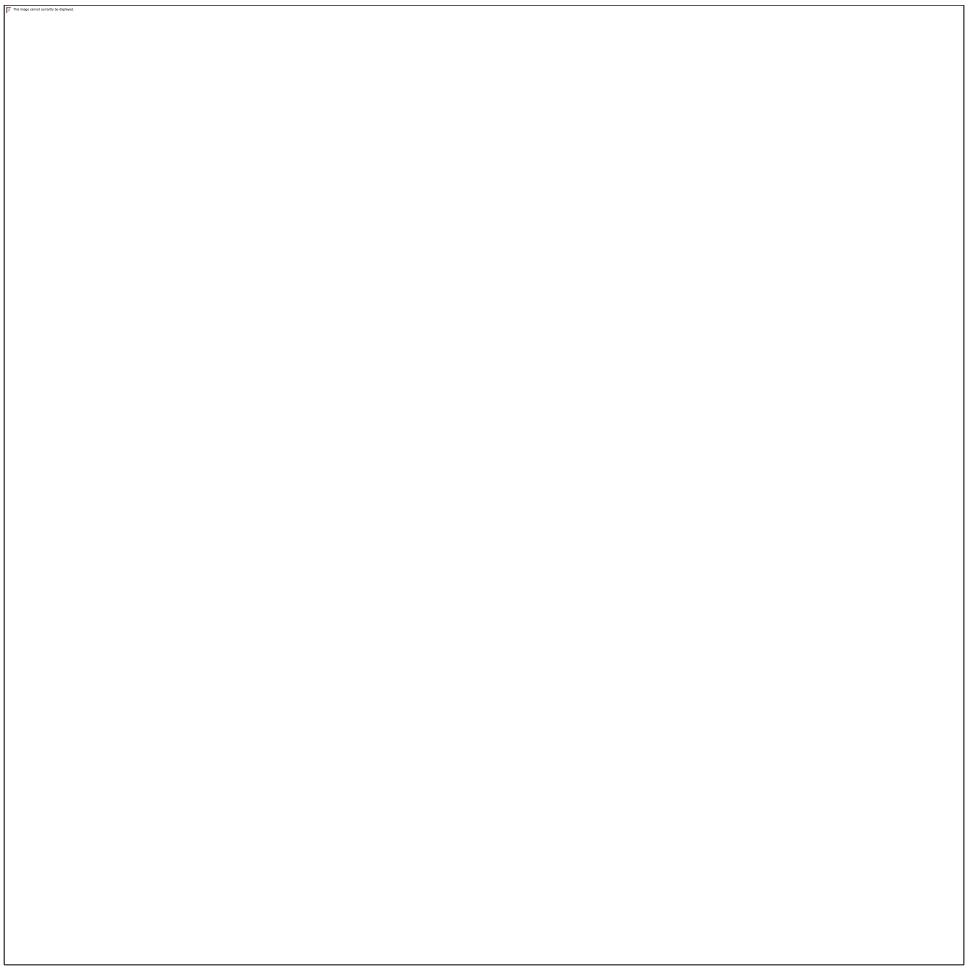

THE EFFICACY OF *RHIZOBIUM* (*LEGUMINOSARUM* BV *TRIFOLLII*) ISOLATES ON THEIR SYMBIOTIC RELATIONSHIP IN SUGARBEAN (*PHASELOUS VULGARIS*) PRODUCTION.

BY

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B213285B

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APPROVAL FORM

Title of dissertation: **"THE EFFICACY OF *RHIZOBIUM* (*LEGUMINOSARUM* BV *TRIFOLLII*) ISOLATES ON THEIR SYMBIOTIC RELATIONSHIP IN SUGAR BEAN (*PHASEOLUS VULGARIS*) PRODUCTION."**

The undersigned certify that they have read the dissertation and it is suitable for submission to the Faculty of Science, and checked for conformity with the Faculty.

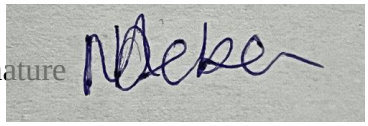
Signature of student  Date: **20/06 /25**

Signature of Supervisor …E. Z……. Date: **20/06 /25**

Signature of Department Chairperson:  Date: 09/10/25

DECLARATION

I DEKE MIRANDA (B213285B) declare that this research herein is my own work and has not been plagiarized from another source(s) without acknowledgement of the concerned author(s) either electronically or otherwise.

Signature  Date

Signature of Supervisor …. E.Z…. Date: **20/06/25**

Signature of Department Chairperson:  Date: 09/10/25

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DEDICATION

I dedicate this work to my mother, Mrs Deke and my siblings Rejoice, Yolanda and Kudakwashe Deke. I did it for you my dearest and compassionate family.

LIST OF ABBREVIATIONS

1. **BNF** - Biological Nitrogen Fixation
2. **SPRL** - Soil Productivity Research Laboratory
3. **RCBD** - Randomized Complete Block Design
4. **ANOVA** - Analysis of Variance
5. **LSD** - Least Significant Difference
6. **CIAT** - Centro Internacional de Agricultura Tropical
7. **NPK** - Nitrogen, Phosphorus, Potassium

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ABSTRACT

Legumes and *Rhizobia* have a mutualistic connection that is essential to sustainable farming methods, especially when it comes to increasing crop yield and soil fertility. The effectiveness of isolates of *Rhizobium leguminosarum bv. trifolii* in enhancing sugar bean *(Phaseolus vulgar*is) growth and yield is examined in this work. Under controlled settings, the main goal is to assess how different isolates affect nodulation and overall plant performance.Eight treatments were used in completely randomized block design (RCBD) greenhouse trials. Before being planted, sugarbean seeds were treated with several isolates of R. *leguminosarum*, and a negative control group was not inoculated. A commercial strain was given to a positive control group. To guarantee statistical correctness, the experimental setting contained replications three times. Nodulation was one of the primary criteria evaluated, where its quantity and shape results indicated significant differences among the *R. leguminosarum* isolates regarding their symbiotic efficiency. Some isolates led to a marked increase in nodulation, which positively correlated with enhanced plant growth. Furthermore, plants inoculated with effective isolates exhibited improved root length, plant height indicating better photosynthetic performance and resilience against biotic stressors .For sustainable agriculture, the mutualistic relationship between *Rhizobia* and legumes is essential.These results highlight how crucial it is to choose the right *Rhizobium* strains in order to maximize crop production. Different isolates varying levels of effectiveness raise the possibility that certain strains are more adapted to particular soil types or environmental circumstances. This study emphasizes how biological nitrogen fixation may be used as a sustainable substitute for artificial fertilizers, thereby improving soil health and lessening environmental impact.In conclusion, strengthening the symbiotic interaction between isolates of *Rhizobium leguminosarum bv. trifolii* and sugar beans offers a practical way to raise crop yields and encourage sustainable farming methods. Future inoculation plans can benefit from the knowledge gathered from this study, which will support sustainable growth in legume farming and food security.

CHAPTER 1

INTRODUCTION

1.0 Background of the study

Gram-negative soil-borne bacteria called *Rhizobium* contain a number of advantageous strains that aid in biological nitrogen fixation (BNF) in legume crops. Its capacity to establish a symbiotic association with legumes for biological nitrogen fixing makes this species unique. Frans (2015) asserts that *Rhizobi*a fix nitrogen to support the development of legumes. Nitrogen is a gas that makes up 70% of the air and is useless to plants. Nitrate or ammonium (NH4+) are the two ionic forms of nitrogen that plants require. Only *Rhizobia* can do this process. In many areas, the production of sugar beans (*Phaseolus vulgaris*) is crucial for both food security and revenue generating. The nitrogen fixation process, which improves plant development and yield, depends heavily on rhizobium bacteria (Somasegaran, 2012). Nonetheless, the native *Rhizobium*'s symbiotic effectiveness

In many parts of the world, sugar beans is a staple meal that provides millions of people with vital minerals and protein. It is essential for food security since it is grown in a variety of agro-ecological zones (FAO, 2021). However, soil fertility is a common problem for sugar bean cultivation, particularly in regions where nutrient depletion has resulted from conventional agricultural methods. Farmers can increase crop yields and improve soil health by utilizing *R. leguminosarum's* innate capacity to fix nitrogen (Vance, 2019).

