Bindura University of Science Education

Evaluation of wheat (*Triticum aestivum*) straw and maize (Zea *mays*) stover as carrier material in Rhizobia inoculant production

BERTHA. BETHEL.CHINYANDURA

B210814B

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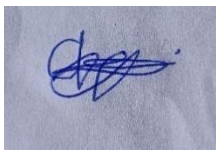
APPROVAL FORM

The undersigned certify that they have read the dissertation titled _Evaluation of wheat (*Triticumaestivum*) straw and maize (Zea *mays*) stover as carrier material in Rhizobia inoculant production.‘ and confirm that it is suitable for submission to the Biological Sciences Department, Faculty of Science and Engineering, for assessment.

Signature of student:  Date: 05 / 09 / 2025

Signature of Supervisor:  Date: 05/09/25

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DECLARATION

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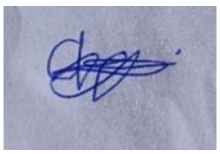
Signature:  Date: 05 / 09 / 2025

Supervisor

I, Mr. P. Munosiyei, declare that I have supervised this thesis and I am satisfied that it can be submitted to the Biological Sciences Department, Faculty of Science and Engineering, at Bindura University of Science Education.

Signature:  Date: 05/09/25

DEDICATION

I dedicate this work to my family.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

BNF : Biological Nitrogen Fixation

SPRL : Soil Productivity Research Laboratory

YEM : Yeast Mannitol Agar

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ABSTRACT

This study focused on the production of legume inoculants using the post-harvest waste product of maize and wheat straw. The aim of this study was to investigate the efficacy of maize stover and wheat straw as potential carrier materials in comparison to bagasse for the production of *Rhizobium* inoculant biofertiliser by evaluating their physiochemical properties and viable cell count. Carrier materials were characterized for some key physicochemical characteristics before the inoculation with the Rhizobia strain MAR 1495.Wheat straw exhibited poor physicochemical properties whereas bagasse and maize had good qualities. The survival of Rhizobia MAR 1495 strain was determined in carrier materials. The carrier materials were inoculated with the Rhizobia strain MAR 1495 and incubated at temperature ranges of 25-30°C for a week, followed by cold storage at 4°C for 28 Days. The viability of Rhizobia was monitored by sampling of the carrier materials every 7 days for 28 days where pH, viable cell count and spores were checked. A complete randomized design with 3 treatments replicated 12 times for each carrier material was used in this research. The study revealed that pH changes in carrier materials varied significantly over 28 days. While no notable differences were observed in the first two weeks (Days 7 and 14), significant variations ($P < 0.05$) emerged by Day 21 and persisted through Day 28. Bagasse and maize stover maintained similar, near-neutral pH levels (7.38–7.56), whereas wheat straw exhibited a consistently lower pH (6.69–6.79).The study revealed that while initial viable cell counts showed no significant difference ($p=0.586$) among carrier materials on Day 7, significant variations emerged from Day 14 onward ($p=0.004$, $p<0.001$, $p=0.002$). Bagasse and maize stover maintained comparable cell counts from Day 14 to Day 28, demonstrating better microbial stability. In contrast, wheat straw exhibited the lowest viable cell counts at Days 21 and 28, with a sharp decline coinciding with a drop in pH. In conclusion, maize stover and bagasse emerged as the most suitable carrier materials due to their favorable physicochemical properties such as neutral pH, high water-holding capacity, and sustained microbial viability and ability to sustain high viable cell counts. Wheat straw, while less effective, may still be viable with further modifications.

CONTENTS

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND OF STUDY

Organic waste recycling, a long-standing and advantageous idea that has been largely overlooked in recent years, has gained support in sustainable agriculture (Bellarby et al., 2014). Due to a number of variables, including increased fertilizer production that is closely linked to greenhouse emissions, conventional agricultural methods have recently been the primary cause of climate change, global warming, and environmental damage (Di Benedetto et al., 2017). On the other hand, increase in population has in the past decade increased demand for food by humans and their livestock. Most arable lands under crop production are not fertile enough to produce sufficient yields to meet the demands of the fast-growing population. It is, however, unfortunate that the imprudent, continuous usage of synthetic fertilizers and chemicals has turned to have some negative impacts on both the terrestrial and aquatic inhabiting organisms. This has therefore led to searches for alternative, sustainable measures to reduce synthetic fertilizer usage in the agricultural sector. Renewable energy is becoming a favourable alternative because fossil fuels have several negative effects on the environment.

Worldwide research is still ongoing to identify reliable alternative crop fertilization mechanisms that will promote sustainable production of agricultural produce, with soil microbiota drawing many microbiologists attention (Bender et al.; 2016). Soil microbiota is integral to the development of sustainable agricultural methods. Therefore, the rhizosphere of plants has been the center of attention for decades now, to create novel alternatives to the current synthetic fertilizer.

Numerous studies have investigated rhizobacteria, which are microorganisms that inhabit plant roots and the rhizosphere, for their ability to enhance plant growth through biochemical processes such as phytohormone production, siderophore release, phosphate solubilization, and biological nitrogen fixation, all aimed at boosting plant development and increasing yields (Vassey, 2003). Microorganism like *Rhizobium,* phosphate solubilizing bacteria and mycorrhizae are some of the rhizobacteria that have been successfully isolated from the rhizosphere of plants and used in formulation of beneficial biofertilizer for sustainable crop development using different carrier materials (Kumar and Patel 2018). Reban, (2008) defines biofertilizers as carrier-based inoculants or products containing effective microorganisms which when applied to soil, seeds or surface of plants colonize the rhizosphere or the internal tissues and induce plant growth to occur. Incorporation of microorganisms enables easy handling, long term storage and effectiveness of the biofertilizer. The classification of biofertilizers is based on the type of microorganisms to be incorporated into the carrier material of the fertilizer and these include microorganisms like algae, fungi and a very wide range of bacteria from different families.

