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Potential of biochar-based inoculant in enhancing rhizobia survival and grain yield of cowpea (*Vigna unguiculata* (L.) Walp.)

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Abstract

The need for low-cost carrier materials is paramount for rhizobium inoculants production, especially in sub-Saharan Africa. This research studied different feedstocks biochar as potential carriers for inoculant production. Coconut coir (CC), coconut shell (CS), rice husk (RH), and sugarcane bagasse (SB) biochars were used as carriers, and their performance was compared to that of standard industrial peat (control). The biochar and peat carriers were inoculated with two elite *Bradyrhizobium* strains such as KNUST 1002 and KNUST 1006 and the reference strain BR 3267 (where BR is *Bradyrhizobium*). The survival rate of the rhizobia was monitored by determining the number of cells, pH, and moisture content for 24 weeks. In addition, the effectiveness of different inoculated carriers was tested in cowpea (*Vigna unguiculata* (L.) Walp) under field conditions via a randomized complete block design with four replications. CC and SB enhanced *Bradyrhizobium* survival for up to 16 and 20 weeks, respectively, whereas CS and RH promoted strain survival for up to 8 weeks. The viable cell count was highest in the peat-based inoculant during the 24-week period. BR 3267 in CC and SB significantly ($p < 0.001$) increased the nodule dry weight of cowpea compared to uninoculated control. The highest grain yield (1774 kg ha^{-1}) was recorded for CC, which was 16% greater than the grain yield of the peat-based inoculant (1524 kg ha^{-1}), although this difference was not significant. CC biochar is a potential carrier for inoculant production in Ghana.

Plain Language Summary

There are organisms in the soil that support plant growth by supplying nitrogen. The degree to which they supply nitrogen differ from organism to organism. Researchers usually extract the best organisms from soil and reintroduce them to the crops. The

Abbreviations: BNF, biological nitrogen fixation; BR, *Bradyrhizobium*; CC, coconut coir; cfu, colony forming unit; CS, coconut shell; EC, electrical conductivity; RH, rice husk; SB, sugarcane baggase; SSA, sub-Saharan Africa; WHC, water-holding capacity.

extracted organisms are usually kept in a medium that is carbon rich and can protect the organisms. This medium is called carrier. The industry based carrier is peat, which is nonrenewable and mining it can contribute to climate change. Agricultural waste such as rice husk, coconut coir, and others that otherwise are environmental nuisance can be converted into such carrier. Such carrier is less expensive and support local business and reduce cost for farmers. Our work identified that these agricultural waste when burned under limited oxygen are called biochar. And these biochar promoted the lifespan of the organisms.

1 | INTRODUCTION

Food security and malnutrition remain major challenges in sub-Saharan Africa (SSA), although several advances in agriculture have been made in SSA in recent years (Sanchez & Swaminathan, 2005). This problem is often associated with declining soil fertility, particularly low soil N and P levels caused by biological and environmental factors. Continuous cultivation without nutrient replenishment has also resulted in a decline in soil fertility, leading to a low yield of crops (Manyong et al., 2001). Chemical fertilizers, which are known to be one of the means for improving crop productivity (Morris et al., 2007; World Bank, 2008), are considered costly and, as such, make it difficult for smallholder farmers to afford them (Druilhe & Barreiro-Hurlé, 2012). Furthermore, the accessibility of mineral fertilizers in most farming communities in SSA remains a challenge. The intensive misuse of chemical fertilizers in agriculture has led equally to a series of environmental and health problems (Abhiram et al., 2023; Cameron et al., 2013; Richter & Roelcke, 2000; Zhu et al., 2000). Alternative inexpensive and environmentally friendly materials (Albareda et al., 2009) affordable to smallholder farmers are therefore needed to solve soil fertility problems and improve yield. One such solution is the biological nitrogen fixation (BNF). BNF is a key source of nitrogen (N), particularly for legume farmers. BNF in legume cropping systems offers farmers the chance to apply little or no fertilizer and therefore potentially could play a greater role in sustainable grain legume production (Albareda et al., 2009). The practice of inoculating grain legumes with highly superior rhizobium strains has been established as an essential means of promoting BNF and ultimately increasing grain yields (Unkovich & Pate, 2000). Ulzen et al. (2016) reported that *Bradyrhizobium* inoculants increased the grain yields of soybean and cowpea in northern Ghana. Inoculation with effective native strains also increased the grain yields of field-grown cowpea and groundnut in the Sudan savanna agroecological zone of Ghana (Osei et al., 2020). Mulas et al. (2015) also reported that inoculation with indigenous rhizobium strains increased yields of common bean (*Phaseolus vulgaris* L.) in northern Spain. The need for rhizobium inoculants remains paramount, hence higher demand for inoculants in the agricultural industry (Elnahal et al., 2022; Kaminsky et al., 2019; O'Callaghan et al., 2022). The actual quantity of inoculants produced worldwide is unknown but it has been previously estimated to be 2000 tonnes (Rebah et al., 2007). However, the most serious challenge facing the development of commercial inoculants is ensuring a consistent survival rate of the rhizobia cells (Hale et al., 2014). David et al. (2002) reported that the characteristics of a high-quality inoculant are related to the properties of the carrier. A good carrier material should have the ability to improve rhizobia survival, increase shelf-life of inoculant, and good adhesion properties.