According to research, rhizobial strains' efficacy can differ greatly depending on a number of variables, such as the strain's suitability for the host plant, the surrounding environment, and the properties of the soil (Norton et al., 2018). For example, adequate nitrogen fixation, which directly affects sugar bean development factors including plant height, biomass, and pod output, depends on efficient nodulation. Research has indicated that the efficacy of distinct *R. leguminosarum* isolates in fostering certain development characteristics might vary (Zahran, 2020). The symbiotic interaction between sugar beans and rhizobia improves several physiological functions of the plant in addition to nitrogen fixation. Effective rhizobial strains can boost drought tolerance, increase disease resistance, and improve nutrient absorption (Khan et al., 2021). These

Despite the recognized significance of *R. leguminosarum* in legume cultivation, comprehensive studies evaluating specific isolates' impact on sugar bean production are limited. Previous research has often focused on generalized findings, lacking detailed assessments of individual isolates and their performance under varying conditions (Jones et al., 2022). This study aims to fill that gap by systematically investigating the efficacy of various R. l*eguminosarum* isolates and their symbiotic relationship with sugar bean production.

Future agricultural research and practices targeted at maximizing the advantages of biological nitrogen fixing can also be informed by a knowledge of the dynamics of the rhizobia-sugar bean relationship. The study emphasizes the importance of rhizobia inoculation in legume cultivation and promotes ecologically sound, sustainable farming methods that increase agricultural output over the long run. This study is extremely pertinent to the current agricultural difficulties because of the growing demand for sustainable food production systems brought on by the world's population growth.

1.2 Problem statement.

From a survey that was carried out at Chemistry Research Institution (2021) at Grasslands in Marondera, most soils in Zimbabwe are acidic with varying degrees of the acidity from slightly acidic which is preferred for crop production to very acidic which is toxic to crop production and affects the uptake of nutrients. It is impossible to overlook the problematic impacts of chemical fertilizers when sustainable agriculture is the worldwide goal. Commercially available sugar bean *Rhizobium* strains are alien and do not fare well in acidic soils. Farmers have suffered significant losses as a result of the poor output of sugar beans. In order to improve the production of sugar beans, it is important to develop native Rhizobium strains capable of flourish in acidic soils. The sugar bean, or *Phaseolus vulgaris*, is a staple crop in Zimbabwe that supplies many communities with their main supply of protein in addition to critical nutrients. However, the majority of the commercial strains of Rhizobium used to grow sugar beans are foreign strains that have shown little success in acidic soils. This discrepancy has led to

The challenges posed by the inability of these exotic *Rhizobium* strains to thrive in acidic conditions underscore the urgent need for developing indigenous strains that can effectively colonize the roots of sugar beans. Indigenous *Rhizobium* strains are likely to be better adapted to

local soil conditions, which could result in enhanced symbiotic relationships with sugar beans, thereby improving nitrogen fixation and overall plant health (Zahran, 2020).

Research has shown that utilizing indigenous strains can lead to better growth performance and increased yields, which are critical for enhancing food security and supporting local economies. By isolating and characterizing indigenous *Rhizobium* strains that can thrive in acidic environments, this study aims to contribute to the improvement of sugar bean production in Zimbabwe. The anticipated outcomes include not only higher crop yields but also the promotion of sustainable agricultural practices that reduce reliance on chemical fertilizers and enhance soil health.

Additionally, the adaption of native Rhizobium strains could provide a strategy to enhance soil nutrient cycling, creating a more robust ecosystem that benefits crops and the environment. This strategy highlights the significance of local solutions to agricultural problems and is in line with international sustainability goals.

However, growing Zimbabwe's output of sugar beans depends on the creation and use of native Rhizobium strains suited for acidic soils. In order to promote sustainable crop production, this research aims to offer insightful information that may guide agricultural practices and policy. This project intends to increase food security and local farmers' economic stability by addressing the unique requirements of growing sugar beans on acidic soils, opening the door to a more sustainable agricultural future.

1.3 Significance of study

Historically, Sugar bean (*Phaseolus vulgaris)*was the earliest known legume in which the *Rhizobium* symbiosis was discovered (Varma, Tripathi & Prasad 2020). Grain legumes are globally recognized as an essential source of food and feed proteins, (Food and Agriculture Organization of the United Nations, 1993) and have become very important in human nutrition and as a feed for domestic animals for example pigs and donkeys. The significance of legumes to these systems encompasses benefits beyond their nitrogen (N) fixing capabilities but also their ability in reducing the cycles of diseases and pests affecting other crops (Ronald and Roy, 2012). For a farmer, the crop provides nitrogen to the soil which is often inadequate. While dry seed and fresh pod of specific landraces attract a high market price. Owing to the high cost and negative

environmental footprints of these synthetic fertilizers, it has become necessary to explore safer and sustainable biological means to meet legume N demand by identifying indigenous soil micro symbionts that can improve the efficiency of the legume-*Rhizobium* symbiosis.

Indigenous *Rhizobium* strains play a important role dyring growth and development of legume plants. The use of sugar bean *Rhizobium* strains plays a big role in farming as it leads to an increase in production. Therefore, farmers can make more profit from the sugar bean production, hence increased productivity. Utilizing biological nitrogen fixation through effective *R. leguminosarum* strains aligns with sustainable agricultural practices. This study advocates for reduced reliance on chemical fertilizers, which can lead to environmental degradation. Sinha et al. (2021) emphasize the importance of sustainable practices that maintain long-term soil fertility.

The development of effective management strategies and an increased understanding of the role of *Rhizobia* in food security is essential to enhance food production. This research contributes to the growing body of knowledge about the interactions between legumes and their symbiotic partners. By focusing on *R. leguminosarum* isolates, the study provides insights into the specificity of these interactions and their implications for crop management. The findings can inspire further research into other leguminous crops and their associated *Rhizobia* (Zahran, 2018; Dhananjaya et al., 2019).

The use of indigenous *Rhizobium* strains can reduce the need for synthetic nitrogen fertilizers, thereby promoting sustainable agricultural practices and minimizing environmental impacts (James and Prasad, 2012) . With the global population rising, the demand for sustainable food sources is escalating. Legumes, especially sugar beans, are crucial for food security due to their high protein content and ability to enrich soil nitrogen levels. Optimizing the use of *R. leguminosarum* isolates can enhance legume production, which is vital for meeting nutritional needs in developing areas (Bock et al., 2018; Kyei-Boahen et al., 2019).

Utilizing efficient *Rhizobium* strains can improve sugar bean yield and quality, leading to increased incomes for farmers and improved food security. Enhancing sugar bean yields through effective inoculation directly benefits farmers economically. Increased yields translate into higher incomes, especially for smallholder farmers who depend on legume cultivation. Research by González et

al. (2020) and Muthoni et al. (2021) illustrates how *Rhizobia*l inoculation can serve as a practical, cost-effective agricultural strategy.

1.4 Aim of the study

Identify the most effective indigenous *Rhizobium* isolates for promoting sugar bean growth.

1.5 Objectives of the project.

1. To determine the plant biomass weight, nodule count and nodule weight of sugar bean (*P.vulgaris)* .

2. To determine the nitrogen fixation efficiency of different *Rhizobium leguminosarum* isolates in common bean (*Phaseolus vulgaris*).

3. Evaluate the efficacy of different *Rhizobium* isolates in sugar bean production

1.6 Research questions

1. What is the plant biomass, nodule count and weight of sugar beans symbiotically associated with *Rhizobium?*
2. How do different *Rhizobium leguminosarum* isolates affect nitrogen fixation in common bean ?
3. Which *Rhizobium* strain will give the best performance in sugarbean?

1.7Hypothesis

H_0 (null hypothesis)

- Indigenous *Rhizobium* isolates and commercial strains do not perform the same in sugarbean production.

H_1 (alternative Hypothesis)

- Indigenous *Rhizobium* isolates and commercial strains perform differently in sugar bean production.

1.8 Limitations of the study

The study was conducted solely at SPRL in Marondera, which may limit the generalizability of the findings to other regions with different soil types, climatic conditions, and environmental factors. The efficacy of *Rhizobium* isolates tested may not represent the full diversity of *Rhizobium* species that exist or their interactions with other sugar bean cultivars. The availability of financial, technological, or human resources might have limited the number of isolates tested or the scale of the experiment. This study primarily focused on plant growth, without a comprehensive analysis of soil health indicators such as microbial diversity, organic matter content, and nutrient profiles. Understanding the broader impacts of *Rhizobium* on soil health is critical for assessing the full benefits of these symbiotic relationships.

