

Municipal Solid Waste Compost as Alternative Carrier Material for Rhizobia Inoculants

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ABSTRACT

Biological nitrogen fixation (BNF), mediated by micro-symbionts (Rhizobia spp.), remains the primary source of N in the arable soils of sub-Saharan Africa (SSA). Rhizobia inoculants are delivered securely to legume seeds, through carrier materials, in order to augment the BNF processes in legume systems. In SSA, peat is the primary carrier material used for this purpose; whose natural reserves are quickly dwindling. It is, therefore, imperative that alternative organic materials, with comparative potential for providing similar services, are evaluated. The objective of this study was to assess the potency of municipal solid waste compost (MSW) as replacements in admixtures of peat material, as carriers of rhizobia inoculants for administering to legume seeds prior to planting. A laboratory study was conducted for duration of 270 days (2016 – 2017), at the College of Agricultural and Environmental Sciences, Makerere University in Uganda. Treatments included five carrier formulations with municipal solid waste compost (MSW) as the base material (w/w); namely 100:0, 60:40, 40:60, 20:80 and 0:100 MSW to peat (Pt), ratios with Bradyrhizobium diazoefficiens USDA strain of rhizobia. Result revealed

that all carrier formulations maintained a high *B. diazoefficiens* population $>1 \times 10^9$ CFU/g for the first 120 days of storage. By the 270th day of storage, the best performing carrier formulation was pure municipal solid waste compost (100:0) with *B. diazoefficiens* population of 2.08×10^9 CFU/g. This was followed by the pure peat carrier (0:100) with *B. diazoefficiens* population of 1.57×10^9 CFU/g, which was not significantly different from municipal solid waste compost carrier. The least performing carrier combination was 40:60 (MSW: Pt) with *B. diazoefficiens* population of 3.08×10^8 CFU/g. From the results, the municipal solid waste compost is an effective replacement for peat in rhizobia inoculant formulation; however, the inherently high alkaline pH (8.96) of municipal solid waste compost needs adjustment to the recommended optimal range (6.0 to 7.0). Overall, the 60:40 admixture is the nearest to the optimal alternative carrier formulation, to substitute for peat as the conventional carrier in the production of rhizobia inoculants in Uganda.

Keywords: BNF, Bradyrhizobium diazoefficiens

INTRODUCTION

Biological nitrogen fixation (BNF), mediated by Rhizobia spp., remains the primary source of N in the arable soils of sub-Saharan Africa (SSA). Rhizobia inoculants are delivered securely to legume seeds, through carrier materials, in order to augment the BNF processes in legume cropping systems. Carrier materials are used to conserve the bacteria by prolonging their shelf life under specified conditions (Kaljeet *et al.*, 2011).

The optimum quality of a good carrier has been prescribed by Ben Rebah *et al.* (2007) to be high in organic C with optimal N contents, near neutral pH and of high moisture holding capacity. Additionally, the material should be non-toxic, inexpensive and accessible all year-round (Ben Rebah *et al.*, 2007). Also, it should be easy to process, free from lump forming, and easy to sterilize by autoclaving or gamma-irradiation (Somasegaran and Hoben, 1994).

No single inoculant is known to possess all these quality characteristics; however, a good inoculant should have as many as possible of these features (Bashan *et al.*, 2014). Peat-based carriers are the most commonly used materials in rhizobia inoculant industry (Bashan *et al.*, 2014) because they maintain high levels of viable rhizobia populations; attributed to high carbon content, high water-holding capacity and large surface area (Kaljeet *et al.*, 2011). Unfortunately, despite of its endowment with such positive carrier attributes, peat is getting depleted in many countries in sub-Saharan Africa. It is, therefore, imperative to explore other alternative competitive materials that are locally accessible, cost effective and environmentally safe, for commercial inoculant production.

Various materials have been tested as alternative rhizobia inoculant carriers to peat, based on maintenance of high populations of viable bacteria. Examples of these include sawdust (Singh *et al.*, 2014), rice husks + kaolin (Kaljeet *et al.*, 2011), water hyacinth compost (Mohamed and Abdel-Moniem, 2010), biochar (Hale *et al.*, 2015; Hardy and Knigh, 2021) and crop residue composts (Phiromtan *et al.*, 2013). Municipal Solid Waste (MSW), which is in abundant supply in SSA, but cumbersome to dispose of, particularly in urban and peri-urban environments of