Peat is recognized worldwide as a carrier for most commercially available bacterial inoculants (Eudoxie & Alexander, 2011). However, peat occurs naturally and is effectively a nonrenewable resource, and its overuse is of great environmental concern. Peat is known to sequester a large amount of carbon; therefore, mining it for inoculant production as a carrier results in carbon losses. Swystun et al. (2013) reported that the extraction of peat can release nutrients and other minerals into water bodies, thereby causing water quality issues. Furthermore, the lack of peat deposits in Ghana and other SSA countries where inoculant science is emerging is likely to increase inoculant production costs, making it difficult for smallholder farmers to afford. Over the past few years, researchers have been looking for worthwhile and efficient alternatives to peat as an inoculant carrier (Glodowska, 2014). Compost (Wall, 2003), charcoal (Beck, 1991; Crawford & Berryhill, 1983), biochar (Hale et al., 2015), and agro-industrial waste (Rebah et al., 2007) are some of the carriers studied in the past, but none of these have been harnessed in Ghana. The current state of inoculant production in Ghana, which involves the importation of peat to be used as a carrier material, is not only expensive but unsustainable. This allows for research into other alternatives that can compete biologically and economically. The physical and chemical properties of biochar, such as high porosity, sorption capacity, high water-holding capacity (WHC), and nutrient retention, might create suitable habitats for microorganisms, thereby sustaining rhizobia densities and enhancing their survival (Ajeng et al., 2020; Glodowska et al., 2017; Thies & Rilling, 2009). Egamberdieva et al. (2018) reported that the use of biochar as an inoculant carrier increased the survival rate of rhizobia and increased rhizosphere colonization. These results are attributed to high-temperature pyrolysis, which kills other microorganisms. Thus, these characteristics of biochar and their relationships with the survival of microorganisms are important to understand when considering biochar as an inoculant carrier. This study hypothesized that biochar-based carrier has the potential to enhance rhizobia survival and increase grain yield of cowpea (*Vigna unguiculata* (L.) Walp). This study therefore sought to evaluate biochar-based carrier for rhizobia survival and grain yield enhancement. Three strains, one from Brazil and two from the indigenous rhizobia population in Ghana were used in this study. The strains have widely been tested (Osei et al., 2020; Ulzen et al., 2016) and accepted as the best strains for inoculant production in Ghana.

Core Ideas

- The physical and chemical characteristics of feedstocks influence rhizobium survival.
- Coconut coir and sugarcane biochar enhance rhizobia survival up to 20 weeks.
- Coconut-coir- and sugarcane-biochar-based inoculants increased nodulation compared with peat-based inoculants.
- The grain yield of cowpea did not vary significantly with biochar-based or peat-based inoculants.

2 | MATERIALS AND METHODS

2.1 | Study site

The study was carried out at the Kwame Nkrumah University of Science Technology (KNUST) in the Kumasi Metropolis from January to November 2019. The region is located in the semideciduous agroecological zone of Ghana. The study site is characterized by a bimodal rainfall pattern with an annual rainfall of 1500 mm and annual average minimum and maximum temperatures of 21˚C and 31.2˚C, respectively. The major growing season is from April to June, and the minor season is from September until December.

2.2 | Feedstock and pyrolysis conditions

Four feedstocks, coconut shells (CSs), coconut coir (CC), sugarcane bagasse (SB), and rice husk (RH), were obtained and charred at a temperature of 500˚C (Hammes et al., 2006) for 1 h and ground to pass through a set of sieves, 42 mesh (355 µm) and 200 mesh (75 µm), to achieve a particle size less than or equal to 75 µm, as this size makes them suitable for seed coating (Somasagaren & Hoben, 1994).

2.3 | Laboratory analyses of biochar

The pH of the biochars was determined at a ratio of 1:10 (biochar:distilled water), and their moisture contents were calculated as the difference in weight before and after oven drying at 70˚C for 24 h (Somasagaran & Hoben, 1994). Biochar carriers with pH values outside the recommended range of 6.0–7.0 (Somasagaren & Hoben, 1994) were adjusted with 5% HCl to achieve the recommended range. The biochar was dried at 60˚C in an oven (Vanek et al., 2016). The organic carbon content was determined via the Walkey and Black method (Walkley & Black, 1934), whereas the total nitrogen content of the biochar was determined via the Kjeldahl distillation method (Bremner, 1982), the available phosphorus content was determined via the Bray P1 method (Bray & Kurtz, 1945), and the cation exchange capacity was determined by leaching the biochar with 1 *N* ammonium acetate (Black, 1965). Furthermore, the WHC, was estimated by following procedure described by (Robertson, 1999). 100 g of biochar samples were saturated in 100 mL of distilled water and allowed to drain for 24 h through a funnel fitted with cotton at the bottom. The WHC was then estimated by measuring the mass of the water retained in the biochar per gramme of biochar multiplied by 100. The volatile ash content was determined by placing 1.0 g of biochar into a crucible (with lid) and placed in a preheated oven at 900 ± 5˚C. The crucible was removed from the oven and reweighed after cooling to room temperature. Volatile ash content was calculated from the mass loss of the sample (Ronsse et al., 2013). The electrical conductivity (EC) was determined according to the procedures described Corwin and Lesch (2005).

2.4 | Inoculant preparation

The inoculants for this study were prepared at the KNUST soil microbiology laboratory as previously described by Ulzen et al. (2016) following the method of Somasegaran and Hoben (1994). Fifteen grams of each carrier material was bagged and sterilized via gamma radiation at the Ghana Atomic Energy Commission. The selected isolates (i.e., *Bradyrhizobium* strains KNUST 1002, 1006, and BR 3267 [where BR is *Bradyrhizobium*]) obtained from the Soil

Microbiology Laboratory of the Department of Crop and Soil Sciences, KNUST-Kumasi, were multiplied in 50 mL yeast extract broth culture until a cell load of 1.0×10^9 cfu/mL (where cfu is colony forming unit) was obtained. Each sterilized carrier material was inoculated with approximately 15 mL broth cultures of each of the selected strains and cured in an orbital incubator for 1 week. Inoculants were then stored in a 4°C refrigerator and cell numbers monitored.