1.9 Delimitations of the Study

Only sugar bean (Phaseolus vulgaris) was selected for the study, excluding other legume species that may interact differently with *Rhizobium* isolates. The study was conducted in a greenhouse facility with controlled conditions to minimize external variability and isolate the effect of *Rhizobium* inoculation. The study focused on specific parameters, such as nodule formation, leaf length and yield, without addressing other ecological or economic factors. Only a select number of Rhizobium isolates, identified based on prior research or availability, were included in the study, excluding other potentially beneficial strains. The study was conducted within a defined period, likely a single growing season, to provide a snapshot of the symbiotic relationship and its impact on bean production.

1.91 Definition of terms

Rhizobium: A genus of soil bacteria that form symbiotic relationships with legumes, infecting their roots and forming nodules where biological nitrogen fixation occurs.

Nodule: A specialized structure formed on the roots of legumes as a result of *Rhizobium* infection, where nitrogen fixation takes place.

Inoculation: The deliberate introduction of *Rhizobium* bacteria to the seeds or soil to promote nodule formation and nitrogen fixation.

Efficacy: The ability of *Rhizobium* isolates to effectively establish a symbiotic relationship with sugar bean plants and enhance their growth and production.

Isolates: Specific strains of *Rhizobium* bacteria that have been isolated and identified for experimental or practical use.

SPRL (Soil Productivity Research Laboratory): A research facility in Marondera, Zimbabwe is a government-owned facility that produces inoculants and conducts research in rhizobiology.

Phaseolus vulgaris: The scientific name for the common bean, which includes sugar beans. It is a legume crop widely cultivated for its edible seeds and pods.

The taxonomy for the common bean, Phaseolus vulgaris, is as follows:

- **Kingdom**: Plantae
- **Phylum**: Anthophyta
- **Class**: Dicotyledoneae
- **Order**: Fabales
- **Family**: Fabaceae
- **Genus**: Phaseolus

.

CHAPTER 2

LITERATURE REVIEW

2.1 Symbiotic Nitrogen Fixation

The relationship between legumes and *Rhizobia*, particularly '*Rhizobium* leguminosarum', is critical for sustainable agricultural practices, especially in the cultivation of sugar bean (*Phaseolus vulgaris)*. This literature review examines the efficacy of '*R. leguminosarum*' isolates in enhancing the symbiotic relationship with sugar bean production, focusing on nodulation, nitrogen fixation, and overall crop yield.

Symbiotic Nitrogen Fixation, the process of biological nitrogen fixation is fundamental to the success of leguminous crops, including sugar beans. *'R. leguminosarum*' forms nodules on the roots of legume plants, facilitating the conversion of atmospheric nitrogen into ammonia, which the plant can readily utilize (Vance, 2019). This symbiotic relationship is particularly beneficial in nutrient-deficient soils, where chemical fertilizers are either unavailable or economically unfeasible. Research indicates that effective nodulation is crucial for maximizing nitrogen fixation, which directly correlates with plant growth and yield (Zahran, 2020). The impact of R. *leguminosarum* on soil health is well-established. Nannipieri et al. (2017) highlight that introducing rhizobia enhances microbial diversity and activity, promoting better nutrient cycling and soil structure. Blagodatskaya et al. (2016) indicate that R. *leguminosarum* contributes to increased soil organic matter, which is essential for sustainable agricultural practices. Research by Rousk et al. (2016) and Sinha et al. (2021) further supports the positive effects of rhizobia on various soil health parameters.

Nitrogen is vital for plant development, and many legumes have evolved to form partnerships with nitrogen-fixing bacteria. Research by Badran et al. (2019) indicates that the relationship between legumes and *Rhizobium* significantly enhances soil fertility and crop yields. Studies by Abdou et al. (2016) confirm that *R. leguminosarum* effectively nodulates sugar beans, leading to notable

increases in biomass and pod production. Further investigations by Rojas et al. (2020) and Reddy et al. (2017) have demonstrated the positive effects of *R. leguminosarum* on nitrogen fixation in various leguminous plants.

2.2 Isolation and Characterization of *Rhizobium* Strains

The isolation of effective *Rhizobium* strains from local environments is crucial for optimizing legume production. Zahran (2018) emphasizes that locally adapted strains often outperform commercial varieties due to their compatibility with local soil conditions. Studies by Duncan et al. (2021) and Dhananjaya et al. (2019) support this notion, showing that indigenous *R. leguminosarum* strains can lead to improved yields in specific agro-ecological contexts.

The effectiveness of *R. leguminosarum* isolates can vary significantly based on genetic and environmental factors. Not all rhizobial strains are equally effective in symbiotic interactions; some may perform better under specific soil conditions or with particular legume varieties (Norton et al., 2020). For example, studies have shown that indigenous strains often exhibit better adaptation to local soil conditions compared to exotic strains, leading to enhanced nodulation and nitrogen fixation in sugar beans (Matsumoto et al., 2021). This highlights the importance of selecting appropriate isolates for specific agro-ecological zones to optimize sugar bean production.

Soil acidity is a critical factor influencing the efficacy of R*hizobial* strains. In Zimbabwe, most soils are acidic, which poses challenges for the survival and activity of R. l*eguminosarum* (Hirsch et al., 2020). Research indicates that many exotic rhizobial strains struggle to thrive in acidic environments, leading to poor nodulation and, consequently, diminished nitrogen fixation (Khan et al., 2021). Conversely, indigenous strains may possess adaptive mechanisms that enable them to function effectively in such conditions. Identifying and utilizing these native strains could significantly improve sugar bean yields in acidic soils.

2.3 Impact on Sugar Bean Yield

Numerous studies have quantitatively assessed the impact of *R. leguminosarum* on sugar bean yields. Adebayo et al. (2018) found that inoculation with specific R. *leguminosarum* isolates led to a 35% increase in sugar bean yield. Similarly, research by Omer et al. (2019) demonstrated that effective *R. leguminosarum* strains significantly enhanced both yield and protein content in sugar

beans. Additional studies by Alvi et al. (2020) and Khuong et al. (2021) corroborate these findings, showing considerable yield improvements through rhizobial inoculation.

The symbiotic relationship between *R. leguminosarum* and sugar bean has direct implications for crop yield and quality. Effective nitrogen fixation enhances not only plant growth but also the nutritional value of the beans produced (Zahran, 2020). Studies have shown that sugar beans inoculated with effective *Rhizobial* strains exhibit increased biomass accumulation, higher pod yield, and improved seed quality compared to those that are not inoculated (Norton et al., 2020). Furthermore, the use of effective rhizobial isolates can reduce the need for chemical fertilizers, thereby promoting sustainable agricultural practices.

However, the efficacy of *Rhizobium* l*eguminosarum* isolates in enhancing the symbiotic relationship with sugar bean production is influenced by factors such as strain variability, soil pH, and environmental conditions. Indigenous rhizobial strains, in particular, offer promising potential for improving sugar bean yields in acidic soils prevalent in Zimbabwe. This literature review underscores the importance of further research into the isolation and characterization of effective rhizobial strains to optimize sugar bean production and contribute to sustainable agricultural practices.

Similarly, Khan et al. (2021) investigated the impact of various R. leguminosarum strains on sugar bean plants grown in varying soil pH levels. Their findings indicated that indigenous strains were better adapted to acidic soils and resulted in higher biomass accumulation and pod yield, emphasizing the importance of selecting appropriate *Rhizobial* isolates based on soil conditions. Norton et al. (2020) also explored the performance of indigenous rhizobia in various agro ecological zones in Zimbabwe. Their research revealed that local strains not only improved nodulation rates but also showed better resilience to environmental stressors, such as soil acidity and drought conditions. This study underscores the potential of indigenous *Rhizobi*al strains to enhance sugar bean production in challenging agricultural environments.

Numerous studies have demonstrated the positive impact of effective *Rhizobium* inoculation on sugar bean yields. For instance, a trial conducted by Vance (2019) showed that sugar beans inoculated with effective *R. leguminosarum* strains exhibited a significant increase in pod yield and seed quality compared to non-inoculated controls. This finding aligns with the broader

understanding that enhanced nitrogen fixation directly correlates with improvements in crop yield and nutritional quality.

