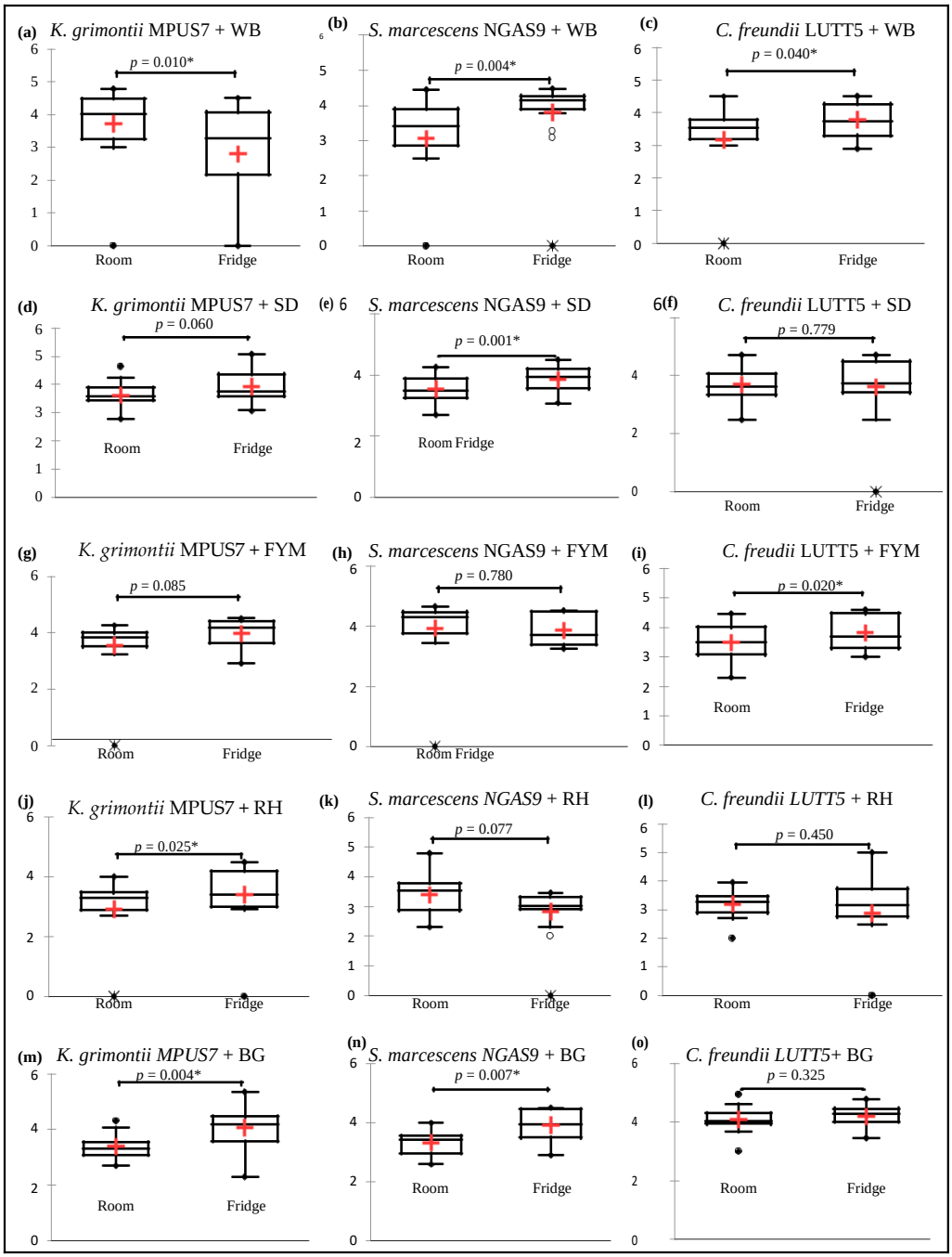

Figure 3. Comparative evaluation of the stability and viability of *K. grimontii* MPUS7, *S. marcescens* NGAS9 and *C. freundii* LUTT5, formulated in wheat bran (WB), sawdust (SD) and farmyard manure (FYM), rice husks (RH) and bagasse (BG) under room and refrigerated storage. Values are means of three replicates ± standard deviation. The y-axes represent the Log CFU g^{-1} of the biofertilizer formulations. * Significantly different at $p < 0.05$. Significant differences in inoculant means between room and refrigerated storage were obtained for *K. grimontii* MPUS7 + WB (**a**), *S. marcescens* NGAS9 + WB (**b**), *C. freundii* LUTT5 + WB (**c**), *S. marcescens* NGAS9 + SD (**e**), *C. freundii* LUTT5 + FYM (**i**), *K. grimontii* MPUS7 + RH (**j**), *K. grimontii* MPUS 7 + BG (**m**), and *S. marcescens* NGAS9 + BG (**n**). No significant differences were observed for the mean inoculant numbers between room and refrigerated storage for *K. grimonti*i MPUS7 + SD (**d**), *C. freundii* LUTT5 + SD (**f**), *K. grimontii* MPUS7 + FYM (**g**), *S. marcescens* NGAS9 + FYM (**h**), *S. marcescens* NGAS9 + RH (**k**), *C. freundii* LUTT5 + RH (**l**), and *C. freundii* LUTT5 + BG (**o**).