2.5 | *Bradyrhizobium* survival study

The survival rates of the *Bradyrhizobium* strains (KNUST 1002, 1006, and BR 3267; *Bradyrhizobium yuanmingense*) in the different carrier materials were assessed by determining the viable cell numbers, pH, and moisture content every 4 weeks for 24 weeks. The number of viable cells in the inoculant was estimated via the drop plate technique as described by Zuberer (1994). Viable cells within the range of 30–300 colonies per plate were counted with a colony counter, and the number of rhizobium colonies was recorded and estimated via the formula described in Equation (1).

$$\text{CFU/mL} = \text{Average no. of colonies counted} \times \frac{1000}{\text{aliquot plated}} \times \frac{1}{\text{dilution factor}} \quad (1)$$

2.6 | Assessment of inoculated carriers under field conditions

The biochar-based inoculants were evaluated under field conditions at the plantation section of the Department of Crop and Soil Sciences, KNUST, Kumasi. The experiment used a 5 (carrier treatments including the uninoculated control) × 3 (*Bradyrhizobium* strains) factorial design plus an absolute control. The treatment combinations were arranged in a randomized complete block design and were replicated four times. The treatments consisted of four different biochar carriers and one control (peat). The carriers were inoculated with three different *Bradyrhizobium* strains (KNUST 1002, KNUST 1006, and BR 3267; *B. yuanmingense*).

The field was plowed to a depth of 15 cm and harrowed before planting. The field was divided into plots, and each plot measured 3 m × 4 m with 1- and 2-m alleys between plots and blocks, respectively. Three seeds of the cowpea (*V. unguiculata* (L.) Walp) cultivar Padi-Tuya were planted per hole at 60 cm × 20 cm and thinned to two after 1 week of emergence. The cultivar is the erect type and matures within 64–67 days. Padi-Tuya is widely grown by farmers in the study location, as it is moderately resistant to insects and *Striga hermonthica*, a parasitic weed

2.7 | Data collection

Nodule dry weight was determined at 50% flowering. This was accomplished by drying the washed nodules and the shoots in an oven at 60°C for 72 h, and their dry weights were measured and recorded accordingly.

The second sampling was performed at physiological maturity, where cowpea pods were harvested from the inner rows, excluding the border rows. The harvested pods were air dried for 3 days until constant moisture was attained, after which they were threshed, and the seed weight was determined to estimate the grain yield per hectare (Okogun et al., 2005). The seed moisture content was adjusted to 12% at the time of estimating the grain yield per hectare using a moisture tester (John Deere Grain Moisture Tester GT–5300).

2.8 | Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) via GenStat statistical software version 12 (VSN International, 2012). Significant differences were assessed at the 5% ($p = 0.05$) probability level, and the means were separated using the Tukey's honestly significant difference test. Viable cell count data were logarithm transformed (Kihara et al., 2011) before subjecting to ANOVA.

3 | RESULTS

3.1 | Physical and chemical characteristics of the carriers

The physical and chemical properties of the biochars used as carriers varied among the measured parameters (Table 1). The initial pH of the biochars ranged from 9.12 to 10.08, and each was then adjusted to 6.8. Peat had more N and P but lower percentage K than the other carrier types. The WHC of peat was greater than that of the other carriers.

3.2 | *Bradyrhizobium* survival study

The results obtained for the viable cells count of the biochar carriers and peat (control) showed a declining trend over the 24 weeks study period (Figure 1A–C). After 12 weeks, *Bradyrhizobium* cells were viable only in SB and CC biochars for all three strains tested. The number of viable cells recorded for KNUST 1002 in SB and CC biochars were 6.7 $\log_{10}$cfu/g and 4.1 $\log_{10}$cfu/g, respectively, whereas those of KNUST 1006 were 4.6 $\log_{10}$cfu/g and 3.6 $\log_{10}$cfu/g, respectively. The standard strain BR 3267 also had values of 6.1 $\log_{10}$cfu/g and 4.2 $\log_{10}$cfu/g for the SB and CC biochars, respectively.

TABLE 1 Physical and chemical characteristics of the carriers.

Carrier types	$pH_{(i)}$	$pH_{(f)}$	Total N (%)	% P	%K	%Ca	%Mg	%Na	%OC	%WHC	Ash (%)	EC (mS/cm)
CC	9.14	6.80	0.44	0.42	5.95	0.61	0.29	0.2	48.43	69.5	13	2.5
SB	9.12	6.80	0.58	0.52	2.57	0.41	0.15	0.14	53.36	73.5	10	2.1
CS	10.08	6.80	0.35	0.46	3.01	0.36	0.03	0.1	57.14	63.2	4	1.2
RH	9.73	6.80	0.40	0.39	2.1	0.44	0.12	0.06	24.07	35.3	56	0.5
Peat (control)	8.41	6.80	0.51	0.55	1.6	2.64	0.1	0.04	50.75	80.5	12	0.3

Abbreviations: CC, coconut coir; CS, coconut shell; EC, electrical conductivity; OC, organic carbon; pH(f), final pH; pH(i), initial pH; RH, rice husk; SB, sugarcane bagasse; WHC, water-holding capacity.