Rhizobia are beneficial soil bacteria that form a symbiotic relationship with leguminous plants, enabling the plants to fix atmospheric nitrogen and convert it into a form that is easily utilized by plants (Baipai et al., 2014). As stated by Bharti et al., 2017, inoculating legume seeds such as *Glycine max (*soya beans) and *Pisumsativum* (peas) with rhizobia enhances nitrogen fixation, leading to improved plant growth, higher yields, and reduced reliance on synthetic fertilizers. Bashan (2014) and Tabassum (2017) affirmed that the application of biofertilizers has achieved global prominence, with the use of agricultural waste as carrier materials emerging as a preferred option due to their wide availability and cost-effectiveness. Nevertheless, the viability and efficacy of rhizobacteria inoculants in biofertilizer production remain heavily influenced by the type and quality of carrier material chosen by the producer

According to Kumar and Patel (2018), carrier materials are essential components of rhizobia inoculants, as they provide a suitable environment for the survival, storage, and effective delivery of rhizobia to the plant roots. In Zimbabwe historically, bagasse, a dry pulp byproduct from the crushing of sugar cane, was used and is still being used in the manufacturing of legume bio inoculants at the Soil Productivity Research Laboratory (SPRL), one of the organizations authorized to make legume bio fertilizer.

1.2 PROBLEM STATEMENT

Traditionally, carrier materials such as peat, vermiculate, and clay have been used in the production of rhizobia inoculants (Di Benedetto et al., 2017). However, these materials have limitations, including high cost, environmental concerns, and the potential for carrying pathogens. Bagasse is slowly becoming scarce to continuously sustain the bio fertilizer production as a result of its use as energy for boilers and electricity production in the south lowveld where it is produced. The higher demand and, cost in transportation and purchasing of bagasse from Chiredzi triangle to the SPRL research station for rhizobia production are major challenges to the effective production process

To date a number of carrier materials have been tried and tested and for most of them their physiochemical properties and viable cell count were not suitable for the survival and growth of the microorganisms. Therefore, intense researches to find the perfect material to develop stable, functionally reliable biofertilizer inoculants are being done globally (Shaikh, 2016). Sugarcane bagasse being one of the carrier materials approved to be effective, but little light has been shed on the usage of carrier materials from other members of the grass family. Maize stover and wheat straw are viewed as alternative carrier material for the production of bio inoculants that are environmentally friendly, but there has been no published studies evaluating their potential as carrier material for bio fertilizers. No sufficient published studies have been done on maize stover and wheat straw, two of the most abundantly available agricultural waste worldwide. Maize stover and wheat straw are viewed as alternative carrier material for the production of bio inoculants that are environmentally friendly, but there has been no published studies evaluating their potential as carrier material for bio fertilizers.

1.3 AIM

This study aims to investigate the efficacy of maize stover and wheat straw as potential carrier materials for the production of *Rhizobium* inoculant biofertilizer by evaluating their physiochemical properties and viable cell count.

1.4 OBJECTIVES

The objectives of this study are:

1. To determine the physiochemical properties of maize stover and wheat straw that may make them suitable carrier material in legumes biofertilizer production.
2. To determine the viable cell count produced when maize stover, wheat straw and bagasse are used as carrier materials.
3. To compare the physiochemical properties and viable cell count of maize stover, wheat straw and bagasse as carrier materials.

1.5 RESEARCH QUESTIONS

This study aims to answer the following questions:

1. What are the key physicochemical properties of maize stover and wheat straw that may make them suitable carrier material in legumes biofertilizer production?
2. What are the viable cell counts produced by when each of the three carrier materials (maize stover, wheat straw and bagasse) are used as carrier materials for biofertilizer production?
3. Which carrier material (maize stover, wheat straw or bagasse) demonstrates the best overall suitability (physiochemical properties and viable cell count) for legumes biofertilizer production?

1.6 HYPOTHESIS

H_0 there is no significance difference in water holding capacity,moisture inheretent and pH and viable cell count between maize stover, bagasse and wheat straw.

H_1 There is significance difference in water holding capacity,moisture inheretent and viable cell count between maize stover

1.7 SIGNIFICANCE OF THE STUDY

The findings of this project will contribute to the understanding of the suitability of wheat straw and maize stover as carrier materials in rhizobia inoculant production. This knowledge can inform agricultural practices and promote the development of sustainable and environmentally friendly approaches to enhance plant-microbe interactions and improve crop productivity. This study gives room for development of alternative carrier materials that are readily available in place of bagasse which will resolve the problem of over dependence on bagasse whose demand is now very high and now difficult to acquire from the supplier. Evaluating different carrier materials helps determine which materials provide optimal conditions for bacterial survival and viability. This information is essential for ensuring the effectiveness of rhizobium inoculants when applied to agricultural fields. Evaluating these materials helps explore their potential value as eco-friendly carrier options for rhizobium strains.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

Since they absorb inert nitrogen from the atmosphere and convert it into protein through rhizobia activity in a carbon-free manner, legumes are an important part of all agrarian systems worldwide and are especially appealing to low input systems of agriculture (Howieson and Dilworth, 2016).

The agricultural sector is increasingly recognizing the potential of the symbiotic association between leguminous plant roots and Rhizobium bacteria as a sustainable alternative to synthetic fertilizers (Arora et al., 2016). This relationship facilitates Biological Nitrogen Fixation (BNF), wherein Rhizobia convert atmospheric nitrogen into amino acids that are subsequently assimilated into plant proteins (Date and Roughley, 1977). Given that nitrogen-based fertilizers represent one of the highest input costs in agriculture, BNF offers a promising and cost-effective alternative. Consequently, the method is gaining traction as a viable substitute for conventional nitrogen-enriched fertilizers. However, rhizobia are not universally present in all soils, and even when present, their populations may be insufficient to ensure effective nitrogen fixation (Somasegaran and Hoben, 1992). To optimize this symbiosis, the application of reliable legume inoculants is essential for achieving successful nodulation. This process, known as ―inoculation,‖ involves introducing beneficial rhizobia to seeds or soil (Burton, 1979).