SSA, still remains unexplored for this purpose. A number of studies have been conducted to determine the quality of MSW compost for use in agricultural production in Uganda (Amoding *et al.*, 2011; Komakech *et al.*, 2014; Kabasiita *et al.*, 2022). Kabasiita *et al.* (2022) characterised MSW compost in 12 municipal composting sites in Uganda and noted that nutrient endowments were generally low. In addition, MSW compost has been reported to have alkaline pH, to levels that may be non-conducive to rhizobia inoculant production (Kabasiita *et al.*, 2022). Hence, considering that peat is highly acidic and requires liming to raise the pH to favourable levels for rhizobia, MSW compost could be mixed with reduced quantities of peat carrier to obviate costly lime requirements. The objective of this study was to assess the potency of municipal solid waste (MSW) compost as replacements in admixtures of peat materials as carriers of rhizobia inoculant production.

MATERIALS AND METHODS

A laboratory study was conducted at Makerere University in Uganda, for a duration of 270 days during 2016 to 2017. Treatments included sterilised carrier formulations of MSW compost, admixed with peat (Pt) in ratios (w/w) in the order of 100:0, 60:40, 40:60, 20:80 and 0:100. These formulations were selected after adjusting a range of (MSW: Pt) carrier admixtures to rhizobia conducive pH values (see below). The treatments were laid out in a completely randomised design (CRD) and replicated three times.

Carrier Preparation

Two types of carrier materials, namely MSW compost and peat (Pt) were used in this study. The MSW was obtained from the Mukono Municipal Composting site in Central Uganda (latitude: 0° 21' 11.99" N and longitude: 32° 45' 19.01" E). Mukono municipal composting site is one of the 12 municipal composting sites under the World Bank Clean Development Mechanism (CDM) programme that finances low-income countries' greenhouse gas (GHG) reduction mechanism, including composting. Windrow composting is the technique practiced at Mukono municipal composting site (Kabasiita *et al.*, 2022).

Peat soil used in this study was obtained from Kabale district in South-western Uganda (latitude: -1° 14' 54.85" S and longitude: 29° 59' 23.75" E). Separately with MSW, the materials were air dried, screened through a 5 mm sieve; and later ground to a very fine powder texture using a hammer mill.

Pre-study pH tests were taken on MSW compost : peat ratios, 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100 based on the pH suitable for rhizobia inoculant production (Somasegaran and Hoben, 1994). From these tested formulations, five MSW: Pt carrier formulations namely; 100:0, 60:40, 40:60, 20:80 and 0:100 was selected as treatment formulations for the study.

Preliminary analytical test results (Table 1) showed that sole peat (Pt) (0:100) was strongly acidic (pH = 3.91) and contained high organic C (C = 16.59%); while MSW compost was fairly alkaline (pH = 8.96) with organic C content of 4.63%. Therefore, peat soil was limed by incubating with different quantities of limestone (CaCO₃), namely 4, 5, 6, 7 and 8 grammes each in different 250 ml beakers containing 10 g of peat, with 90 ml of distilled water. The quantities

of limestone were based on the recommended procedures by (Somasegaran and Hoben, 1994). The setups were managed in triplicates.

The mixture in each beaker was stirred using a magnetic stirrer, and the pH monitored using a pH electrode, for three days until stable results were obtained (Somasegaran and Hoben, 1994). The peat (Pt) carrier formulation, in which 6 g of CaCO_3 was added, achieved a pH of 6.62 and was used for to raise the pH of peat. Several researchers have observed calcium or magnesium carbonate to be the most effective reagent to raise the pH of peat to the desired level, for rhizobia survival (Somasegaran and Hoben, 1994; Tittabutr *et al.*, 2012; Thirumal *et al.*, 2017).

In order to determine how much $\text{Al}_2(\text{SO}_4)_3$ that would be sufficient to neutralise the alkalinity of MSW to the pH suitable for the rhizobia inoculants, the pH of the MSW compost was lowered by adding different quantities of $\text{Al}_2(\text{SO}_4)_3$, namely 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2 and 3 g, each in a different 250 ml beaker containing 10 g of MSW compost with 90 ml of distilled water, replicated 3 times.

The mixtures were stirred using a magnetic stirrer and the pH monitored using a pH glass electrode for three days, until stable pH values were obtained. The MSW formulation to which 0.3 g of $\text{Al}_2(\text{SO}_4)_3$ was added achieved a pH of 6.77 and was selected for use in lowering the pH of MSW. Therefore, the final pH used in the study was 6.77 and 6.62 for pure MSW (100:0) and pure peat (0:100), respectively.