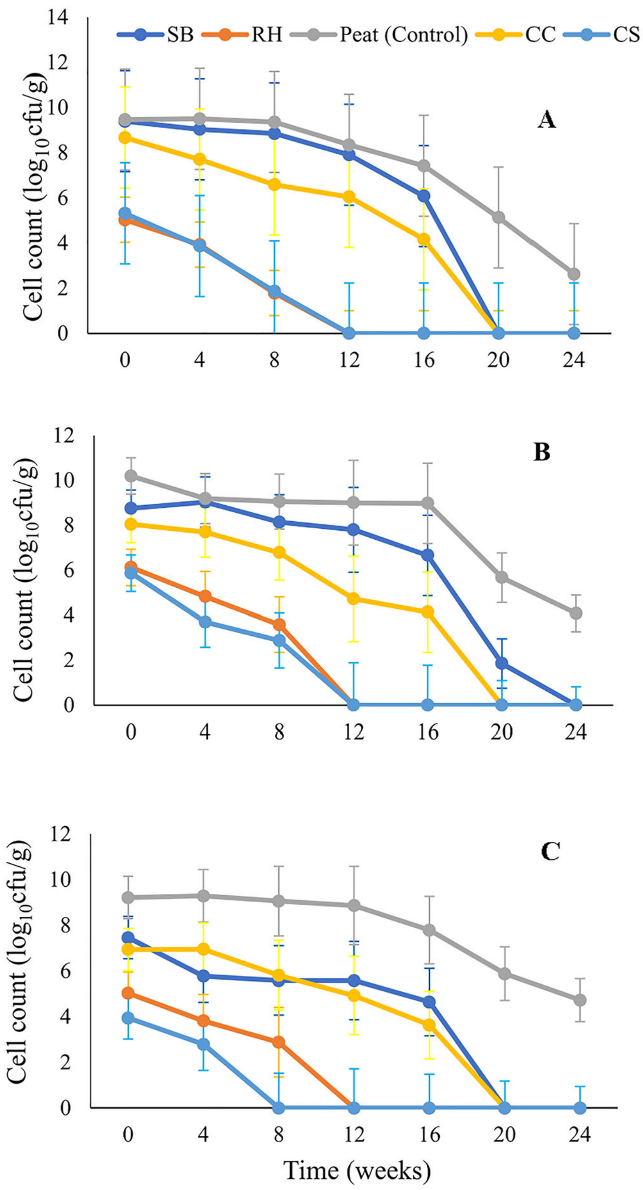

FIGURE 1 Population dynamics of *Bradyrhizobium* strains, BR 3267 (A), KNUST 1002 (B), and KNUST 1006 (C) in five different carrier materials as influenced by time. Bars denote the standard error of means. CC, coconut coir; CS, coconut shell; RH, rice husk; SB, sugarcane bagasse.

The inoculation of SB with the *Bradyrhizobium* strain BR 3267had the highest initial count (Week 0) of 9.46 log_{10}cfu/g, which declined to 6.08 log_{10}cfu/g in Week 16. The cell count recorded for CC biochar inoculated with *Bradyrhizobium* strain BR 3267 at the initial stage was 8.67 log_{10}cfu/g, which also declined to 4.16 log_{10}cfu/g by Week 16 (Figure 1A). A sudden decline in the number viable of cells was observed in both the CS and RH biochars inoculated with KNUST 1002, KNUST 1006, or BR 3267, with both carriers recording zero viable cell counts after12 weeks.

Bradyrhizobium strain KNUST 1002 with an initial cell count of 8.76 log_{10}cfu/g in SB biochar sustained viable cells of up to 1.86 log_{10}cfu/g by Week 20, whereas the same strain in CC biochar with an initial cell count of 8.04 log_{10}cfu/g declined to 0.00 log_{10}cfu/g by Week 20 (Figure 1B).

The viable cell count was highest in peat throughout the incubation period and was able to sustain cell counts for up to 24 weeks for the KNUST 1002, KNUST 1006, and BR 3267 strains tested (Figure 1A–C).

3.3 | pH and moisture content of the biochar-based inoculants

There were fluctuations in the pH readings for the different carrier-based inoculants. The SB biochar inoculated with the *Bradyrhizobium* strain KNUST 1002 presented an increase in pH from 6.31 to 7.88 from 0 to 24 weeks (Figure 2A). For CC biochar inoculated with *Bradyrhizobium* strain KNUST 1002, the initial pH of 6.93 remained stable until Week 16, after which it increased to 8.5 at Week 24 (Figure 2A). CS biochar inoculated with *Bradyrhizobium* strain KNUST 1002, on the other hand, exhibited a fluctuation in pH throughout the study. An initial pH of 9.26 was reported at Week 0, which decreased to 7.92 at Week 4 and then to 8.55 at Week 12. Furthermore, inoculation of RH biochar with the *Bradyrhizobium* strain KNUST 1002 resulted in a pH of 9.26 at Week 0, which decreased to 7.38 at Week 8 but increased to 8.67 at Week 24. The pH for peat (control) inoculated with *Bradyrhizobium* strain KNUST 1002 was stable from 0 to 12 weeks and ranged from 6.92 to 7.30. The pH, however, increased from Weeks 16 to 24 in the range of 7.72–8.15 (Figure 2A).