2.2 NATURE OF RHIZOBIA

The ability of the Rhizobiabacteria to form nodules on the top roots of some leguminous plants was the initial factor in their discovery and characterisation (Somasegaran and Hoben, 1992). Because the Rhizobia found in the nodules' leghemoglobin fix nitrogen, these nodules frequently led to increased plant growth. Burton (1982) also noted that the classification of Rhizobia was based on the ability of leguminous plants to induce nodulation, independent of nitrogen fixation. Rhizobia are rod-shaped, non-sporulating, aerobic to microaerophilic gram-negative bacteria that take three to eight hours to grow. Both solid and static liquid media surfaces can support the growth of these microorganisms, as long as there is a sufficient surface area for growth (Howieson and Dilworth, 2016). When producing bioinoculants, it is advised to grow in submerged culture in fermenters with aeration to maximise the production of viable cells (Burton 1984).

In the nodules of the leguminous plants, the enzymatic mechanism of the root nodulating bacteria, like *Bradyrhizobium*, converts atmospheric di-nitrogen (N2) to ammonia (NH3). Legumes typically have nodules on their roots, but they can also occasionally be found on their stems (Howieson and Dilworth, 2016). According to Chao (1984), the majority of Rhizobia bacteria in groundnuts, soy, and other nodulating legumes are located at the centre of the nodules in the green, red, or brown leghemoglobin. These motile, rod-shaped, pleomorphic bacteria are classified as slow-growing alkaline-producing bacteria and fast-growing acid-producing bacteria (Burton et al., 1984).

2.3 RHIZOBIUM SPECIES AND STRAINS

Rhizobia are a specialized group of soil bacteria known for their capacity to enhance legume growth through the formation of root nodules, a process termed nodulation. This symbiotic interaction is highly host-specific, as only Rhizobium strains compatible with a particular legume species can effectively induce nodule development. Due to the substantial agronomic and economic benefits associated with enhanced nitrogen fixation, rhizobial inoculants are commercially produced in many countries. These inoculants consist of rhizobia strains that have been isolated from plant nodules and subsequently cultured under laboratory conditions for mass application.

Optimal nitrogen fixation in legumes often requires specific strains of Rhizobia tailored to particular host species. Based on their growth rates, Rhizobium species are typically classified as either fast or slow growers (Burton, 1984). Fast-growing species such as *Rhizobium meliloti, R. trifolii, R. phaseoli*, and *R. leguminosarum* are commonly isolated from nodules on sesbania, leucaena, alfalfa, chickpea, and clover. These strains generally form visible colonies on solid media—such as yeast extract mannitol agar (YMA)—within 4 to 5 days of incubation at 28°C. In contrast, slow-growing strains like *Bradyrhizobium japonicum*, typically associated with cowpea and soybean nodules, require 6 to 8 days under the same conditions to produce visible colonies. Once isolated, these pure colonies are preserved in slant bottles under refrigeration for long-term maintenance of viable Rhizobial strain

2.4 *RHIZOBIUM* INOCULANTS AND NUTRITIONAL REQUIREMENTS

Formulations of advantageous microorganisms prepared with an appropriate and user-friendly carrier material are known as bio-inoculants (Burton, 1982). Rhizobia needs growth factors, minerals, and an energy source. Since sucrose is widely accessible, effective, and reasonably priced, it is one of the most popular energy sources for carbon for rapidly growing rhizobial strains. Rhizobium strains within a species can vary in their capacity to use carbohydrates, according to Graham and Parker (1964). Thus, it's critical to confirm that the chosen Rhizobium strains are able to use the fermenter medium's carbohydrate. Mannitol and glycerol are preferred by certain manufacturers as energy sources for Rhizobium production. According to Burton (1984), pentose and hexose sugars like arabinose or xylose are preferred by slow-growing Rhizobia. Sucrose-induced growth in submerged culture was found to be comparable to that of other sugars or glycerol (Howieson and Dilworth, 2016).

Several factors, including the composition of the basal medium, the nitrogen source, oxidation, reduction potential, sterilization method, and inoculum size, affect the ability of microorganisms to metabolize specific carbohydrates (Reban et al., 2002). Although most Rhizobia can assimilate nitrogen in the form of ammonium or nitrate ions, their growth is generally enhanced in media enriched with low molecular weight amino acids, such as those derived from plant extracts, yeast, alfalfa, cabbage, wheat straw, maize steep liquor, and hydrolyzed casein (Burton, 1982). *Rhizobium spp*. typically require a pH of 6.0 to 7.5, a temperature of 25–30°C, and access to particular nutrients, including root exudates and nitrogen-free media supplemented with specific carbohydrates. In contrast, *Pseudomonas fluorescens* strains thrive under slightly different but overlapping conditions, favoring a pH of 6.5 to 7.5, temperatures between 25–30°C, and nutrients such as sucrose, peptone, and trace amounts of salts.

2.5 CARRIER MATERIALS

For centuries, microbial inoculation has been used to improve crops. According to Brahmaprakash et al. (2012), carrier materials that promote microbial growth and efficiently transport the microbes to the rhizosphere improve the effectiveness of inoculants. A solid or liquid-based medium called carrier material is used to move live microorganisms from the lab to the rhizosphere of plants while giving microbial inoculants a favourable environment for growth and development (Howieson and

Dilworth, 2016). Because of their long-term preservation and ease of handling, carrier-based bacterial inoculants, also known as biofertilizers, are very effective (Fred et al., 1932).

According to Gade et al. (2014), carriers could come from organic sources like compost, biogas slurry, crushed maize cob, biochar peat, etc. When choosing which carrier types to use during the production of inoculations, the availability and cost of these carriers are crucial considerations. Bagasse, the fibrous residue left over after sugarcane juice is extracted, is a potential carrier material with a variety of agricultural applications. Its natural moisture content ranges from 40 to 50 percent, though processing methods may alter this. For optimal handling and storage, bagasse may need to be dried to a moisture content of 10–15%. Despite lacking essential nutrients, bagasse can be improved with additions such as potassium and phosphorus. Bagasse is helpful for maintaining soil moisture and encouraging the growth of beneficial microorganisms due to its relatively high water-holding capacity, which is approximately two to five times its dry weight in water. Bagasse typically has a pH of 6.0 to 8.0, which is neutral to slightly acidic and promotes plant growth and microbial activity. All things considered, bagasse's many benefits make it an excellent choice for enhancing soil quality and encouraging sustainable farming practices. Due to the growing scarcity of bagasse, maize stover and wheat straw are being considered as carrier materials.