Carrier Characterisation

The carrier formulations slated for the study, were analysed for total organic carbon (OC), total N, Bray 1 P, lead (Pb), Cadmium (Cd), Nickel (Ni) and Iron (Fe). Total organic carbon was analysed by the Walkley and Black method; and total N was analysed by the Kjeldhal technique, as described in by Okalebo *et al.* (2002).

For the heavy metals, 5 g of the air-dried samples were extracted in 50 ml of 1% ethylenediaminetetraacetic acid (EDTA). The filtrate was then aspirated into an air-acetylene flame in the atomic absorption spectrophotometer (AAS), and Pb, Cd, Ni and Fe, were read off at their specific wavelengths. The carrier formulations' analytical results are presented in Table 1. The results revealed low concentrations of heavy metals (Fe, Pb and Cd) in the materials used in the different carrier formulations (Westfall *et al.*, 2005).

Table 1: Chemical characteristics of the carrier materials (w/w) used in the study

MSW: Peat w/w	pH (H_2O)	Total N %	Organic carbon	C/N ratio	Fe $\mu\text{g g}^{-1}$	Pb	Cd	Ni
100:0	8.96	0.59	4.63	7.85	148.00	0.02	0.01	nd
60:40	7.44	1.01	10.84	10.75	52.30	nd	nd	nd
40:60	6.99	1.24	12.28	9.90	42.30	nd	nd	nd
20:80	6.38	1.27	11.89	9.59	66.20	nd	nd	nd
0:100	3.91	1.55	16.59	10.70	38.20	nd	nd	nd

nd = not detected

Rhizobia Strain and the Inoculant

Rhizobium strain, *Bradyrhizobium diazoefficiens* (USDA 110), a slow-growing (Somasegaran and Hoben, 1994) and commonly used commercial strain for soybean (*Glycin max*) production in Uganda, was used in this study. The control treatment had no *B. diazoefficiens* inoculation in the respective carrier formulations. The inoculants were stored at room temperature, post preparation, for a period of 270 days (9 months).

A pure slant culture of the *B. diazoefficiens* (USDA 110), was cultured in yeast extract-mannitol agar plates, supplemented with Congo red (25 mg L⁻¹), according to Somasegaran and Hoben (1994). A loop-full of the cultured strain was aseptically transferred into a sterilized 250 ml Erlenmeyer flask, containing 100 ml yeast extract-mannitol broth, consisting of 1 g of Mannitol, 5 g of K₂HPO₄, 2 g of MgSO₄·7H₂O, 1 g of NaCl, 0.4 g of yeast extract and distilled in 1 L of water (Somasegaran and Hoben, 1994). The flasks were then placed on a rotary shaker, at 127 revolutions min.⁻¹, at 28 °C for 7 days. The resulting broth culture was used as the starter culture for the larger volume of broth, contained in 4 L glass fermenters.

The formulations were then packed into autoclavable polyethylene bags, each containing 50 g of the carrier. The bags were partially sealed and double-sterilised in an autoclave for 60 minutes, at 121 °C; with a waiting period of about 24 hours between the first and second sterilisation step. Sterility of the materials was determined by setting up carrier formulation + yeast mannitol broth (YMB) blanks as according to Somasegaran and Hoben (1994).

After cooling at 28 °C, the bags were completely heat-sealed in a sterile hood, and each aseptically injected with 16 ml of broth culture. The injection points on the bags were immediately sealed, using plastic labelling pieces of tape, to maintain the moisture content at 60 %. Then, the broth was worked into the carrier formulations, by kneading the bags until the liquid inoculum was uniformly absorbed by the carrier formulations.

The bags were labelled to indicate the treatment label; the strain used and the date of preparation. All the carrier formulations were then incubated in the dark, at room temperature (about 28 °C) for 14 days. Two weeks is the latent period that rhizobia is expected to reach their maximum population after inoculation (Somasegaran and Hoben, 1994). Counting of viable of rhizobia cells in the bagged carrier formulations was done at an interval of 30 days, continued throughout the storage period (270 days).

Data Collection

Prior to commencement of the study, the pH of the carrier formulations was determined in the MSW-Pt admixtures. During the study, the following data were determined, viz. moisture content, viable rhizobia colony and contaminant counts as described below.

pH of Carrier Formulations:

The pH of the carrier formulations was determined prior to the experiment, using the glass electrode procedure. This was done by taking samples of about 10 g of the MSW and peat compost mixtures (w/w) from, viz. 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, and 0:100 into a 500 ml beaker containing 90 ml of distilled water each, in

triplicates. The mixture was stirred using a magnetic stirrer and the pH monitored using a pH electrode three times after a day's interval until stable results were obtained. The pH results for the carrier formulations are presented in Table 2.