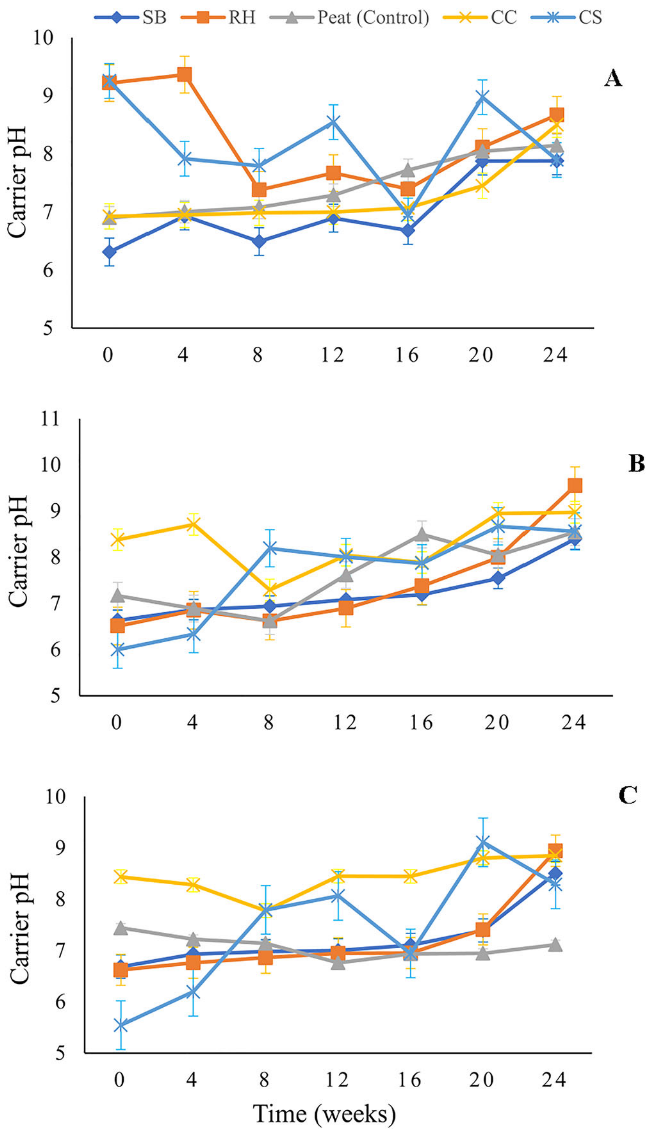

FIGURE 2 The pH of biochar-based carrier inoculated with *Bradyrhizobium* strain KNUST 1002 (A), KNUST 1006 (B), and BR 3267 (C) over time. Bars denote the standard error of means. CC, coconut coir; CS, coconut shell; RH, rice husk; SB, sugarcane bagasse.

For *Bradyrhizobium* strain KNUST 1006 (Figure 2B) in SB biochar, a pH of 6.64 was recorded at Week 0, which further increased to 8.40 at Week 24. CC biochar inoculated with *Bradyrhizobium* strain KNUST 1006 also had a pH of 6.51 at Week 0, which increased to 9.55 at Week 24. Moreover, the pH of RH biochar inoculated with *Bradyrhizobium* strain KNUST 1006 was 8.38, which decreased to 7.30 at Week 8 and increased again to 8.98 at Week 24. Similarly, *Bradyrhizobium* ststrain KNUST 1006 in CS biochar was 6.0, which increased to 8.56 at Week 24. The initial pH of the peat (control) inoculated with the *Bradyrhizobium* strain (KNUST 1006) also decreased from Week 4 to 6.89 and then decreased to 6.62 at Week 8. An increase in pH for peat inoculated with *Bradyrhizobium* strain KNUST 1006 was observed during Weeks 8–24 (Figure 2B).

Additionally, SB and CC biochars inoculated with *Bradyrhizobium* strain BR 3267 presented statistically similar initial pH values of 6.69 and 6.62, respectively. An increase in pH was recorded for SB (8.51) and CC (8.95) at Week 24 (Figure 2C). A pH of 8.44 was recorded for RH biochar inoculated with *Bradyrhizobium* strain BR 3267, which further increased to 8.85 at Week 24. Moreover, the CS biochar inoculated with the *Bradyrhizobium* strain BR 3267 (Figure 2C) also resulted in a lower initial pH of 5.45, which increased to 8.29 at Week 24. The pH of the peat inoculated with the same strain was also 7.44 at Week 0 but decreased to 7.12 at Week 24 (Figure 2C).

The moisture content of the biochar-based and peat-based inoculants were also within the acceptable range (35%–50%) (Figure 3A–C) but decreased slightly during the study period. The inoculation of SB with the *Bradyrhizobium* strain KNUST 1002 resulted in the highest moisture count of 23.7% at the end of the study period (Week 24) (Figure 3A). Low moisture content values (17.5% and 17.9%) were recorded for the CS and RH biochars inoculated with *Bradyrhizobium* strains KNUST 1006 and BR 3267, respectively (Figure 3B,C). However, the moisture content of the peat ranged from 43.8% to 20.5% (Weeks 0 - 24), which represented 53% moisture loss when inoculated with *Bradyrhizobium* strain BR 3267. The moisture loss for SB biochar inoculated with *Bradyrhizobium* strain BR 3267 was 43%. Both CC and RH biochars inoculated with *Bradyrhizobium* strain KNUST 1002 also resulted in moisture loss rates of 49.7% and 41.3%, respectively.

3.4 | Soil physicochemical properties and most probable number of rhizobia at the study site

The results of the physical and chemical properties of the soil at the study location are presented in Table 2. The results of the soil tests indicated that the samples from the study location contained low concentrations of nitrogen, phosphorus, potassium, and calcium. The soil pH status was slightly acidic. The indigenous rhizobia count was low (7.5 cells g^{-1} soil).