Measurements that were taken;

- ✓ Plant height
- ✓ Nodule weight
- ✓ Root length
- ✓ Biomass fresh weight
- ✓ Root fresh weight

In the greenhouse experiment, plant height, nodule weight, and root length were systematically measured to assess the efficiency of *Rhizobium* inoculation in sugar bean plants. Plant height was recorded using a ruler, measuring from the base of the stem to the highest point of the plant after a specified growth period, ensuring consistency in measurement time across all samples. Nodule weight was determined by carefully harvesting the root systems, rinsing them to remove soil, and counting the nodules formed. These nodules were then weighed using a precision balance to obtain accurate measurements of their total biomass. Root length was assessed by gently removing the plants from their pots, rinsing the roots, and laying them out straight on a measuring tape, where the total length was recorded. Each parameter was documented in a data sheet, allowing for comparative analysis of the effectiveness of different Rhizobium isolates on sugar bean growth.

CHAPTER 3

MATERIALS AND METHODS

3.1Study site;

The study was conducted at the Soil Productivity and Research Laboratory, at Grasslands Research Station . It is located 70km peg along Harare – Mutare road in Marondera. SPRL is a research center in Marondera,

Zimbabwe. It is a facility that is owned by the government that produces inoculants and conducts research in Rhizobiology. Soil Productivity Research Laboratory a department under the Chemistry and Soils Research Institute (CSRI) of the Department of Research and Specialist Services (DR& SS) under the Ministry of Agriculture, Mechanization and Irrigation Development. Marondera receives an average rainfall of 800 -1000mm and temperature of 7-18^{O}C in winter.

3.2Collection of Indigenous Rhizobium isolates

The selected *Rhizobium* isolates were obtained from the laboratory where they were stored for future development. Data acquisition involved a meticulous review of the laboratory records and also databases that shows strains that have been isolated before, their origins, characteristics and any previous experimental results. *Rhizobium* isolates were archived in a -80^{0}C freezer inside slant bottles in the Microbiology laboratory. The *Rhizobium* isolates opted for the research were IMFF 133, IMFF 202, IMFF 3, IMFF 4, IMFF 2 and IMFF146. The isolates were thoroughly selected and collected ensuring low exposure to environmental temperatures to maintain viability.

3.2.1 Purification of the Indigenous Rhizobium isolates

Once collected, the isolates were inoculated onto Yeast Mannitol Agar with Congo red to obtain sterile cultures. The inoculation process included streaking of a loopful frozen culture onto the culture media with Congo red. These agar plates were incubated at optimal temperatures for *Rhizobium* growth at 28^{0}C for 48 hours. Yeast Mannitol Agar with Congo red was used because the *Rhizobium* isolates used produces a unique colour change in YEMA with Congo red. If the

Rhizobia culture is contaminated it takes in the Congo red colour after incubation as shown in Fig 2.4 (a).

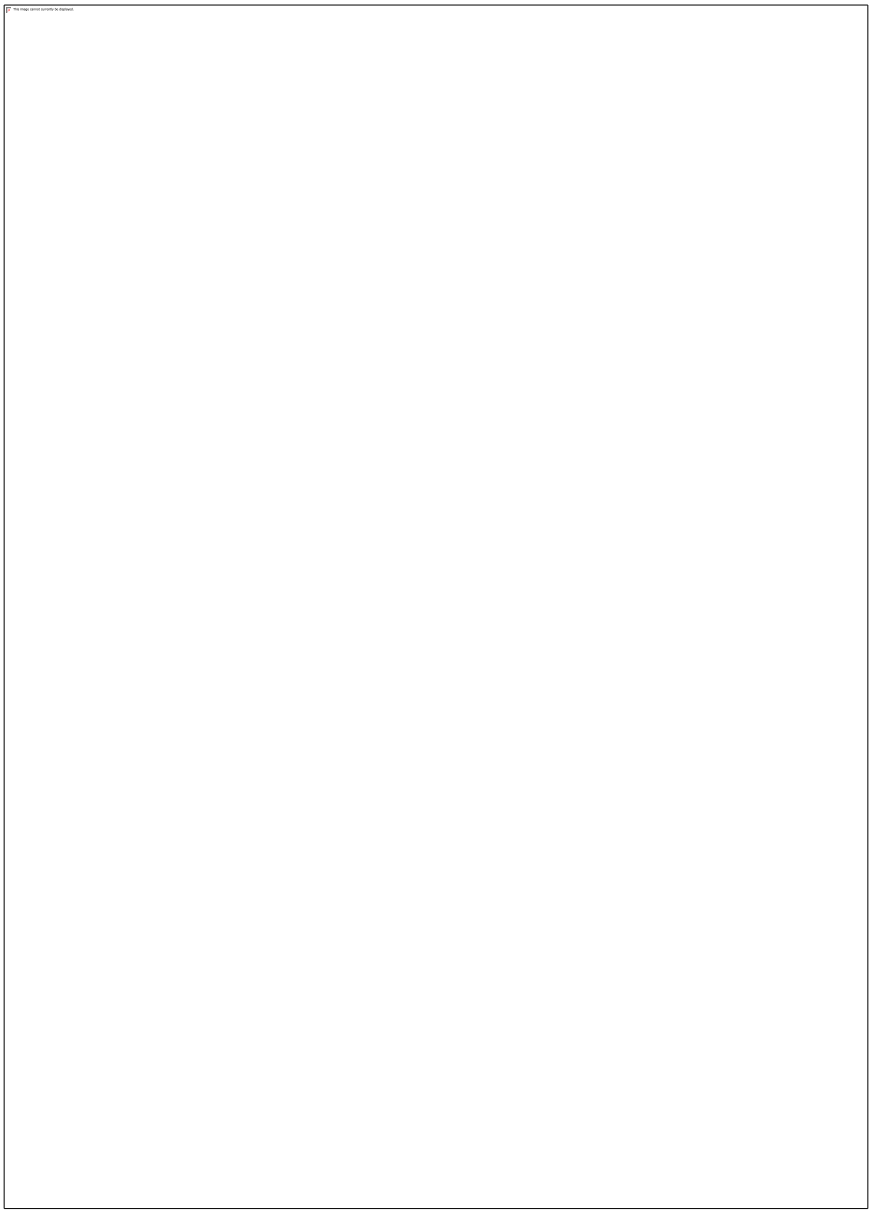

Fig 3.2: Streaking of *Rhizobium* cultures on Yeast Mannitol Agar with Congo red.

3.2.3 Preparation of Yeast Mannitol Agar with Congo red

iPreparation of Yeast Extract Mannitol agar was done by measuring 0.18g calcium carbonate($CaCO_3$), 10g Mannitol, 15g agar, distilled water, 10ml solution P (10g di-potassium orthophosphate into 200ml of water), 1ml solution D (ferric chloride dissolved into 200ml distilled water), 10ml solution Q (10g Magnesium sulphate and 5g sodium chloride into 500ml of distilled water.) into a 2-liter flask. Boiling for 1hour and adding Congo red indicator (used to check for contaminants in the bacteria, if the red color). After adding Congo red, autoclave for 30 minutes at temperature of 120°C (Somasegaran, P and Heinz J. 2012).