Table 2: pH values of municipal solid waste compost to peat carrier formulations before the start of the study

Rhizobia carrier formulation		pH
MSW compost (g)	Peat (g)	
100	0	8.96
90	10	8.45
80	20	8.08
70	30	7.57
60	40	7.44
50	50	7.28
40	60	6.99
30	70	6.78
20	80	6.38
10	90	5.38
0	100	3.91

MSW = Municipal solid waste

Moisture Content:

Moisture content was determined at 14-day intervals, and subsequently at 30 days intervals until after 270 days, using the Gravimetric Method. By this procedure, 10 g of each carrier sample were dried in duplicates at 70 °C for 48 hours (Somasegaran and Hoben, 1994) . Moisture content was then calculated on dry weight basis using Equation 3.

$$M (\%) = \frac{(W_1 - W_2) \times 100}{W_1} \dots\dots\dots \text{Equation 1}$$

Where: M = Moisture content (%); W1 = weight of carrier before drying; and W2 = weight of carrier after drying at 70 °C.

Viable Rhizobia Colonies and Contaminant Counts:

The number of viable cells per bag was determined at 14 days after inoculation, and thereafter, at 30 days intervals for 270 days, using a ten-fold dilution series, with sterile water to 10^{-7} (Somasegaran and Hoben, 1994). Carrier samples from the bags were selected randomly, by removing three inoculum packets from each bag, without replacement.

The ten-fold step dilution series (10^{-1} through 10^{-7}); comprising of tubes 1 to 7, each tube containing 9 ml of distilled water for dilution of the inoculum. During dilution, 1 ml of the inoculum was transferred into tube 1, using calibrated Pasteur pipettes. This was followed by mixing it thoroughly by removing 1 ml of the inoculum and expelling it back into the tube, with sufficient vigour to effect a thorough mixing. The expulsion was repeated 5 times into the tube

and then 1 ml of the inoculum from tube 1 was transferred into tube 2. This process was repeated until the dilution series was completed up to tube 7.

The Drop Plate Method was used and the last three dilutions (10^{-7} , 10^{-6} and 10^{-5}), were used to determine the population of viable cells. Four drops from each dilution were introduced into Congo Red Yeast Extract Mannitol Agar (YEMA) plates, using calibrated Pasteur pipettes. The drops, upon absorption onto the Congo Red YEMA plates, were incubated at 28 to 30 °C for 5 days. From the four drops, the numbers of rhizobia colonies formed were determined using a colony counter. The resulting Colony Forming Unit (CFU) per gramme was calculated using Equation 1:

$$\text{CFU/g} = \frac{1\text{ml} \times \text{average number of colonies per drop} \times \text{DF}}{\text{volume of one drop}} \dots\dots\dots \text{Equation 2}$$

The volume of one drop was obtained by determining the volume of 20 drops and then dividing the total volume by 20. The colonies that absorbed Congo red dye were considered to be contaminants according to Somasegaran and Hoben (1994), and their counts were also determined using a colony counter from the same three drops. The contaminants per gramme were calculated using Equation 2:

$$\text{Contaminants/g} = \frac{1 \text{ ml} \times \text{number of contaminants per drop} \times \text{DF}}{\text{Volume of one drop}} \dots\dots\dots \text{Equation 3}$$

Where: DF = Dilution Factor

Statistical Analysis

pH Data:

The pH data were first transformed into H^+ ion concentration using $-\log_{10}[\text{H}^+]$ before being subjected to statistical analysis. A one-way analysis of variance (ANOVA) tests using GenStat 12th Edition software, were carried out on pH data collected during the preliminary tests.

Viable Rhizobia Colony and Contaminant Counts:

Viable rhizobia colony counts and contaminant data were normalised by $\log_{10}$ transformation, to conform to the requirements of analysis of variance. Carrier formulation moisture content data were converted to percentage, and, thereafter, also subjected to analysis of variance (ANOVA) using GenStat, 12th Edition software. Significant treatment means were separated using the Least Significant Difference method (LSD) at 5% level of significance. Correlation analysis was done for rhizobia viable colony counts, contaminants and moisture content data.

