Bindura University of Science Education

Assessing indigenous *rhizobium* isolates for enhanced plant growth and biomass on cowpea (*Vigina Anguiculata*) in acidic sandy loam soils (pH 4.5).

By
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A research project submitted in partial fulfilment of the requirements for the Bachelor of Science Honours Degree in Biological Sciences.

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Approval form

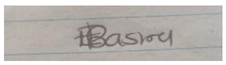
Signature of student Date: 16/06/2025

Signature of Supervisor: E.Zingoni Date:26/09/2025

Signature of Chairperson of Department: Date 16/06/2025

Declaration

I, Evbu Basira 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: 16/06/2025

Supervisor

I, Emmanuel Zingoni, 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: E.Z Date:16/06/2025

Dedication

I dedicate this project to my mother Jenifa Basira.

Acknowledgements

I would like to express to express my sincere gratitude to my supervisor, Mr E. Zingoni for his invaluable guidance, continuous support, and encouragement thought this project. I am also thankful to the faculty and staff of Soil Productivity Research Laboratory for providing the necessary resources and supportive environment. I also want to give special thanks to my peers Stewart Makono and Miranda Deke who helped me with decisions and motivation. Finally, I am deeply grateful to my family for their unwavering love and support.

List of Abbreviations

BNF: Biological Nitrogen Fertilizer

DR&S: Department of Research and Specialist

N2Africa: Nitrogen for Africa

SPRL: Soil Productivity Research Laboratory

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ABSTRACT

The growing demand for sustainable agricultural practices has emphasized the importance of harnessing beneficial soil microorganisms, such as rhizobia, to improve legume productivity, especially under challenging soil conditions. This study was done to assess the growth-promoting potential of indigenous rhizobial isolates on cowpea (*Vigna unguiculata*) grown in acidic soil conditions under greenhouse conditions at Soil Productivity research Laboratory (SPRL) Marondera. Seven treatments were evaluated, including five native isolates (M1 to M5), a commercial inoculant (COM 276), and an uninoculated negative control (ZERO). Each treatment was assessed for its effect on key agronomic parameters such as nodule number, root length, and total plant biomass. The results revealed significant differences ($p < 0.001$) among treatments for all parameters measured. Notably, isolates M2, M1, and M4 demonstrated superior performance, with M2 producing the highest nodule number (39.67), and M1 exhibiting the longest root length (20.98 cm) and high dry biomass (2.83 g). The uninoculated control showed minimal growth and no nodulation, showing the necessity of rhizobial inoculation for successful cowpea cultivation under low-pH conditions. High coefficients of variation, particularly in root-related traits, suggest underlying biological variability and possible microenvironmental effects within the greenhouse. These findings underscore the agronomic value of indigenous rhizobia adapted to local soil conditions and their potential as biofertilizers for improving cowpea productivity. The study recommends further molecular characterization, field validation, and the development of inoculant formulations to enhance adoption and application in acid-prone agricultural regions.

Contents

CHAPTER 1

INTRODUCTION

1.0 Background of the study

The production of cowpea (*Vigina Anguiculata*) has been limited due to high cost associated with synthetic fertilizers since some smallholder farmers cannot afford to buy it. According to Matoso et al (2018), the ability of cowpea to fix Nitrogen depends on the characteristics of the rhizosphere (Hamza and Alebeio, 2017). Cowpea form root nodules with the bacteria that lives in the soil and the bacteria are called rhizobia. The bacteria which are in the root nodule can fix nitrogen from the air in the form that can be used by cowpea and the other part of the Nitrogen is used to make protein in the grain and the other is left behind in falling leaves so that the fertility oof the soil can be improved (Njeru and Nyanga, 2020). Cowpea is one of the droughts tolerant legumes therefore soil fertility should be enhanced so as to increase the production of cowpea (N2Africa, 2015). Rhizobial inoculants are reported to influence crop growth, yield and nutrient uptake by different mechanisms (Moussa et al., 2016). Cameiro Da Silver at el (2018), mentioned that the inoculants fix Nitrogen, help promoting free living nitrogen-fixing bacteria, increase supply of nutrients such as phosphorus and iron, produce hormones, enhance other beneficial bacteria or fungi control bacterial and fungal diseases, and help in controlling insect pest. Most small holder farmers are located in acid sandy soils derived from granite which are acidic and they will end up producing low yields (Horn, Gibrehohiwot and Hussein, 2016). This study is going to focus on the indigenous isolates performance when exposed to sandy loamy at pH 4.5 which are the soils commonly found in Zimbabwe.