Unlike *S. marcescens* NGAS9 (Figure 3b) and *C. freundii* LUTT5 (Figure 3c), the number of viable cells of *K. grimontii* MPUS7 in the WB formulation was significantly higher under room storage (3.7 ± 1.1 log CFU g^{-1}) than refrigerated conditions (2.8 ± 1.7 log CFU g^{-1}) (Figure 3a). Similar observations were also made for *S. marcescens* in FYM (Figure 3h) and RH (Figure 3k). These results demonstrate that these formulations can easily be integrated into existing agricultural distribution systems that lack refrigeration facilities [44].

3.2.3. Comparative Effects of Carrier Materials on the Viability of Biofertilizer Formations

The viability of the biofertilizer inoculants in different carrier materials under room and refrigerated conditions were also evaluated using the students' t-test for independent variables. The comparative effects of the studied carrier materials on the stability of the inoculants are shown in Table 3. Under room conditions, the WB, SD, FYM, and RH formulations almost equally supported the viability and stability of the three inoculants. However, for the BG formulations, significant differences ($p \leq 0.0001$) were noted for the population of the three inoculants and this formulation seemed to support the growth of *C. freundii* LUTT5 better than *K. grimontii* MPUS7 and *S. marcescens* NGAS9 under the same storage conditions. This may be related to the physiological differences between these rhizobacterial species coupled with carrier-based factors [11,26,30,51]. Under refrigeration, however, significant differences of inoculant populations were only noted for the WB formulations ($p = 0.038$) while the rest of the carrier-based formulations equally supported and maintained the viability of all three biofertilizer inoculants during the experimental period. These results are probably due to the differences in the physicochemical properties of the carrier materials [11,30,51]. Despite having the greatest nutrient contents among the studied carriers, FYM did not significantly influence the stability of inoculants probably because it was also more alkaline (pH 8.38) and had a higher MC (15.8%) since these properties are not suitable for inoculant survival and viability [28,29,34].

Table 3. Comparative evaluation of the viability of *K. grimontii* MPUS7, *S. marcescens* NGAS9 and *C. freundii* LUTT5 (log CFU g^{-1}) in different carrier materials under room and refrigerated storage.

Storage Conditions	*K. grimontii MPUS7*	*S. marcescens NGAS9*	*C. freundii LUTT5*	*p* Value
Room Conditions (25 ± 2°C)				
Wheat Bran	3.721 ± 1.107 ^{a}	3.055 ± 1.265 ^{a}	3.168 ± 1.267 ^{a}	0.285
Sawdust	3.626 ± 0.464 ^{a}	3.544 ± 0.414 ^{a}	3.697 ± 0.599 ^{a}	0.702
Farm Yard Manure	3.548 ± 0.962 ^{a}	3.935 ± 1.077 ^{a}	3.495 ± 0.694 ^{a}	0.372
Rice Husks	2.921 ± 1.159 ^{a}	3.385 ± 0.670 ^{a}	3.202 ± 0.463 ^{a}	0.302
Bagasse	3.398 ± 0.418 ^{b}	3.351 ± 0.404 ^{b}	4.094 ± 0.424 ^{a}	**<0.0001**
Refrigerated Conditions (8°C)				
Wheat Bran	2.799 ± 1.694 ^{b}	3.806 ± 1.056 ^{a}	3.792 ± 0.515 ^{a}	**0.038**
Sawdust	3.934 ± 0.506 ^{a}	3.850 ± 0.478 ^{a}	3.607 ± 1.125 ^{a}	0.482
Farm Yard Manure	3.977 ± 0.509 ^{a}	3.868 ± 0.512 ^{a}	3.825 ± 0.561 ^{a}	0.702
Rice Husks	3.401 ± 1.039 ^{a}	2.829 ± 0.826 ^{a}	2.855 ± 1.553 ^{a}	0.351
Bagasse	4.071 ± 0.755 ^{a}	3.927 ± 0.516 ^{a}	4.187 ± 0.353 ^{a}	0.456

Values are means of three replicates ± standard deviation of the mean. Values with the same letter superscripts in the same row are not significantly different ($p > 0.05$; *t*-test for paired samples).

The choice of carrier material is one of the factors that affects the efficacy of biofertilizer formulations [11,30,51], and can greatly enhance the shelf life and performance of microbial inoculants [12,35,40]. When selecting carrier materials for biofertilizer formulation, it is necessary to use those that can support high cell numbers to achieve longer shelf lives and increase their usability. The present study investigated was on various agricultural wastes as carriers for biofertilizer formulation because they are rich sources of nutrients for microbial growth and are readily available [10]. This not only makes them cheap but also contributes to the reduction of pollution by putting the agricultural wastes into

reuse [38]. These materials were also selected because of their solid nature which makes them compatible with the application technologies for conventional fertilizers.

The overall observations showed that the numbers of viable cells significantly declined in all bio-formulations during the incubation period, similar to previous observations [52]. Such declines may be attributed to the depletion of nutrients, moisture, and autolysis of cells [10,11,41]. At the moment, various efforts in the biofertilizer technology are aimed at selecting carriers and developing novel formulations that can support high inoculant populations and plant growth [25,39], and this study provides the baseline regarding the survivability of various inoculants in organic carrier materials.

4. Conclusions

Except for OC, OM and K quantities, the carrier materials had significantly different physicochemical properties which can greatly influence their suitability as carriers for inoculants. Most of the rhizobacterial formulations could be stored for up to 6 months under both room and refrigerated conditions. Nevertheless, the viability and stability of the inoculants in the formulations seemed better under refrigeration. The carrier materials and storage temperatures both influenced inoculant stability and viability. This study sets a good platform for further explorations into the potential of the formulations for the biofertilization of potatoes and other crops under controlled and field experiments.

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