RESULTS

Viable Rhizobia Colony Counts

At 14 to 120 Days of Storage:

All the carrier formulations maintained significantly ($p < 0.05$) high viable *B. diazoefficiens* populations; greater than 1×10^9 CFU/g (Figure 1). However, at 270 days, all carrier formulations had declined from 1×10^9 to 1×10^8 CFU/g, with the exception of pure MSW

compost carrier (100:0) and pure peat carrier (0:100), both of which retained a population of 2.08×10^9 CFU/g and 1.57×10^9 CFU/g, respectively (Table 3).

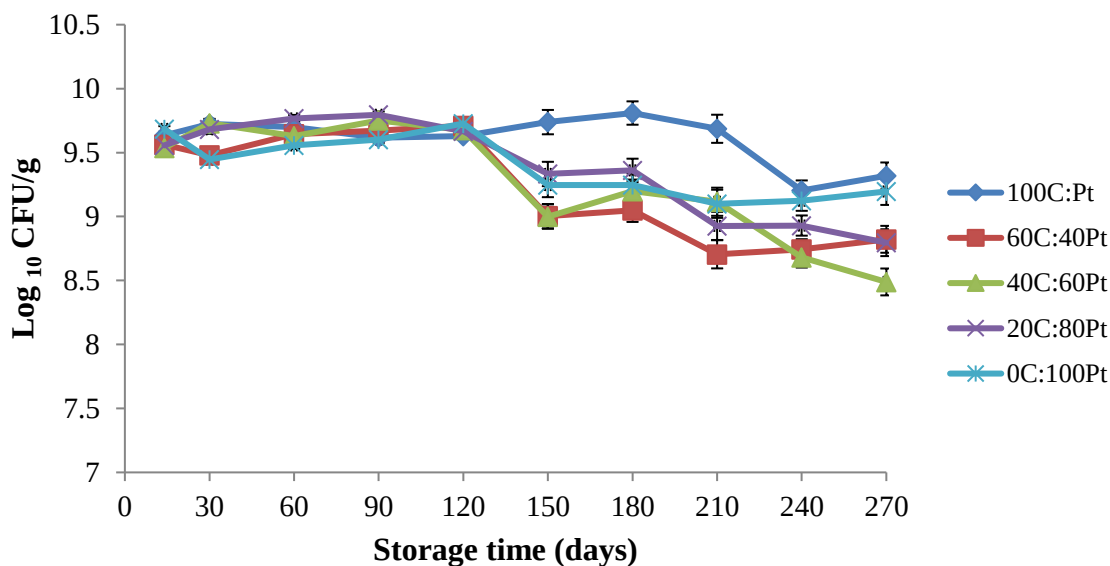


Figure 1: Viable *B. diazoefficiens* USDA 110 in different municipal solid waste compost and peat carrier formulations during rhizobia inoculants storage. C = Municipal Solid Waste compost carrier, Pt = Peat carrier.

There was no change in rhizobia viable populations in the control bags kept at room temperatures.

At 270 Days of Storage:

Carrier formulations differed significantly ($P < 0.05$) in retention of viable cells of *B. diazoefficiens* at 270 days of storage (Table 3). Pure MSW compost carrier 100:0 (MSW: Pt) still maintained the highest rhizobia population, with 2.08×10^9 CFU/g, although this was not significantly different ($p > 0.05$) from that of pure peat carrier 0:100 (MSW: Pt) with 1.57×10^9 CFU/g. The lowest viable population were presented by formulation 40:60 with 3.08×10^8 , although this was again not significantly different ($p > 0.05$) from 60:40 and 20:80 (MSW: Pt) carrier formulations over the same storage period.

Table 3: Rhizobia viable colony counts in carrier formulations at 270 days of storage

Carrier formulation (MSW: Pt)	CFU/g	Log ₁₀ CFU/g
100:0	2.08×10^9	9.3177
60:40	6.64×10^8	8.8221
40:60	3.08×10^8	8.4888
20:80	6.25×10^8	8.7957
0:100	1.57×10^9	9.1959
LSD _{0.05}		0.345
CV (%)		2.3

MSW = Municipal Solid Waste, Pt = peat

Moisture Content

There was significant decrease ($P < 0.05$) in moisture retention in the different carrier formulations (Figure 2). Pure peat carrier, 0:100 (MSW: Pt), retained moisture the most; followed by 20:80, 40:60 and 60:40 (MSW: Pt). Also, increase in MSW compost at the expense of its Pt counterpart, reduced moisture retention in the carrier admixture. Besides, moisture content in all the carrier formulations decreased during the period of inoculants storage. Drastic decline in moisture content was observed most, especially in pure municipal solid waste compost 100:0.