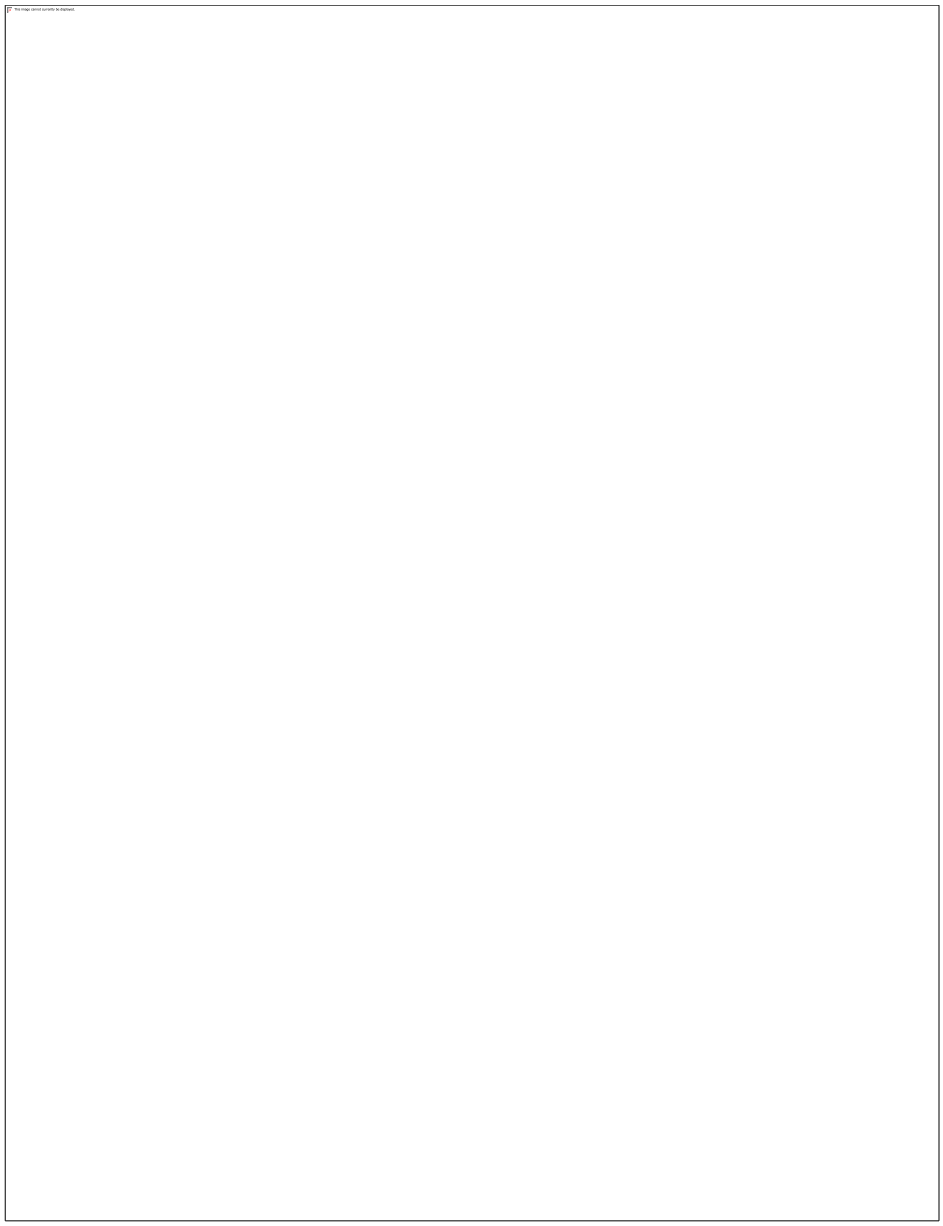

Fig 3.2.2 autoclave machine being closed after putting YMA with Congo red plates inside to autoclave.

3.2.4 Preparation of Standardized Inoculum

Preparation of Yeast Extract Mannitol Broth by measuring 0.18g calcium carbonate, 10g Mannitol, 15g agar, distilled water, 10ml solution P, 10ml solution Q into a 2-liter flask. Autoclaving for 30 minutes at temperature of 121°C. YMB was used as a carrier material of *Rhizobium* cultures. After preparation of the Inoculum .100ml of the solution was dispensed into

each of 640ml bottles and the bottles were autoclaved at 121^0C for 30 minutes. After autoclaving, each bottles were inoculated with *Rhizobium* isolates.

3.2.5Pot preparation

Sand soil was gathered from a river in Grasslands, Marondera. The sand soil was then cleaned under running water to remove dirty as well as debris until the water in sand becomes transparent. After washing, the soil was filtered through a 2mm sieve, this was done to eliminate small rocks and other particles that may often present in sand soil. The sand soil was repacked again in sacks and were taken to an autoclaving machine. The soil was processed through an autoclave at 121^0C for 1 hour. Autoclaving the sand soil was done to eliminate microorganisms that can compete with sugar bean for resources or that may cause diseases. It was done also to minimize the risk of contamination from microorganisms that can affect the experiment results. After autoclaving, the soil was removed from the sacs. The pots were washed with running water and dried. After drying them they were sterilized using 70% alcohol. Small pieces of sacs were cut and put at the base of the pots for drainage purposes. The sand was then weighed and packed in all of the 24 pots. This weighing was done to ensure that all plants were planted in the same amount of soil Figure (3.2.5). After packing the pots with sand were placed in the greenhouse for experiment. Labels were put and pots were put such that there were completely randomized.

Fig 3.2.5 shows labelled pots with sand soil placed in a glasshouse.

3.2.6 Greenhouse experiment (Glasshouse)

The experiment was carried out as a Completely randomized design with 8 treatments replicated three times under greenhouse conditions. This design allows for robust statistical

analysis while controlling for spatial variability within the greenhouse environment. Each experimental unit consisted of one pot containing bean plants subjected to the designated treatment protocol. The treatments contained isolates except for the negative and positive controls. Six *Rhizobium* isolates were used, IMFFF 133, IMFF 146, IMFF 2, IMFF 202, IMFF 3 and IMFF 4.

Table 3.2.6 Showing treatments, number of replications and isolates for Glasshouse experiment.

Treatment	Replications	Isolates
1	3	IMFF 133
2	3	IMFF 146
3	3	IMFF 2
4	3	IMFF 202
5	3	IMFF 3
6	3	IMFF 4
7	3	POSITIVE CONTROL (commercial strain)
8	3	NEGATIVE CONTROL(zero)

3.2.7 Source of sugar bean Rhizobium isolates seeds and the sowing of the seeds

Indigenous Rhizobium isolates were obtained from SPRL, Grasslands in Marondera. The seeds were purchased from certified agricultural suppliers. Genetic integrity was maintained by selecting uniform, high-quality seeds. Following planting, the soil was carefully watered. Holes were made inside each pot with a depth of about 1-2 inches and 3 -4 inches apart to allow for growth. In each hole sugar bean seed was placed and was covered gently with soil and pat down lightly. Three holes were made in each pot. Soon after germination the 6 x3 of the pots were inoculated with 100ml of Yeast Mannitol Broth with Rhizobium isolates. The other pots(1x3) acted as a negative control so nothing was put in it. The last pots (1x3) acted as positive control and commercial strain was administered to it. Since all the 8 treatments were replicated 3 times it resulted in 24 pots. Distilled water was used through the experiment daily. Distilled water is free from contaminants and dissolved minerals. Nitrogen free solution was also used in the experiment because it helps prevent the addition of excess nitrogen to the soil. 200ml of distilled water was used to maintain consistent soil moisture and also it avoids

overwatering. The volume is often sufficient to ensure nutrients in the soil are accessible to the plants without leaching them .

Fig 3.2.7 Greenhouse setup after planting sugar bean seeds.

Growth Parameter Assessment

Throughout the eight-week growing period, multiple non-destructive growth parameters were regularly monitored to track developmental patterns across treatments. Plant height was measured weekly from the soil surface to the apical growing point using a standard measuring tape. Additional parameters including leaf number, plant height, and visual health assessments were recorded at regular intervals to provide comprehensive growth data. Although plants did not reach the reproductive stage within the experimental timeframe, vegetative developmental stages were carefully documented.

3.2.8 Harvesting of the common bean (Sugar bean) plant

This study examined common beans (Phaseolus vulgaris L.) in a greenhouse setting with eight distinct treatments replicated three times. Though the eight-week experimental period did not allow plants to reach maturity for bean production, the comprehensive assessment of plant growth parameters offers significant data on early developmental stages and biomass accumulation patterns. At the conclusion of the eight-week experimental period, plants were harvested for detailed analysis. Each plant was carefully removed from its pot to preserve intact root systems for examination. This process required gentle loosening of the sand soil around the pot edges, followed by careful inversion and removal of the entire root-soil mass. Following harvest, plants underwent a systematic cleaning process to remove soil particles without damaging plant tissues. Roots were submerged in clean water and gently agitated to dislodge soil particles.