2.6 INOCULANT STRAIN SELECTION

In order to efficiently infect the root hair cells and provide an abundance of nitrogen to the crops, legume inoculants—preparations of live Rhizobium bacteria—are applied to the seeds of leguminous plants or directly into the soil (Somasegaran and Hoben, 1992). These inoculants are designed especially for seeds because they are the simplest and most practical way to contract the radicle, infect the seed as soon as it begins to germinate, and encourage the formation of nodules (Burton, 1982 and Somasegaran et al., 1982).

One of the most crucial stages in the production of legume inoculants is strain selection because the selection procedure ultimately determines the strain's efficacy (Burton, 1982). Burton (1979) states that the potential inoculant manufacturer should use Rhizobium cultures that are already available and have been proven to be effective on the legumes for which they will create inoculants. These cultures can also be used as benchmarks to assess novel strains or isolates. The following criteria are taken into consideration when choosing strains before inoculant production, per Somasegaran and Hoben's (1992) Handbook of Rhizobia: the strain's capacity to grow well in media and to produce efficient N-fixing nodules on the legume plant for which the inoculum is advised Additionally, the strains should be able to increase leguminous crop yields in a variety of soil types and unfavourable weather conditions. In addition to being highly persistent, effective strains should have a high capacity for competitive infection and soil population growth. According to Howieson and Dilworth (2016), good strains should be able to tolerate soil stressors such as high levels of manganese and aluminium, as well as a variety of host genotypes or cultivars.

When there are many other highly contagious native strains in the rhizosphere, the Rhizobium strain that can infect and dominate the formation of nodules on a specific host is regarded as highly competitive (Chao, 1984). The most widely used inoculants are seed inoculants, though it is not always possible to apply them directly to the seeds because they are frequently coated in chemicals that are toxic to Rhizobia and are meant to protect them from insects, pests, and diseases. Rhizobia applied to seed planted in hot, dry soils may die before the seeds germinate, despite the fact that it may seem simple and convenient to apply the inoculum. Large peat granules can therefore be used to directly add inoculants to the soil; however, peat can be somewhat costly, and most soil inoculations require specialised knowledge and equipment in addition to large amounts of inoculum (Burton, 1982).

According to Somasegaran (1994), seed inoculants for legumes come in a variety of forms, such as liquid or broth, bottles or agar slants, peat or powdered solid base, oil-fried form, lyophilised or freeze-dried powder, and polyacrylamides. The most dependable inoculum is one that is based on peat or moist powder (Burton, 1979). But the only way to determine a legume inoculant's actual quality is to see how well it works in the soil and climate in which it will be applied. Similar-looking inoculants can differ significantly in how many live, effective Rhizobia they produce and, as a result, how well they induce nodulation (Kumar, 2011). Peat and biochar as carrier materials for the production of legume inoculants have been the subject of the majority of recent studies. The most popular solid carrier for creating legume inoculants is peat. Because Rhizobia in peat carriers stay viable longer on the seed and in the package, it is also the most reliable (Howieson and Dilworth, 2016). However, many countries lack access to high-quality peat, and because it is somewhat expensive, it is not economically viable for large-scale production (Chao, 1984).

Coriander husks, rice husks, wheat bran, sugarcane bagasse, compost manure, and agricultural wastes are some of the materials that have also been utilised (Goyal et al., 1966 and Arora et al., 2016). Although the quality of the carrier material cannot fully reveal much about its ability to keep the inoculum cells viable, it has been useful to characterise these materials to check their ability to house Rhizobia. Only after the viable inoculum has been placed in the material and allowed to grow under careful observation for at least six months can it be determined whether the carrier quality is good (Date and Roughley, 1977).

In selecting suitable carrier materials for the production of legume inoculants, several critical characteristics must be considered. These include high absorption capacity, ease of drying and grinding, non-toxicity to Rhizobia, absence of abrasive minerals, low soluble salt content, ease of sterilization, and consistent availability at a reasonable cost (Somasegaran & Hoben, 1994). A variety of substances—such as bagasse, sugarcane filter mud, coir dust, coal, lignite, charcoal, various compost blends, clays, and minerals like apatite and vermiculite—have been extensively studied and deemed appropriate for this purpose (Burton, 1979; Paczkowski & Berryhill, 1979).

CHAPTER 3: MATERIALS AND METHODS

3.1 STUDY SITE

This study was carried out at the Soil Productivity Research Laboratory, located at the Grasslands Research Institute in Marondera in Mashonaland East Province of Zimbabwe, 68km to the south east of Harare because that is where bagasse is received from Chiredzi. The research station is in natural region II of Zimbabwean ecological regions. The area is characterized by acidic deep brown fine loamy, kaolinitic thermic soils derived from granite.

3.2 SOURCE OF RESEARCH MATERIAL

Maize Stover was collected from a harvested field at Grasslands Research institute in Marondera for free. Wheat straw was collected from Kudenga farm in Marondera. Sugarcane bagasse was purchased and transported from Chiredzi, at one of the sugarcane milling companies.

Figure 1. Harvested maize residue stalks

Figure 2. Harvested wheat straw

3.3 EXPERIMENTAL DESIGN

A completely randomized design (CRD) with 3 treatments replicated 12 times for each carrier material was used in this research. Bagasse, wheat straw and maize stover are the treatments. Bagasse carrier material was the control. The research was done under controlled temperatures. Random selection of three packets on each carrier material was done and checked for pH and viable cell count.

3.4 RESEARCH PROCEDURE

Before inoculation with Rhizobia (MAR 1495), inherent moisture content and water holding capacity of wheat straw and maize stover were determined using the gravimetric method. Rhizobia (MAR 1495) was introduced into all the carrier materials.

3.4.1 CARRIER MATERIAL PREPARATION

The long maize stover from mature and dry maize and wheat straw together with their leaves and ears were separately hand chopped into smaller pieces to allow them to dry up before the milling process. These small pieces were sun and oven dried and as soon as they dried up, they were subjected to grinding by an electrical grinder. After grinding each of the carrier powdery material products was sieved through a 2mm sieve to get a finer carrier material. The larger residues after sieving were ground again until they produced fine powder that could pass through the 2mm sieve. Sugarcane bagasse that was already milled upon purchasing was also sieved using the 2mm sieving dishes and the coarse material that did not pass through the sieve was disposed.