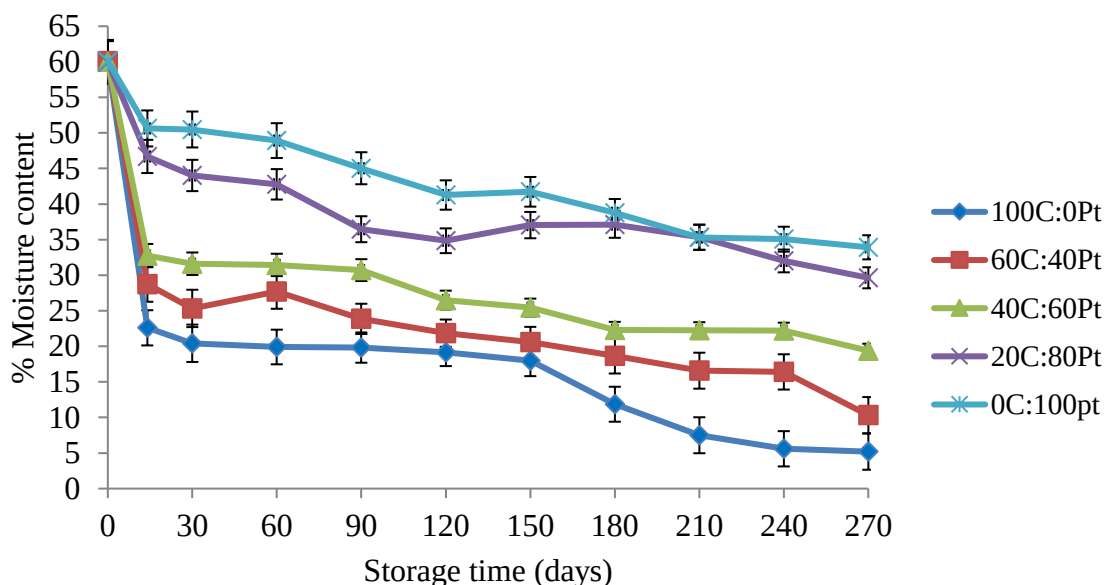


Figure 2: Changes in moisture content (%) in different municipal solid waste to peat carrier formulations (w/w) during storage.

Parameter Correlations

There was a weak negative correlation ($r = -0.423$) between *B. diazoefficiens* colony counts and contaminant population. On the other hand, no correlation was observed between *B. diazoefficiens* population and moisture content.

Table 4: Correlation analysis among *B. diazoefficiens* colony counts, contaminant population and moisture content.

	<i>B. diazoefficiens</i> (Log ₁₀ CFU/g)	Contaminant population (Log ₁₀ Contaminant/g)	Moisture content
<i>B. diazoefficiens</i> (Log ₁₀ CFU/g)	1		
Contaminant population (Log ₁₀ Contaminant/g)	-0.42347	1	
Moisture content	0.064395	-0.0392	1

Contaminants During Storage

There were significant ($P < 0.05$) increases in contaminant population in carrier materials after 120 days of storage (Figure 3). Before 120 days of storage, the contaminant populations in the inoculant were less than 1×10^1 contaminant/g; however, the values rose to 1×10^5 contaminant/g by the 270th day of rhizobia inoculant storage.