3.5 | Nodulation assessment of cowpea affected by different biochar-based carrier inoculants

The interaction effect between carrier type and *Bradyrhizobium* strain was significant ($p < 0.05$) for nodule dry weight (Table 3). The biochar- and peat-based inoculants had higher nodule dry weight than the uninoculated control. The nodule dry weight obtained with the RH carrier, irrespective of strain, was significantly lower than that obtained with the other

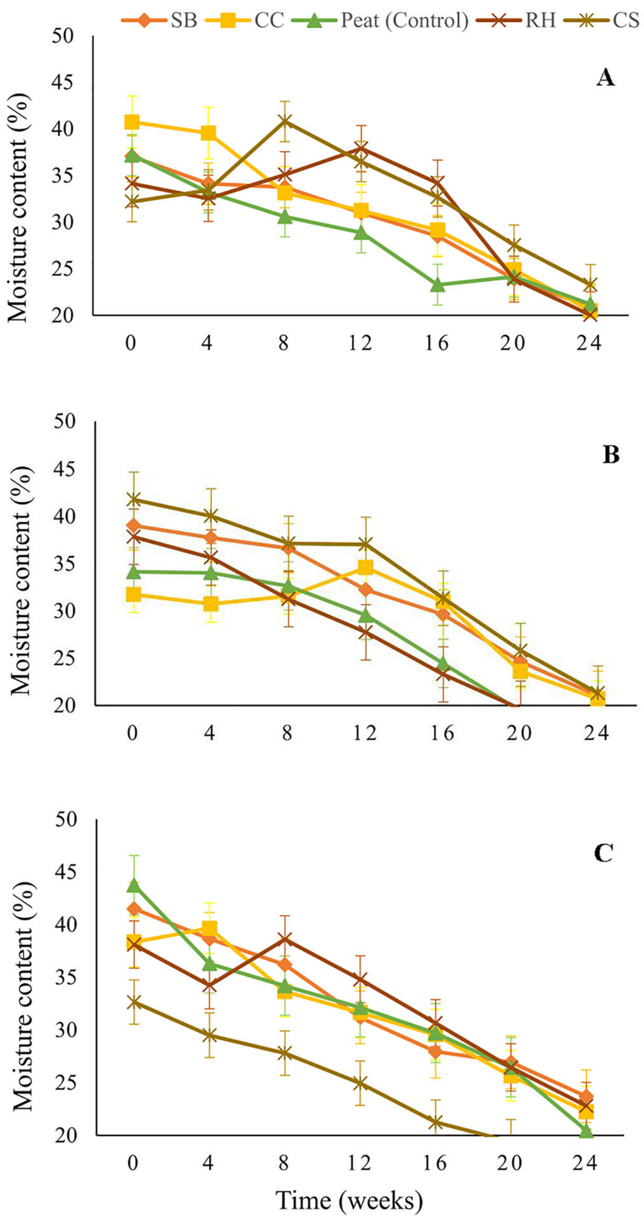

FIGURE 3 The moisture content of biochar-based carrier inoculated with *Bradyrhizobium* strain KNUST 1002 (A), KNUST 1006 (B), and BR 3267 (C) over time. Bars denote the standard error of means. CC, coconut coir; CS, coconut shell; RH, rice husk; SB, sugarcane bagasse.

carriers. For strains KNUST 1002 and KNUST 1006, the peat-based carrier had relatively greater nodule dry weights than the biochar-based carriers.

The treatments had no significant ($p > 0.05$) effect on cowpea grain yield (Table 3). However, except SB inoculated with strain KNUST 1006 and CS inoculated with strain BR 3267, all biochar- and peat-based inoculants had higher grain yields than the uninoculated control. The highest grain yield was recorded for the CC carrier inoculated with *Bradyrhizobium* strain KNUST 1002, which was 16% greater than that of the peat carrier (control) inoculated with the same strain.

TABLE 2 Physicochemical properties and number of rhizobia in the soil at the study site.

Parameter	Value
pH (1:2.5)	6.17
Total nitrogen (%)	0.18
Organic carbon (%)	0.72
Available P (mg kg^{-1})	15.41
Exchangeable K (cmol $_{(+)}$ kg^{-1})	0.23
Exchangeable Ca (cmol $_{(+)}$ kg^{-1})	2.40
Exchangeable Mg (cmol $_{(+)}$ kg^{-1})	0.80
Exchangeable Na (cmol $_{(+)}$ kg^{-1})	0.002
Particle size distribution	
Sand (%)	77.6
Clay (%)	17.2
Silt (%)	5.2
Textural class (%)	Sandy loam
MPN count (rhizobia cells g^{-1} soil)	7.5

Abbreviation: MPN, most probable number.

4 | DISCUSSION

4.1 | Physical and chemical characteristics of the different biochar-based carriers

The results revealed variations in the physical and chemical properties of the carriers, which was supported by earlier findings (Amonette & Joseph, 2009; Hale et al., 2015; Lehman et al., 2011). The properties of biochar have been reported to be linked to its feedstock source and the duration of the pyrolysis (Downie et al., 2009; Mukome et al., 2013). A suitable carrier needs to be inexpensive, high in organic matter content, have a higher WHC (Gaur & Gaind, 1990), readily adjustable pH to 6.0–7.0 (buffering capacity), sterilizable, free of toxic substances, and readily available (Xavier et al., 2004). In this study, the higher initial pH values observed in the biochar carrier types could be attributed to the high temperature of pyrolysis (500°C) and the feedstock used. Lehmann et al. (2011) indicated that the pH of biochar varies between 4 and 12 depending on feedstock type and temperature of the pyrolysis. Moreover, Chen et al. (2006) reported that oxidation of carbon to form carboxyl group results in higher pH values of biochar. However, regarding the relationship of microorganisms to biochar surface, the pH of biochar might be considered, as having an important effect on microbial abundance (Lehmann et al., 2011). Hence, a pH of 6.0–7.0 has been reported to be optimal for *Bradyrhizobia* (Somasagaren & Hoben, 1994). Adjusting the pH of each biochar-based carrier yielded the recommended pH values. This finding indicated that all the biochars used in this study supported *Bradyrhizobium* growth. A report by Kaljeet et al. (2011) indicates that unfavorable pH of a carrier material will

TABLE 3 Influence of carrier type and *Bradyrhizobium* strain on cowpea nodule dry weight and grain yield.