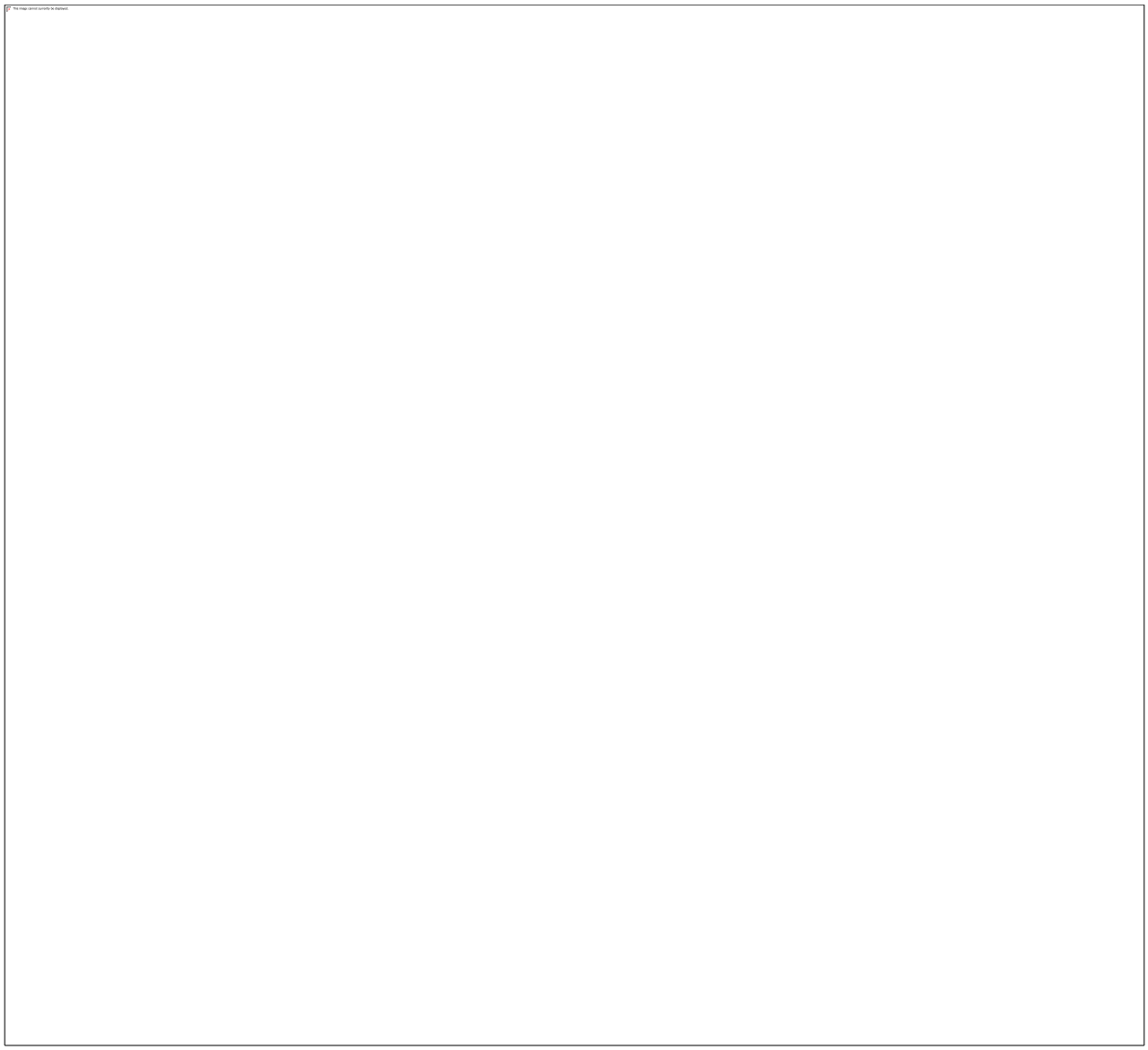

Fig 3.2.8 Shows the washed harvested common bean plants each placed on labelled khaki papers

3.2.9 Fresh Parameter Measurement

Plant Height Determination

Final plant height was measured from the base of the plant at the soil line to the apical meristem using a measuring tape. Measurements were recorded to the nearest millimeter for precision.

Wet Mass Assessment

Immediately following cleaning, total plant wet mass (fresh weight) was determined using a calibrated analytical balance. For detailed analysis, plants were separated into component parts (roots, stems, leaves) and each component was weighed separately to quantify biomass distribution patterns. The entire plant (including roots) was weighed using a balance to obtain the wet weight. This measurement reflects the total biomass before any drying occurs.

Fig 3.2.9 shows roots being weighed separately using a balance.

Leaf Length Measurement

Select the largest leaf from each plant and measure its length using a ruler. Record this data, as leaf size is an important indicator of plant health and photosynthetic capacity.

3.3 Root System Analysis

Root systems were carefully spread on a clean surface to assess morphological characteristics. Root length, availability of nodules was documented. The number of nodules present on each plant was counted, as this data is important for assessing the effectiveness of nitrogen-fixing bacteria and overall root health.

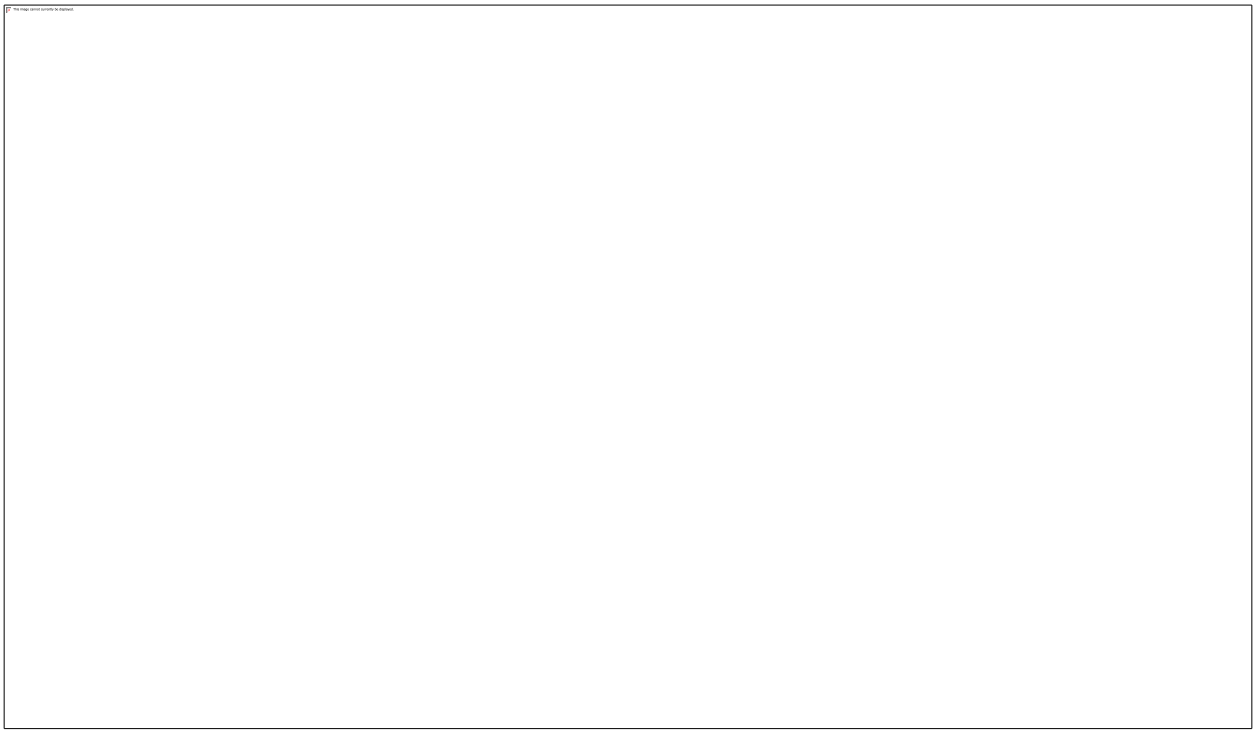

Fig 3.3 shows number of nodules being counted

3.3.1 Drying and Biomass Determination

After fresh parameter measurement, plant materials were prepared for dry weight determination. Each plant component was placed in labeled khaki envelopes. These envelopes were clearly marked with treatment identification, replication number, and sample description to ensure proper data tracking throughout the drying process. Labeled envelopes containing plant materials were arranged in a forced-air drying oven set to 65-70°C. Samples were dried for 72 hours, indicating complete moisture removal. The use of khaki envelopes rather than paper bags provides better durability during the drying process while still allowing moisture to escape efficiently.

Dry Weight Measurement

Following the drying period, envelopes were removed from the oven and were allowed to cool without reabsorbing atmospheric moisture. Once cooled to room temperature, dried plant materials were carefully removed from envelopes and weighed using a precision analytical balance. Weights were recorded to the nearest 0.01g to ensure accuracy.

3.3.2 Data Analysis and Parameter Calculation

Collected data for each parameter (plant height, fresh weights, dry weights) were organized by treatment and replication for statistical analysis. Mean values and standard deviations were calculated for each treatment to assess variability and treatment effects. After completing the measurements, compile all data systematically. Wet weight and dry weight of each plant, Number of nodules counted per plant, final height of each plant in cm and leaf length measurements.