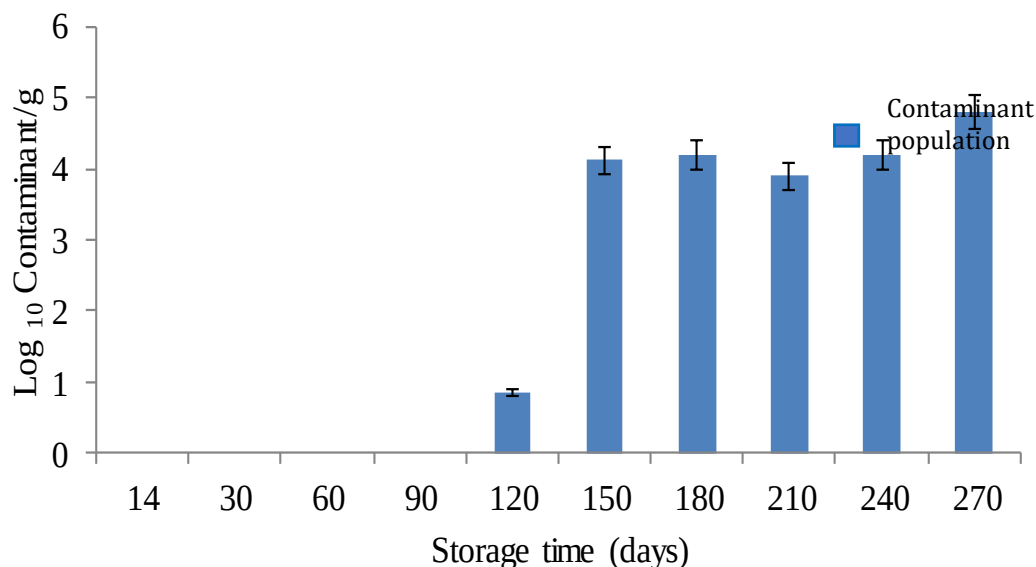


Figure 3: Contaminant population in the MSW: Pt carrier formulations over the storage period.

DISCUSSION

Viable Rhizobia Count at Various Stages of Storage

At 14 to 120 Days of Storage:

The high levels of viable colonies of *B. diazoefficiens* USDA 110 strain maintained by various MSW: Pt carrier formulations during inoculants storage (Figure 1) could be attributed to the inherent chemical properties of the carrier materials (Table 1), which provided conducive conditions for the survival of *B. diazoefficiens* in the inoculants.

The pH of pure peat was found to be strongly acidic ($p = 3.91$); and was thus subjected to neutralization treatment using calcium carbonate (Somasegaran and Hoben 1994) to a pH value near neutral (pH 6.62). Somasegaran and Hoben (1994) concluded that rhizobia inoculants remain reasonably viable when maintained at a pH near neutral (6.0 -7.0).

In contrast, the pH of the MSW compost was found to be way alkaline (pH = 8.96), and was thus subjected to acidification treatment using $Al_2(SO_4)_3$, that lowered the pH to a value of 6.77. The pH of the remaining carrier formulations (MSW: Pt), namely 60:40, 40:60 and 20:80 varied in pH in the order of 7.44, 6.99 and 6.38, respectively. The overall conservation of survival of rhizobia activity in all MSW: Pt carrier formulations, was therefore caused in part by adjustment

of the pH values to near the recommended values; in addition to the favourable ambient conditions.

The carbon contents in the various carrier formulations also remained fairly high (Table 1). This could also have contributed to the survival of *B. diazoefficiens* USDA 110 in the carrier formulations advanced by various literatures (Yadav and Chandra, 2014; Hardy and Knigh, 2021). The maximum organic carbon content was recorded in peat (0:100) with a value of 16.59%. This was followed by the (MSW: Pt) formulations in the order of 40:60, 20:80, 60:40 and 100:0; with C = 12.28, 11.89, 10.84 and 4.63%, respectively. *Bradyrhizobia diazoefficiens* USDA 110 strain are organolithotrophic organisms that require sufficient organic carbon source for metabolism. According to Yadav and Chandra (2014), for carrier materials to maintain viable microbial cells throughout storage, they should have high carbon contents and near neutral pH. Hardy and Knigh (2021) also noted that high carbon in the carrier contributed to the survival of *Rhizobium leguminosarum* in biochar carrier material.

Apart from the chemical properties of the carrier materials outlined above, the texture of the carrier formulations may also have contributed to the high population of *B. diazoefficiens* in the carrier formulations. In the present study, dried peat and MSW compost carriers were ground to a very fine powder; which in turn increased the specific surface area of the particles and enabled nutrients to spread more uniformly, and easily adhered to microorganisms (Somasegaran and Hoben, 1994). The very fine powdery structure also could have enabled the carrier formulations not to form lumps during inoculant preparation. Somasegaran and Hoben (1994) stated that a good carrier material should be free of lumps.

Strict sterilization of the carrier materials could also have partly contributed to high rhizobia populations during storage of the inocula. Carrier formulations in the study were sterilized twice consecutively for 60 minutes at 121 °C, with a waiting period of 24 hours. Abd El-Fattah *et al.* (2013) noted that the population of *Azotobacter chroococcum* was higher in sterilized carriers, than in unsterilized carrier materials. Tittabutr *et al.* (2012) also noted the highest population of *Bradyrhizobium sp.* PRC008 in sterilized peat after 6 months of storage. Sterilization of carriers eliminates microbial contaminants, and hence extends shelf life of the inoculants (Abd El-Fattah *et al.*, 2013).