1.1 Problem statement

According to DR and SS about 70% of the farming soils are acidic and they are sandy soils and these are the same soils which are being used by cowpea farmers. Acidic sandy loamy soils prevalent in Zimbabwe pose significant challenges to plant growth, leading to reduced crop yields and food insecurity. Despite the potential of indigenous microbes which are being used to enhance plant growth, limited research exists on their diversity, optimal application methods, and

Interactions with acidic soil conditions (Nkomo, 2015). The country's Agricultural sector is also facing a problem in the infertility of soil and these are the same soils which are being used to cowpea production. Smallholder farmers, who constitute the majority, lack access to microbial innoculants which are effective and affordable. Current microbial inoculants used in Zimbabwe are often imported, expensive, and unsuited to local conditions, resulting in poor performance in cowpea production. Local research institutions are also lacking the capacity to develop and produce effective microbial inoculants. Again, the Department of research and specialist services carry out a study and found that the imported microbial inoculants are not well suited to local conditions such as soil pH, presence of indigenous microbial communities and temperature and they end up giving negative results. When the imported microbial innoculants have been affected by environmental conditions in the country it leads to reduced plant growth and productivity, higher production cost and inefficient nutrient uptake. Furthermore, reliance on imported innoculants is affecting the development of local microbial inoculant production capacity. Carvalho et al, (2017) said that indigenous rhizobial isolates have higher chances of success since they are from that area.

Policy and regulatory frameworks supporting microbial inoculant use in Zimbabwean agriculture are inadequate, hindering their adoption. Limited awareness and knowledge among farmers, extension agents, and policymakers further constrains the potential benefits. Zimbabwe's regulatory frameworks microbial innoculants in Agriculture are woefully inadequate, hindering their adoption and limiting the country's agricultural potential The lack of clear guidelines, standards and protocols for registration, and labelling rhizobium innoculants creates uncertainty and confusion.

Zimbabwe's economic challenges, characterized by high inflation rates, currency fluctuations, and limited financial resources, severely limit investment in agricultural research and development. This constraint hinders the development of innovative solutions which address pressing agricultural issues, such as soil acidity, drought tolerance, and pest management. Insufficient funding for ARD institutions and programs results in inadequate infrastructure, outdated equipment, and brain drain, as skilled researchers seek opportunities elsewhere. Zimbabwe's agricultural sector is struggling with improving productivity, competitiveness, and food security. Enhanced investment in ARD is crucial to unlock the sector's potential and drive economic growth.

Another issue is the issue of climate change which is exacerbating soil acidity, posing significant threats to agricultural productivity and ecosystem health. Rising temperatures and altered precipitation patterns alter soil moisture levels, leading to increased leaching of essential nutrients and accumulation of acidic compounds (Farooq et al., 2017). Enhanced atmospheric carbon dioxide concentrations stimulate plant growth, but also increase soil respiration, releasing more carbon dioxide and accelerating soil acidification. Warmer temperatures are also accelerating microbial activity by breaking down organic matter and releasing acidic byproducts. Droughts and flood are more frequent due to climate change, exacerbating soil erosion, nutrient depletion, and acidification. These interconnected factors compound soil acidity, reducing fertility, and affecting plant growth, water quality, and biodiversity.

1.2 Aim of the study

The aim of this study is to come up with cowpea rhizobial isolate which is friendly to Zimbabwean soils which are acidic and sandy loam. The production of cowpea (*Vigina Anguiculata*) has been limited due to high cost associated with inorganic fertilizers since some smallholder farmers cannot afford to buy it. According to Bala, Baidoo and Woomer (2016), the ability of cowpea to fix Nitrogen depends on the characteristics of the rhizosphere. Cowpea is one of the droughts tolerant legumes therefore soil fertility should be enhanced so as to increase the production of cowpea. Rhizobial inoculants are reported to influence crop growth, yield and nutrient uptake by different mechanisms. Inoculants fix Nitrogen, help promoting free living nitrogen-fixing bacteria, increase supply of nutrients such as phosphorus and iron, produce hormones, enhance other beneficial bacteria or fungi control bacterial and fungal diseases, and help in controlling insect pest. Most small holder farmers are located in acid sandy loamy soils derived from granite which are acidic and they will end up producing low yields.

1.3 Specific objectives

1. To determine the isolate that will produce the highest number of nodules in sandy loam soils by counting number of nodules per plant after harvesting.

2. To determine an isolate that will produce the longest roots in sandy loam soils by measuring root length at harvesting

3. To determine an isolate that will produce the highest biomass fresh weight in sandy loam soils by weighing oven dried plants after harvesting.