3.4.2 CARRIER MATERIAL CHARACTERIZATION

3.4.2.1 PH ANALYYSIS OF CARRIER MATERIALS

Ten grams of each carrier material were suspended in 200 mL of distilled water in 400 mL glass beakers. The mixtures were stirred using a magnetic stirrer while monitoring the pH with a calibrated pH meter electrode. According to Somasegaran and Hoben (1992), the optimal pH range for inoculant carriers is between 6.5 and 7.0. If the pH fell below 6.5, finely powdered calcium carbonate ($CaCO_3$) was gradually added under continuous stirring until a pH of 6.5 was achieved. The amount of $CaCO_3$ required to neutralize each carrier was recorded and used to extrapolate dosage requirements for large-scale production.

3.4.2.2 DETERMINATION OF INHERETENT MOISTURE LEVELS OF CARRIER MATERIALS

A drying oven was used to measure the inherent moisture level of each carrier material and the weight was recorded. Ten grams of the ground carrier material was carefully weighed on a pre weighed weighing dish and placed in the oven at 70°C for 24 hours. The carrier materials were weighed and returned to the oven. Another weighing at 48h was done to confirm the end point of moisture loss

To calculate the inherent moisture the formula below will be used

$$Inherent\ moisture = \frac{(W1 - W2)}{W1} \times 100$$

Where W1 = Weight of carrier before drying

W2 = Weight of carrier after drying at 70^0C

3.4.2.3 DETERMINATION OF WATER HOLDING CAPACITY OF CARRIER MATERIALS

To determine the water holding capacity of the carrier materials, 50 g of oven-dried carrier was placed in a 500 ml beaker, followed by the gradual addition of 200 ml of water with continuous stirring until saturation was achieved, resulting in a thin slurry. This slurry was then transferred to a pre-weighed measuring cylinder fitted with a drainage hole at the base, covered by a sieve. The setup was left to drain overnight. The final weight of the measuring cylinder containing the saturated carrier material was recorded, and the water holding capacity was calculated using the formula provided by Somasegaran and Hoben (1994) below.

$$Water\ holding\ capacity = \frac{(W3 - W4)}{W2} \times 100$$

Where:

W2 = Weight of carrier after drying at 70^0C W3

= Weight of the drained slurry and beaker

W4 = Weight of the beaker.

3.4.3 PRIMARY PRETREATMENT OF DRIED CARRIER MATERIAL

Initially pH of each carrier materials was taken. Salts, lime and water were added and mixed in a cement mixer for around 4 minutes according to the Handbook for Rhizobia Bagasse-proportions Somasegaran et al., 1992. 3 kilograms of each of the three carrier materials (maize stover, wheat straw and bagasse) was carefully placed into a small rotating mixer individually. The maize stover, bagasse and wheat straw separately were carefully mixed with mineral salts (Mg_2SO_4, K_2HOPO_4 and $NaCl_2$), and water and lime was used for pH adjustment of 6.5 to 7.. Mineral solution of magnesium sulphate, di-potasium orthophosphate and sodium chloride was added to the carrier media to attain the recommended moisture content of about 20% to 30% (Burton, 1979).

Quality control was performed at many different stages of the process, the first was done to check for the purity of the Rhizobia culture before its introduction into the broth media. The bacteria from the agar slant bottles were plated onto Yeast Mannitol Agar (YMA) with Congo red indicator. Presence of contaminants in the Rhizobia culture was seen by absorption of the indicator. The contaminated cultures were sent for further sub-culturing until a pure strain was obtained (Somasegaran and Hoben, 1992).

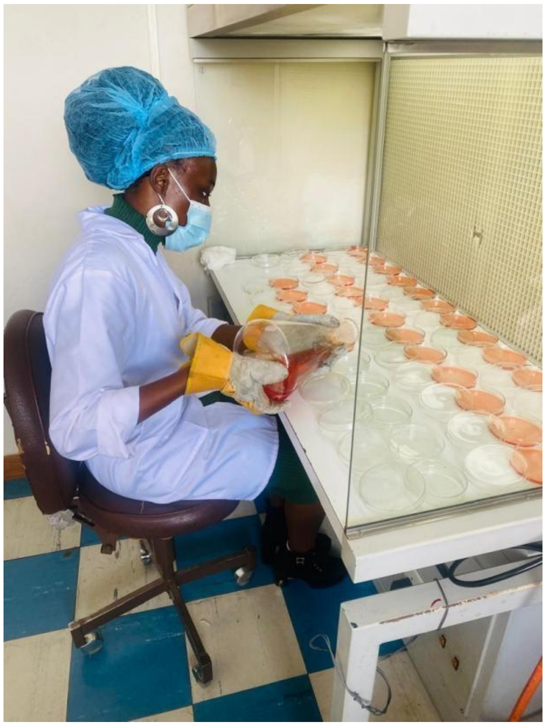

Figure 3. Pouring of agar into plates

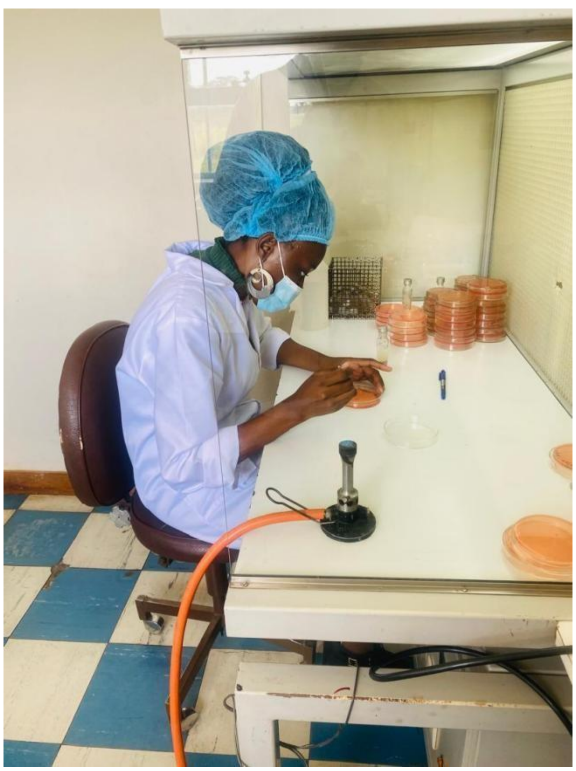

Figure 4. Bacteria from the agar slant bottles being plated onto Yeast Mannitol Agar (YMA) with Congo red indicator

3.4.4 PACKAGING AND STERILIZATION

Packaging of the carrier material was done following the Standard Operating Procedure (SOP) booklet.