3.3.3 Statistical Analysis

The data from the laboratory and greenhouse experiment were entered in Microsoft Excel and analysis of variance (ANOVA) was conducted to determine significant differences among

treatment**s** for each measured parameter. This was done using Fisher ‘s protected Least Significance Different (LSD**)** at 5% level of significance on all significant data. With eight treatments replicated three times, the experimental design provided sufficient statistical power to detect meaningful differences. Where significant treatment effects were identified, appropriate post-hoc tests were employed to determine specific differences between treatment means.

CHAPTER 4

RESULTS

The results from table 1 shows that there was a significance difference in nodule number ($p < 0.001$) as well as root fresh weight ($P < 0.001$). Treatment 4 had the highest biomass weight as compared to the other treatments, whereas treatment 202 outcompeted others in nodule numbers as well as root lengthy. From the studies done there is potential in using the indigenous isolates as shown by our results from the greenhouse as compared to CIAT899 *Rhizobium tropici* from Colombia. There is potential in using treatment 2, 4 and 202 for *Phaseolus vulgaris* as bio fertilizers.

After the investigation from the greenhouse there is need to further investigate our indigenous isolates in different agro ecological regions and compare their response to CIAT899 Rhizobium tropici from Colombia. There is potential in using treatment 2, 4 and 202 for Phaseolus vulgaris as bio fertilizers.After the investigation from the greenhouse there is need to further investigate our indigenous isolates in different agro ecological regions and compare their response to inoculation. There is also need for genetic analysis of these isolates as we have to know their genera and species. Their species are only known from the morphological and biochemical characterization that was done in the laboratory which is not enough.

Table 4.1: Results of Rhizobium isolates on common bean (Phaseolos vulgaris).

Treatment	Fresh weight	Nodule Number	Plant height	Root fresh weight	Root length
133	2.47	29^{c}	12.41	2.92^{ab}	17.72
146	3.16	29.7^{cd}	13.62	7.03^{d}	19.33
2	2.93	47^{e}	13.07	4.93^{c}	21.15
202	2.45	41.3^{de}	9.83	3.87^{bc}	20.08
3	2.86	29.3^{c}	12.52	3.33^{ab}	16.57
4	3.08	31^{cd}	13.46	5.29^{c}	19.58
CIAT899	2.58	16.7^{b}	12.4	3.16^{ab}	19.78
ZERO	2.23	1.0^{a}	11.75	1.81^{a}	10.88
P-value	**0.525**	**<0.001**	**0.206**	**<0.001**	**0.057**
Grand mean	**2.72**	**28.1**	**12.38**	**4.04**	**18.14**
LSD (5%)	**1.052**	**11.98**	**2.847**	**1.532**	**6.119**
SED	**0.608**	**6.92**	**1.645**	**0.885**	**3.535**
CV%	**22.4**	**24.6**	**13.3**	**21.9**	**19.5**

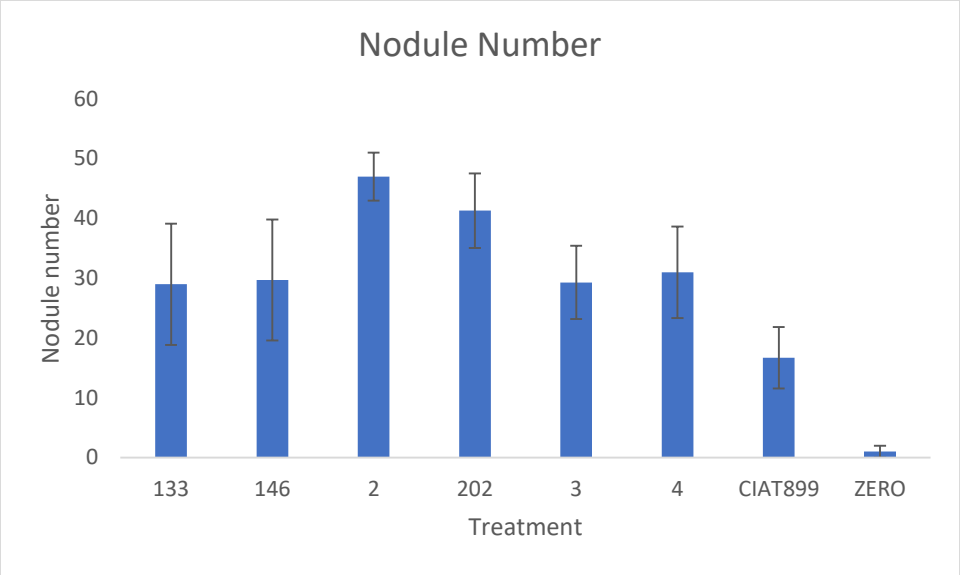

Fig 4.2 Effects of Rhizobium isolates on nodule number of sugar bean.

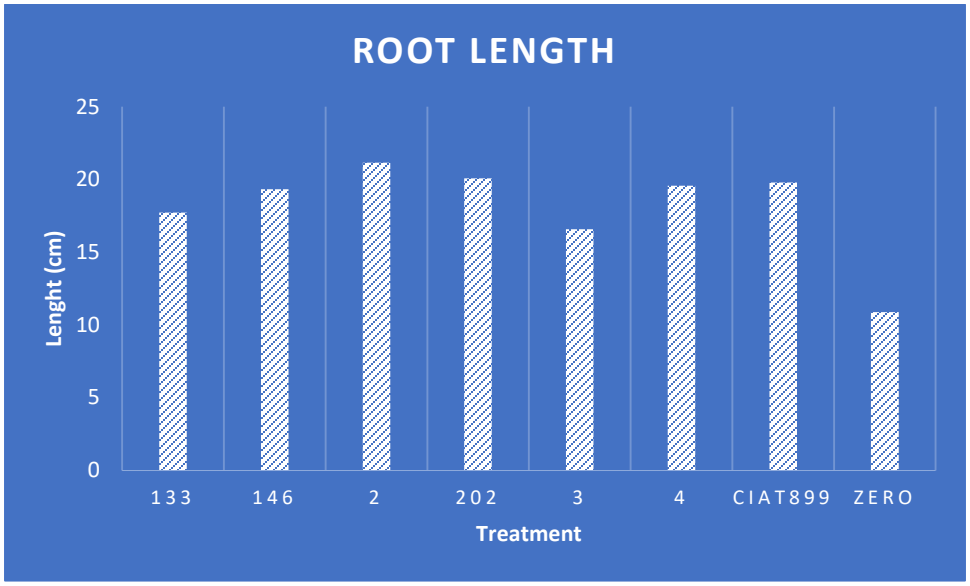

Fig 4.2.1 Effects of Rhizobium isolates inoculates on the root length of sugar beans.

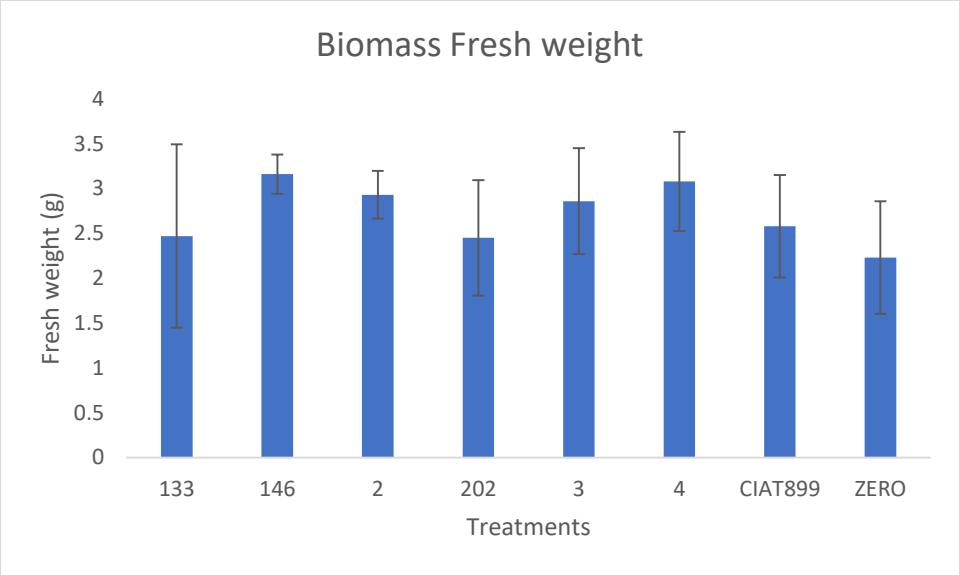

Fig 4.2.2 Effects of Rhizobium isolates inoculates on plant biomass (fresh weight).