	Nodule dry weight (mg plant^{-1})			Grain yield (kg ha^{-1})		
	Strains			Strains		
Carrier types	1002	1006	3267	1002	1006	3267
SB	108.5b	105.5b	130.2a	1428a	1243a	1531a
CC	116.2b	111.8ab	123.0a	1774a	1469a	1458a
CS	149.2a	130.0a	92.0b	1632a	1549a	1306a
RH	58.0c	90.2b	62.5c	1378a	1677a	1514a
Peat (control)	151.3a	128.5a	111.3ba	1524a	1639a	1556a
Grand mean	116.6a	113.2a	103.8a	1547a	1515a	1473a
Uninoculated	38c			1361a		
p-value	<0.001			0.303		
CV	41.2			18.9		

Note: Means followed by different lowercase letters within carrier type for each strain are significantly different based on the Tukey's honestly significant difference test at 5% probability.
Abbreviations: CC, coconut coir; CS, coconut shell; CV, coefficient of variation; RH, rice husk; SB, sugarcane bagasse.

create unsuitable growth conditions for rhizobia, decreasing the number of viable cells. The effects of salinity on rhizobia are very necessary to understand when producing and applying an inoculant. The finding is in agreement with that of Hussain et al. (2002), who evaluated the efficacy of *Rhizobium trifoli* in enhancing the salt tolerance of *Trifolium alexandrinum* (berseem or Egyptian clover) in loamy soil and concluded that the total green and dry matter yields as well as the total root dry weight of berseem decreased with increasing salinity. Another study by Singleton et al. (1982) revealed that when the pH of a culture solution was raised from 1.2 to 6.7 mScm^{-1} or 13 mScm^{-1}, all rhizobium strains and species being tested decreased with several strains failing to grow at the latter value. The EC value obtained in this study did not surpass the EC values suggested by Singleton et al. (1982) and, as such, can be concluded as not having a direct effect on rhizobia survival for this studyy.

Moisture holding capacity is also another factor affecting *Bradyrhizobium* survival. A high moisture level is necessary because moisture is lost during storage and the survival of rhizobia in a carrier is affected by low moisture levels (Somasagaren & Hoben, 1994). Several authors have indicated that a moisture content of 35%–50% should be the target range for rhizobium inoculants (Denardin & Freire, 2000; Mishra, 2002). The moisture content range of the biochar carriers evaluated in this study agreed with the minimum value as opined by these authors. Therefore, it could be reported that, under favorable conditions, biochar-based carriers can create a more suitable moisture environment to support bacterial growth.

The ash content varied among the different biochars, ranging from 4% to 56%, with RH carrier recording the highest (56%) among all the carrier types. A similar observation was made by Enders et al. (2012), who reported higher ash content values for RH biochar. From their study, they attributed high ash content to the partial change in the composition promoted by a possible interaction between organic and inorganic constituents during feedstock pyrolysis.

The availability of organic carbon is the key factor regulating microbial activities. The C content of biochars ranges from 33.0% to 82.7%, and the N content ranges from 0.1% to 6.0% (Jha et al., 2010; Spokas et al., 2012). The results from this study revealed that the carriers had favorable nutrient levels that could also have a significant effect on bacteroides population abundance and viability.

4.2 | *Bradyrhizobium* survival

The results revealed that the biochar-based carriers tested were able to support all the *Bradyrhizobia* strains over time but differed in their population abundance, which could be attributed to feedstock type (Lehmann et al., 2011) as well as the physical and chemical characteristics of the biochar. SB carrier was observed to be the most appropriate to support *Bradyrhizobium* strain KNUST 1002 survival up to Week 20, which suggests that the strain was more adapted to the physical and chemical conditions of this carrier. Reports by Mubarik et al. (2012) opined that the optimal pH for *Bradyrhizobia* is between 6 and 7, although a lower optimum pH may be found for strains isolated from soils. This means that the favorable pH of the SB carrier contributed to the survival of *Bradyrhizobium* strain KNUST 1002 for a longer time. The favorable organic carbon (OC) content of SB is in line with Steven et al. (2016), who reported that greater amounts of easily mineralizable carbon in bagasse are likely available to bacteria over time for longer storage, thereby increasing and ensuring their survival over time.

The population of CC–rhizobia inoculant was maintained for up to Week 16, irrespective of the strain inoculated. The favorable WHC recorded by the CC carrier could account for this observation. Pietikainen et al. (2000) reported that CC has a sponge-like matrix, which results in a slow release of moisture, a characteristic that is instrumental for higher survival of *Bradyrhizobium*. However, both RH and CS biochar-based carriers could support *Bradyrhizobium* strain numbers up to Week 8. The low WHC of the RH carrier compared to that of the other carriers could mean that the viability of the bacteria was reduced as the moisture was reduced. This is in line with Ghazi (2017), who reported that elevated WHC of biochar increases survival of soil microbes.

The results of this experiment indicated that peat recorded viable *Bradyrhizobium* cell counts up to Week 24. This finding indicates that *Bradyrhizobium* was metabolically active in the peat throughout the study. A similar observation was reported by Mahdi et al. (2010), who stated that the suitability of various carrier materials for the development of biofertilizer is in the order of peat > lignite > charcoal > soil > RH.