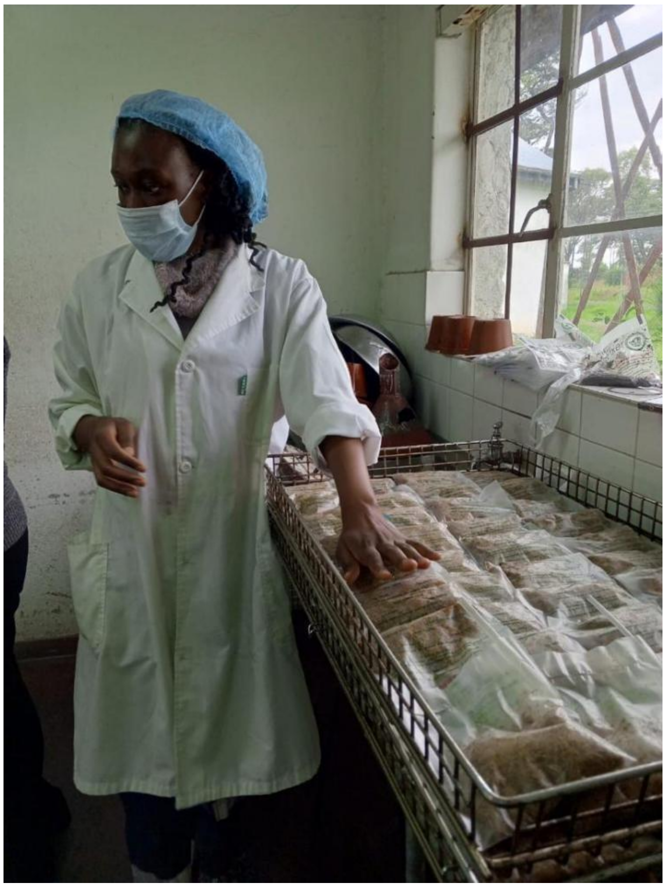

Figure 5. Packed carrier materials arranged in a basket

Using small pre-weighed containers, 85-86g of the carrier was weighed and put into a funnel on a tripod stand, for easy transfer of the carrier into high density polyethylene 100g sachets. These were further partially heat sealed, leaving a1.5cm gap at the top corner using a heat-sealing machine. The sachets were then sent to the next section where straw insertion into the partially sealed carrier material sachets was done.

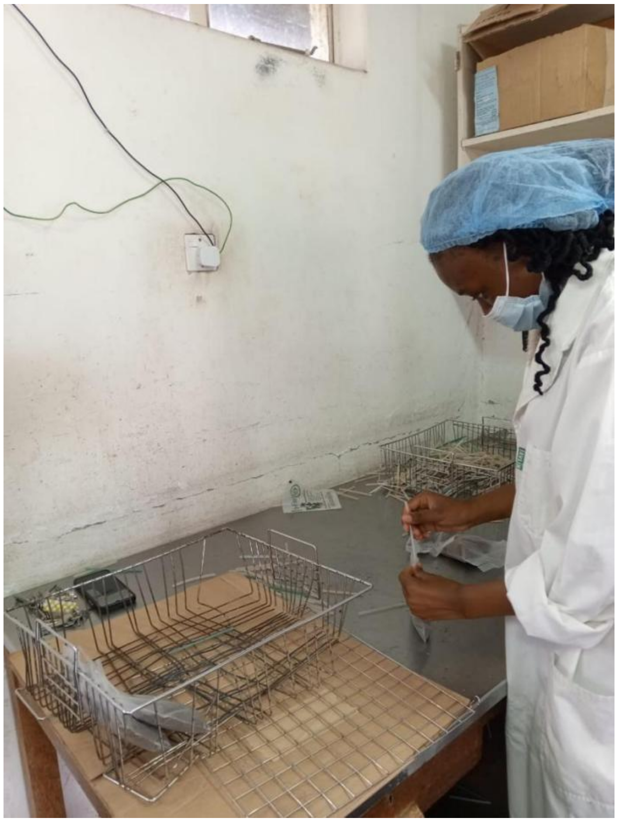

Figure 6. Insertion of straw into the partially sealed packet of bagasse

The straws inserted facilitated gaseous exchange and prevented the packets from bursting during the sterilization stage. The packets were; left to stay overnight so as to allow any bacterial and fungal spore that maybe present in the carrier to germinate. After this, the packets with carrier material were sterilized by autoclaving at 121^0C for an hour. Immediately after sterilization, the packets were resealed whilst they were still hot so as to minimize contamination. Contaminated packets that were not properly sealed were discarded after the overnight cooling process. The carrier packets were placed under ultraviolet (UV) sterilization to make sure that any microorganisms and other live contaminants that may have survived autoclave sterilization would be completely eliminated (Burton,1979). After UV sterilization the carrier packets were ready for inoculation.

3.4.5 INOCULATION OF THE CARRIER MATERIALS WITH RHIZOBIA

Before handling the packets for inoculation, hands were sterilized using 70% ethanol to minimize contamination. The packets were individually surface sterilized using damp cotton wool saturated with 95% ethanol, then immediately passed onto inoculation. Using an automated syringe gauged at 5mls, fitted with a long stainless-steel needle that can be flame sterilized, 15mls of the inoculum was injected into each of the packets of maize stover, wheat straw and bagasse. Immediately after injecting the inoculum into the carrier material, the hole left by the hot flame sterilized needle during the inoculation process was sealed using a quick flamed adhesive waterproof sticker label with the name of the inoculated strain. After the whole inoculation process, the packets was orderly packed into shallow meshed metal baskets labeled with the legume name that is soya bean, Rhizobium strain and number, dates of inoculation and grading after completion of the incubation period. After inoculation and incubation of the rhizobia inoculum into the three types of carrier material quality control for the viability of the Rhizobia was checked by performing serial dilutions after 1week, 2weeks, 3 weeks and 1 month using the Miles and Misra method as stated in Somasegaran and Hoben (1992). The YMA with Congo red plates was divided into eight sections, labelled with two major sections per plate–the 10^6 and 10^7.