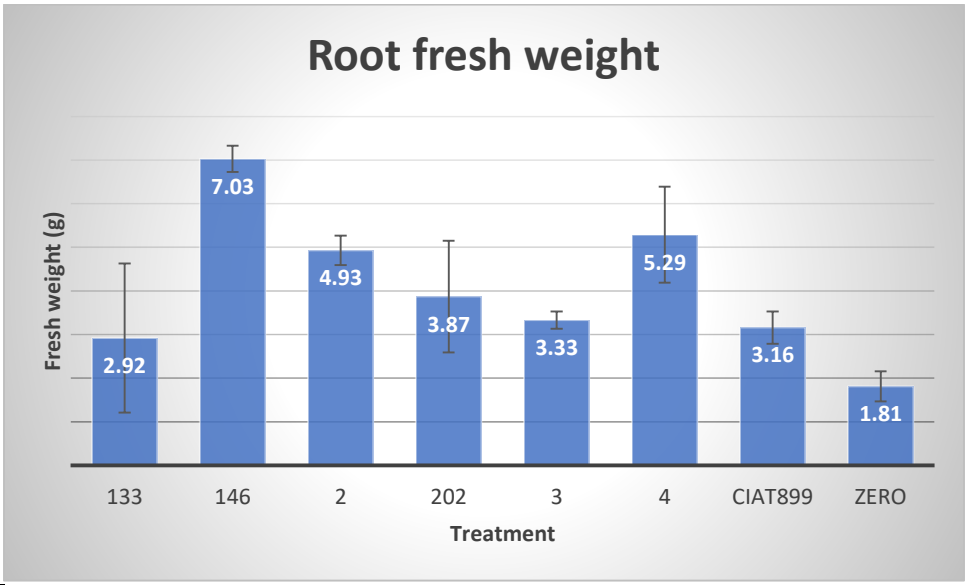

Fig 4. 3 Effects of Rhizobium isolates inoculation on root fresh weight.

CHAPTER 5

DISCUSSION

5.1 Efficacy of Indigenous *Rhizobium* isolates on sugar bean production

The results of our experiment demonstrated that indigenous Rhizobium isolates significantly enhance the symbiotic relationship with sugar bean (Phaseolus vulgaris). This finding aligns with previous research emphasizing the importance of local strains, which are often better adapted to specific soil conditions and environmental factors of their native regions (Bashan et al., 2014). In our research, we established selection criteria for these isolates, focusing on their ability to nodulate effectively and fix nitrogen efficiently under local conditions. Indigenous Rhizobium strains exhibited superior nodulation rates compared to exotic strains, leading to increased nitrogen fixation, which is critical for optimal plant growth. The enhanced nodulation not only facilitates

greater nitrogen availability but also contributes to the overall vigor of sugar bean plants, as evidenced by improved growth parameters such as height, biomass, and pod yield.

The impact of indigenous Rhizobium on growth parameters is multifaceted. By fostering a robust symbiotic relationship, these local isolates promote healthier root systems, which enhance nutrient uptake and improve soil structure. The presence of effective nodules not only supplies nitrogen but also influences other essential nutrient dynamics, creating a more balanced nutrient profile in the soil. This comprehensive improvement in nutrient availability is particularly beneficial in nutrient-deficient soils, where chemical fertilizers may be scarce or economically unfeasible. Over time, the use of indigenous Rhizobium can lead to sustainable agricultural practices, minimizing reliance on synthetic fertilizers that have been linked to soil degradation and water pollution (Hodge & Stewart, 2000). Furthermore, the long-term integration of these local strains into crop management practices is likely to enhance soil fertility and biodiversity, ultimately contributing to improved agricultural resilience in the face of climate change.

In addition to immediate crop yield benefits, the role of indigenous Rhizobium isolates in enhancing soil health cannot be overstated. As these microorganisms establish symbiotic associations with sugar beans, they contribute to the development of a more diverse microbial community in the rhizosphere, which is essential for maintaining soil health. This diversity not only supports nutrient cycling but also promotes the breakdown of organic matter, further enriching the soil. Moreover, the increased nitrogen fixation associated with effective nodulation leads to a gradual buildup of soil nitrogen levels, benefiting subsequent crops in a rotation system (Zahran, 2020). This sustainable approach to nitrogen management aligns with contemporary agricultural goals of reducing chemical inputs while maintaining high productivity levels.

Furthermore, the adaptation of indigenous Rhizobium strains to local climatic and soil conditions enhances their performance in specific agricultural contexts. For example, studies have shown that these strains can exhibit greater resilience to environmental stressors such as drought and soil acidity, which are common challenges in many farming regions (Matsumoto et al., 2021). This adaptability not only improves the immediate growth and yield of sugar beans but also contributes to the long-term viability of cropping systems, particularly in areas prone to climatic variability.

The integration of indigenous Rhizobium into farming practices also supports the principles of agroecology, which emphasizes the importance of biodiversity and ecological balance. By fostering the growth of native microorganisms, farmers can create a more resilient agricultural ecosystem that can better withstand pest pressures and changing environmental conditions. Additionally, the reduction in chemical fertilizer usage not only benefits the environment but can also lead to cost savings for farmers, making the adoption of indigenous Rhizobium a win-win situation for both productivity and sustainability.

Overall, the efficacy of indigenous Rhizobium isolates in enhancing sugar bean production illustrates the critical role of local biodiversity in agricultural systems. By leveraging the unique adaptations of these native strains, farmers can optimize crop yields while promoting environmental sustainability. Future research should continue to explore the interactions between indigenous Rhizobium and other soil microorganisms to fully understand their collective impact on crop health and soil vitality. Emphasizing the use of local strains not only supports sustainable farming practices but also enhances the resilience of agricultural systems in challenging environmental conditions, paving the way for a more sustainable and productive agricultural future.

5.2 Impact on growth Parameters

Quantitative analysis revealed that plants inoculated with indigenous isolates exhibited increased growth parameters, such as plant height, biomass, and nodule number, compared to controls. This observation is consistent with the literature that suggests effective nodulation leads to improved nutrient uptake, particularly nitrogen, which is crucial for the vegetative growth of sugar bean (Zahran, 1999). In this research we discussed the methodology used to measure these growth parameters, emphasizing the need for rigorous experimental design to isolate the effects of *Rhizobium* from other potential variables. The growth of sugar bean was markedly higher in treatments 2, 202, 4 with indigenous *Rhizobium* isolates. This increase can be attributed to enhanced nitrogen fixation, which improves the overall fertility of the soil and supports better pod development (Khan et al., 2018). The data collected during our study corroborate earlier research indicating that effective *Rhizobium* symbiosis can lead to a significant increase in crop yield, making it a viable agricultural strategy for improving food security (Giller, 2001).

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From the study it can be confirmed that Indigenous *Rhizobium* isolates have great impact on sugar bean production. The results from the study proves that *Rhizobium* isolates 2, 202 and 4 works very well in the growth of Sugar bean. This study highlights the significant benefits of indigenous *Rhizobium* isolates on the production of sugar bean (Phaseolus vulgaris). The observed improvements in growth parameters and overall crop health underscore the importance of utilizing local biological resources in sustainable agriculture. The findings advocate for the adoption of indigenous *Rhizobium* as a viable strategy to enhance agricultural productivity while promoting environmental sustainability. By reducing reliance on synthetic fertilizers, these isolates contribute to healthier soils and ecosystems, aligning with global efforts towards sustainable farming.

6.2 Recommendations

- Farmers are encouraged to adopt indigenous *Rhizobium* isolates 2, 202 and 4 as part of their agricultural practices for sugar bean cultivation. Local agricultural extension services can facilitate access to these biological inoculants, providing training on their application and benefits. Demonstration plots can be established to showcase the effectiveness of these isolates in improving yield and soil fertility.

- Awareness campaigns must be implemented to educate farmers and stakeholders about the benefits of using indigenous Rhizobium. Workshops, informational brochures, and community meetings can help disseminate knowledge about sustainable practices and the importance of maintaining soil health.
- Future research should investigate the diversity of indigenous *Rhizobium* strains across different geographic regions. This could help identify the most effective isolates for various environmental conditions, ultimately improving crop yields and sustainability practices.

- As agricultural practices evolve, further research is essential to explore the long-term impacts of these isolates on soil fertility, crop resilience, and overall farm productivity. This will ensure that farmers can sustainably enhance their yields while maintaining soil health. By embracing these strategies and recommendations, we can work towards a more resilient and productive agricultural system, ultimately contributing to food security and environmental stewardship.

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