4.3 | pH and moisture content of the biochar-based inoculants after inoculation

The optimum pH range of inoculants must be between pH 6.0 and 7.0 (Somasegaran & Hoben, 1994). From this study, even though all the carriers were adjusted to the recommended pH level (6.8), the inoculation of the carriers with *Bradyrhizobium* strains resulted in different pH values, which indicates that the activities of *Bradyrhizobia* in the carriers might have influenced the pH values of the carrier types. This confirms the study by Ghazi (2017), who obtainedimproved changes in pH readings for a carrier material and attributed it to the production of metabolites by the bacteria, which then changes the pH of carrier material. In addition, acidic or basic compounds may be released by bacteria cells after their death, which can reduce or increase the pH readings of the carrier material and subsequently decrease the viable rhizobium cell count (Somarathne et al., 2013). Furthermore, activities of bacteria to ensure their survival is of a major concern, which is in agreement with Bazilah et al. (2011), who observed that higher temperatures of 30˚C compared to lower temperatures (10˚C and 20˚C) may caused an increased number of dead bacteria, and this will in turn promote the production of waste by bacteria and thereby change the pH drastically.

Additionally, for standard quality assurance of biofertilizers, another parameter used for quality assessment of the biofertilizer other than viable cell count and pH is moisture content (Kaljeet et al., 2011). According to Deaker et al. (2004), moisture contents of 40%–50% proved optimal for growth and survival of a range of rhizobia strains prepared as peat cultures. In this study, all the carriers had favorable moisture content values during the initial assessments. However, loss of moisture was observed in all the biochar-based carrier types during the study period. Nonetheless, Kaljeet et al. (2011) reported that the percentage loss of moisture should not be too high to decrease strain viability. In a research by Dieker et al. (2011), it was reported that rhizobia survive best when changes in the moisturere status of cells are minimized. The findings of this study suggest that reduced moisture conditions of the carrier materials limited *Bradyrhizobium* uptake of certain essential elements that could have increased their mortality.

4.4 | Nodulation and grain yield response of biochar-based inoculants

The positive response of cowpea to biochar-based rhizobium inoculation supports the findings of other researchers, who reported an increase in nodulation after inoculation (Martins et al., 2003; Hungria & Bohrer, 2000; Katulanda, 2011; Albareda et al., 2009). This may be due to the relatively low indigenous cowpea rhizobia population at the study site. Houngnandan et al. (2000) reported that the response to inoculation is likely to occur when the indigenous rhizobia population is less than 5 or 10 rhizobia cells g^{-1} soil. Additionally, Ulzen et al. (2016) also reported significant increases in nodule number when indigenous rhizobia were less than 10 cells g^{-1} soil.

Carrier material may present differential responses to nodulation by rhizobia strains. These observations indicate that the formulation of rhizobium inoculants with various carrier substrates might have enhanced their colonization ability and effectiveness in terms of plant growth and development, as reported by several authors (Cho et al., 2015; Trivedi et al., 2005; Tamreihao et al., 2016). Similarly, Glodowaska et al. (2017) reported the significant effect of the physical properties of biochar on the survival of *Bradyrhizobium japonicum*. Hale et al. (2014) also indicated that biochar materials have physical and chemical properties, which might affect their ability to serve as a carrier and introduce the bacteria to the soil. As such, evaluation of carrier materials must be performed simultaneouslywith the selection of strain to increase the efficiency of rhizobia inoculants (Fernades et al., 2009).

The grain yield of cowpea did not vary significantly among the treatments. For successful inoculation that translates into yield, the introduced strains must be able to outcompete the native population in nodule occupancy. This was not the case in this study as the yield of the control plots did not vary significantly from the treated plots indicating that the indigenous rhizobia though low in numbers may have outcompeted the introduced strains thus affecting N_2 fixation and ultimately yields of cowpea. Other factors such as low soil fertility status particularly N and P mineral nutrients of the study site, which are major constraints limiting legume N_2 fixation and yield (O'Hara, 1988), could have contributed to the no differences in yields observed. Ulzen et al. (2016) demonstrated that rhizobia inoculation can be beneficial to cowpea productivity. This study, however, did not observe significant increase in grain yield with inoculation and therefore confirms the findings of other researchers (de Freitas et al., 2012; Singleton & Tavares, 1986; Sarkodie-Addo et al., 2006; Chemining'wa et al., 2007), who reported nonsignificant yield in inoculated cowpea trials.

5 | CONCLUSION

The study was conducted to test the hypothesis that biochar-based carriers have the potential of enhancing rhizobia survival and yield of cowpea. The findings indicate that biochar-based carriers enhanced the survival and sustained the densities of *Bradyrhizobium* strains but not yield of cowpea. The physical and chemical characteristics of biochar such as moisture and pH affected the initial rhizobia cell count and survival. Generally, CC biochar could be described as the promising carrier materials for sustaining and enhancing survival of *Bradyrhizobium* strains KNUST 1002, KNUST 1006, and BR 3267. The biochar-based rhizobia inoculants achieved between 57% and 81% of the potential yield of Padi-Tuya (2.2 t ha^{-1}) cultivar of cowpea in comparison to the uninoculated, even though it was not significant. Long term and multilocation studies are needed to elucidate the effectiveness of the rhizobia in the biochar-based carrier. The findings have implications for inoculant producers in SSA as it indicates that agricultural waste materials, which otherwise serve as environmental nuisance, can be converted into useful products to reduce the cost of inoculant production.

AUTHOR CONTRIBUTIONS

Winnefred Mensah: Funding acquisition; investigation; methodology; project administration; validation; writing—original draft; writing—review and editing. **Nana Ewusi-Mensah**: Data curation; supervision; writing—original draft; writing—review and editing. **Azumah Ayamah**: Data curation; formal analysis; methodology; supervision; writing—original draft; writing—review and editing. **Jacob Ulzen**: Methodology; software; supervision; writing—original draft; writing—review and editing. **Ophelia Osei Ulzen**: Methodology; supervision; writing—original draft; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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