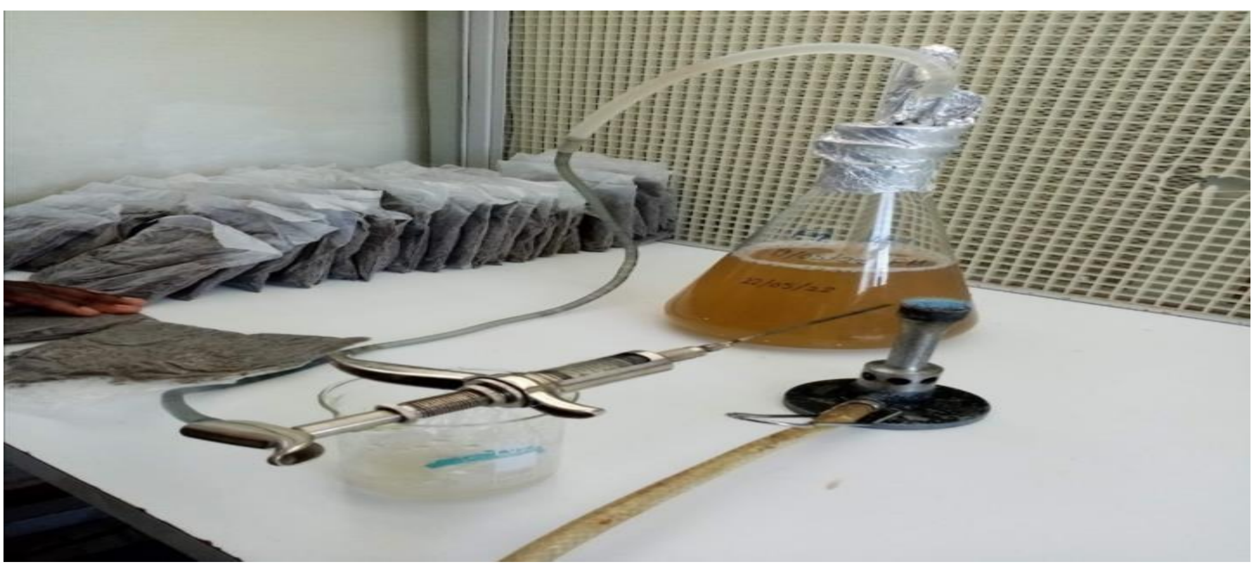

Figure 7. Small scale fermenter for rhizobia inoculation setup in a laminar flow cabinet

3.5 DATA COLLECTION

Three sachets of each carrier material was cut open and checked for *Rhizobi*a cell count, spores and pH from 7 days, 14 days, 21 days and 28 days from each treatment.

3.6 DATA ANALYSIS

One-way Analysis of Variance (ANOVA) was conducted using GenStat (18th edition) to compare pH values and viable cell counts across three carrier materials: bagasse, wheat straw, and maize stover. Mean separation was performed using Fisher's Protected Least Significant Difference (LSD) test at a 5% level of significance. Additionally, comparisons were made regarding the water holding capacity and inherent moisture content among the three materials

CHAPTER 4: RESULTS

4.1 PHYSICOCHEMICAL PROPERTIES OF CARRIER MATERIALS

The physicochemical properties of maize stover, wheat straw and bagasse obtained are provided in Table 4.1.

Table 1. Physicochemical properties for carrier materials characterization.

Carrier type	Initial pH	Moisture content average / %	Water holding capacity average / %
Maize stover	5.66	1.72	72
Wheat straw	3.5	2.02	50
Bagasse	4.70	3.6	69

Carrier materials characterization observations showed that wheat straw had the lowest pH of 3.5 compared to maize stover that had 5.66 and bagasse had 4.70 (Table 4.1). In contrast to the other aforementioned parameters measured, bagasse presented a higher inherent moisture content (3.6 %) compared to wheat straw (2.02 %) and maize straw (1.72%) (Table 1). Maize stover had good water retention qualities of 72% as compared to bagasse 69% and wheat straw 50 % as seen in table 4.1. Basically in comparison to bagasse maize stover had better physicochemical properties and wheat straw had poor qualities.

Table 2: Summary statistical table of pH of the possible carrier over a period of 28 days

Treatment	Day 7	Day 14	Day 21	Day 28
Bagasse	7.32	7.35	7.559^{b}	7.50^{b}
Maize	7.18	7	7.382^{b}	7.48^{b}
Wheat	6.41	6.79	6.687^{a}	6.48^{a}
P-value	**0.284**	**0.531**	**0.02**	**0.019**
Grand mean	**6.97**	**7.05**	**7.209**	**7.15**
lsd	**1.35**	**1.169**	**0.5607**	**0.7**
se	**0.676**	**0.585**	**0.2807**	**0.351**
CV%	**9.7**	**8.3**	**3.9**	**4.9**

There was no significant difference (P>0.05) in the pH between the possible (wheat straw and maize stover) and ideal (bagasse) carrier materials on Day 7 and Day 14. The pH in wheat straw dropped from the initial value of 6.41 and 6.79 at Day 7 and Day 14 respectively. There was significant difference in pH at Day 21 (P=0.02) between the different carrier materials. Bagasse and maize stover had almost the same pH ranging between 7.38 and 7.56. Wheat straw had the lowest pH of 6.69. At Day 28 there was significant difference between the pH (0.019), with bagasse and maize having almost the same pH.

4.2 THE VIABLE CELL COUNT PRODUCED BY MAIZE STOVER, WHEAT STRAW AND BAGASSE AS CARRIER MATERIALS.

The viable cell count from bagasse, wheat straw and maize stover studied over 28 days are shown in Table 4.2.