At 270 Days of Storage:

It was noted only after 120 days of storage, that differences in rhizobia populations were emerged in all carrier formulations, but with a decline of $\log_{10}^1$ CFU/g at 270 days (Table 3). In spite of the fact that the carrier formulations were highly sterilized, contaminants emerged and were detected after 120 days (Figure 1). These could have contributed to the decline in rhizobia population post the first 120 days of inoculants storage.

Decline in rhizobia population could also have been attributed to a decline in nutrient supplies during storage (Phiromtan *et al.*, 2013), as the quality of inoculants declines naturally during storage. According to Herridge *et al.* (2002), the maximum carrier contamination level is usually $< 10^6$ CFU/g. Nevertheless, in the present study, the microbial contaminants were less

than the population of targeted rhizobia and the inoculants were still viable after nine months and could be used in two cropping seasons.

Whereas all the carrier formulations maintained high populations of viable *B. diazoefficiens* cells up to 270 days, pure MSW compost carrier (100:0) had the highest population (Table 3), though not significantly different from peat carrier. This implies that MSW compost is a good substitute for peat for rhizobia inoculant production. This study needs to be repeated extensively with a range of MSW compost, to demystify the diversity of quality of this organic material.

Moisture Content

The significant decline in moisture content over storage time (Figure 2), observed in all the carrier formulations, could have contributed to the decline in *B. diazoefficiens* population after 120 days (Figure 1). Moisture is an important physical parameter that contributes to the survival of rhizobia population (Deaker *et al.*, 2004). Pure peat carrier retained 29 % more moist at the end of the storage period (270 days), than did pure MSW compost. This could be attributed to the structure of peat soil that is characterized by organic coarse particles; mainly fibres (Huat *et al.*, 2011). Fibres are loose, hollow and spongy, thereby enabling peat soil to hold considerable amounts of water (Huat *et al.*, 2011). Municipal solid waste compost maintained the highest rhizobia population and, yet retained the least moisture (Figure 2). This contradicts Arora *et al.* (2014) and Singh *et al.* (2014), who reported that the coriander husk and sawdust carriers, respectively, maintained high rhizobia population due to high moisture retention and low rhizobia population were observed in carriers that retained the least moisture. However, in the present study, result showed no relationship between the high rhizobia population obtained in the carrier formulations and respective moisture content (Table 4). This implies that the high *B. diazoefficiens* population found in the carrier formulation was not attributed to the level of moisture content; perhaps because, moisture was not yet limiting to viability of the microsymbiont.

Population of Contaminant

The contaminant emergence, *hitherto* noted after 120 days of storage (Figure 3), likely resulted into the decline in rhizobia population by $\log_{10}^1$ CFU/g (Figure 1). This implies that there could have been contamination during storage and sampling. This possibly meant that sterilization by autoclaving was not sufficiently effective for solid organic carrier materials like peat and MSW compost. Other ultra-sterilizing techniques such as Tyndallisation could be a competent alternative as it allows spores of the contaminants to germinate, and the vegetative cells destroyed in the subsequent autoclaving (Bashan *et al.*, 2014). However, this approach is reportedly not only time consuming, but also too costly to be afforded by laboratories in developing countries. The best sterilization method by far is by gamma-irradiation (Bashan *et al.*, 2014); but this is minimally carried out in developing countries, again owing to high cost of operationalisation.

Numerous studies have advanced that carrier contamination reduces inoculant shelf life (Deaker *et al.*, 2004; Abd El-Fattah *et al.*, 2013). However, during the present study, the reduction in rhizobia population did not exceed the minimum standard of 1×10^7 CFU/g (Smith,

1992); confirming that the inoculants were still of good quality. This suggests that farmers can store the rhizobia inoculant products at room temperature for up to 270 days (9 months), without observable significant deterioration in inoculant quality. This is a major achievement in Uganda, where shelf life on inoculants is of serious concern to small scale farmers, who are the main producers of most leguminous crops in the country.

CONCLUSION

This study has revealed that municipal solid waste (MSW) compost is an effective replacement of peat soil in serving as a rhizobia carrier, as long as pH is managed to conform to the range recommended for optimum viability of rhizobia cells (pH = 6.0-7.0). All carrier formulation proportions maintained high *B. diazoefficiens* populations at levels greater than 1×10^8 CFU/g for 270 days (9 months) storage period, equivalent to two soybean growing seasons. Pure MSW compost carrier (100:0) maintained the highest *B. diazoefficiens* population; though not significantly different from peat carrier; thus, confirming their equitable competitiveness, differences in original pH notwithstanding.

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