Table 3: Summary statistical table of viable cell count (10^9) of the possible carrier over a period of 28 days

Treatment	Day 7	Day 14	Day 21	Day 28
Bagasse	0.862	0.823^{a}	6.32^{b}	4.126^{b}
Maize	0.819	0.795^{a}	5.84^{b}	4.25^{b}
Wheat	0.726	1.61^{b}	0.6^{a}	3.59^{a}
P-value	**0.586**	**0.004**	**<0.001**	**0.002**
Grand mean	**0.802**	**1.076**	**4.25**	**3.988**
lsd	**0.3151**	**0.393**	**0.702**	**0.2645**

se	**0.1577**	**0.1969**	**0.351**	**0.1324**
CV%	**19.7**	**18.3**	**8.3**	**3.3**

There is no significant difference (p=0.586) in the viable cell counts between the possible and the known carrier materials on Day 7 with a grand mean of 8.02 x (10^8). There was significant difference between viable cell count at Day 14, Day 21 and Day 28 with P-values of 0.004, <0.001 and 0.002 respectively. From Day 14 to Day 28 Bagasse and maize had almost the same viable cell count. Wheat straw had the lowest cell count at Day 21 and Day 28

The viable cell count of wheat staw dropped greatly from Day 21 to Day 28 as the pH also dropped within the same days.

CHAPTER 5 : DISCUSSION, SUMMARY, RECOMMENDATION AND CONCLUSIONS

5.1 DISCUSSION

5.1.1 : PHYSICOCHEMICAL PROPERTIES OF CARRIER MATERIALS

The initial physicochemical characterization revealed distinct differences among the carrier materials (Table 4.1). Maize stover exhibited the highest pH (5.66), followed by bagasse (4.70) and wheat straw (3.5). The neutral to slightly acidic pH of maize stover and bagasse aligns with the optimal pH range (6.0–7.5) for most plant-growth-promoting rhizobacteria (PGPR), which are critical for biofertilizer efficacy (Bashan et al., 2014). In contrast, the low pH of wheat straw (3.5) may inhibit microbial survival, as acidic conditions can disrupt cell membranes and metabolic activity (Malusá et al., 2012).

Moisture content and water-holding capacity are crucial for microbial survival in carrier materials. Bagasse had the highest inherent moisture content (3.6%), while maize stover demonstrated superior water retention (72%) compared to wheat straw (50%). High waterholding capacity is essential for maintaining microbial hydration and nutrient diffusion, which are vital for prolonged microbial viability (Trivedi et al., 2005). The results suggest that maize stover and bagasse are more suitable than wheat straw due to their ability to retain moisture, a key factor in biofertilizer storage and application (Herrmann &Lesueur, 2013).

5.1.2. PH DYNAMICS OVER TIME

The pH of all carrier materials increased over the 28-day incubation period (Table 4.2). Bagasse and maize stover maintained near-neutral pH levels (7.32–7.56 by Day 21), while wheat straw remained more acidic (6.41–6.73). The rise in pH may be attributed to microbial activity, such as ammonia production during organic matter decomposition (Gaind et al., 2006). Statistical analysis indicated significant differences ($p < 0.05$) in pH by Day 21 and Day 28, with bagasse and maize stover showing similar trends. The stability of pH in these materials supports their suitability as carriers, as extreme pH fluctuations can stress microbial populations (Vassilev et al., 2015).

5.1.3 VIABLE CELL COUNTS AND MICROBIAL SURVIVAL

Viable cell counts (Table 4.3) revealed critical trends in microbial survival. By Day 21, bagasse and maize stover showed substantial increases in viable cells (6.32×10^9 and 5.84×10^9 CFU/g, respectively), while wheat straw lagged (0.6×10^9 CFU/g). The low performance of wheat straw may be linked to its acidic pH and inferior water retention, which are suboptimal for microbial proliferation (Bashan et al., 2014). The high cell counts in bagasse and maize stover correlate with their favorable physicochemical properties, underscoring their potential as effective carriers.

The decline in viable cells by Day 28 (e.g., bagasse: 4.12×10^9 CFU/g) suggests nutrient depletion or accumulation of inhibitory metabolites, a common challenge in carrier-based inoculants (Herrmann &Lesueur, 2013). Nevertheless, the counts remained above the minimum threshold (10^6–10^7 CFU/g) required for biofertilizer efficacy (Malusá et al., 2012).

The findings demonstrate that maize stover and bagasse are promising carriers for biofertilizer production, offering stable physicochemical conditions and robust microbial support. These results contribute to sustainable agricultural practices by identifying low-cost, renewable materials for microbial inoculant formulations.

5.2 SUMMARY

Maize stover and bagasse emerged as the most suitable carrier materials due to their favorable physicochemical properties such as neutral pH, high water-holding capacity, and sustained microbial viability and ability to sustain high viable cell counts. Wheat straw, while less effective, may still be viable with further modifications. Future research should explore composite carriers or amendments to enhance microbial survival during storage.

5.3 RECOMMENDATIONS

Based on the findings, the following recommendations are proposed for future research and practical applications. Maize stover and bagasse should be prioritized as carrier materials for biofertilizer production due to their optimal pH, water retention, and ability to support microbial growth. Wheat straw could be improved through pre-treatment methods, such as pH adjustment or moisture enhancement, to increase its suitability as a carrier. Investigate the long-term stability of carrier materials beyond 28 days to assess their durability and performance under field conditions. Explore the impact of environmental factors (e.g., temperature, humidity) on the physicochemical properties and microbial viability of carrier materials. Develop standardized protocols for the preparation and storage of carrier materials to ensure consistency in biofertilizer quality. Conduct field trials to validate the efficacy of biofertilizers produced using these carrier materials in legume cultivation. Increase sample sizes and replicate experiments to enhance the robustness of statistical conclusions. Include additional parameters, such as nutrient content and microbial diversity, to comprehensively evaluate carrier material performance. By addressing these recommendations, future studies can optimize the use of agricultural residues as carrier materials, contributing to sustainable biofertilizer production and improved legume crop productivity.

5.4 CONCLUSION

In conclusion, maize stover and bagasse emerged as the most suitable carrier materials due to their favorable physicochemical properties such as neutral pH, high water-holding capacity, and sustained microbial viability and ability to sustain high viable cell counts. Wheat straw, while less effective, may still be viable with further modifications. Future research should explore composite carriers or amendments to enhance microbial survival during storage.

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