4. To determine an isolate will produce the highest biomass dry weight in sandy loam soils by weighing at harvesting.

1.4 Research question

1. Which isolate will produce the highest plant nodule in sandy loam soils?

2. Which isolate will produce the longest root length in sandy loamy soils?

3. Which isolate will produce the highest biomass plant fresh weight root length in sandy loam soils?

4. Which isolate will produce the highest root fresh weight in sandy loam soils?

1. 5 Significance of the study

Considering its nutritional benefits and resilience under changing climate, cowpea has been recommended as one of the underutilized matter, carbon and also Nitrogen fixation. This promotes soil fertility and physical characteristics for example infiltration crops with the potential to alleviate food insecurity, malnutrition, and poverty in sub-Saharan Africa. To increase cowpea production which will reduce cost to farmers since it has the dual purpose to feed humans and it's a source of Protein, Vitamin and other vital minerals. The residues can also be used as animal feed. It also increases the soil nutrition value when integrated in crop rotation systems because it builds up the soil organic and tension capacity.

1.6 Hypothesis

H_0 The isolates perform the same in sandy loam soils.

H_1 The isolates perform differently in sandy loam soils.

CHAPTER 2

Literature review

N2Africa was a research project which aimed to improve nitrogen fixation in Africa. The project was launched in 2009 and it was funded by Bill and Mellinda Gates foundation and Howard G. Buffet foundation (Liu et al.,2020). The main objective was to enhance the fixation of nitrogen in legumes at the same time reducing the use of synthetic fertilizers. This project was mainly important in N2Africa where soil fertility was a major problem. In N2Africa strains which were suitable for African climatic conditions. The project also focused on breeding the leguminous plants which were better adapted to African soils (Oruru et al.,2018). New varieties were developed involving conventional breeding techniques. Cowpea was one of the most targeted crops since during the project. N2Africa has got its limitation in Zimbabwe because it focused on a small space of area (Ouma et al.,2016). Again N2Africa (2015), mentioned to it focused on Mudzi, Matobo, Buhera, Chivi, Gwanda and Goromonzi.Comparison on exotic strains vs indigenous isolates was done but the information on the indigenous strains was limited. In addition to that it focused more on regions than soils.

Legumes which are commonly grown in Zimbabwe include Cowpea (*Vigina Anguiculata*). The grain of cowpea contains 25% protein and several vitamins. Kebede and Bekeko (2020), mentioned that cowpea tolerate drought and grow in a variety of soils and also being a legume which fixes nitrogen. Most small-scale farmers grow cowpea and can be grown with other crop because it tolerates shade (Owade, Abong and Mwangombe,2020). Cowpea covers the ground this prevents soil erosion. Maize (*Zea mays)* is a staple good in Zimbabwe. Not all areas in Zimbabwe produce yields in maize. Areas like Buhera and Bocha receive low rainfall so they produce less than average yield in Zea Maize (Nkomo,2015). Cowpea in Zimbabwe in Zimbabwe can be grown either as a monocrop or it can be in association with. Mentioned that the benefits of cropping systems are to improve soil fertility. According to the article N2Africa (2015), legumes such as cowpea, soil fertility benefits of legume to cereal, the duration of legume biomass and residue management is considered to have minimally competitive growth traits for example branching systems and deep tap roots to avoid intra competition.

Cowpea was domesticated in the Southern part of Africa part of the African but it later on spreads to the east Africa west and lastly Asia. Most farmers prefer cowpea because of its weed suppressing ability since it grows covering the ground. The biological nitrogen fixation as the reduction of. According to Fortokun in Matova and Gasura (2017), the process requires sixteen molecules of Adeno Trisphosphate (ADP) and a complex of enzymes so that Nitrogen bonds can be broken and make combination of Nitrogen. The rhizobia can exist in two states either saprophytic or can exist as host legume forming plant derived growth mass on roots called nodules (Horn and Shimelis,2018). The legume can form nodules after communicating with a compatible rhizobium through release of chemical compounds called flavonoids from the roots.

There are also factors which affects Nitrogen fixation for example pH which was included in this study (Matikiti et al ,2015), in low pH soils the growth of a plant can be reduced indirectly by reducing the number of nodules and it affects genes which produce nodules. Failure to nodulate is due to the acidity of the. Olubunmi (2015) mentioned that few rhizobia grow well in the pH between 4.5 to 5. Cowpea (*Vigina Angulculata*) rhizobia have unique characteristic of being able to form nodules from a broad spectrum of tropical legumes (Ondieki et al.,2016).

Nyaga and Njeru carried out a study on the potential assessment of Native Rhizobia to improve cowpea Growth and Production in Semiarid regions of Kenya. The objective of the research was to come out with native rhizobia isolates which will adapt the Kenyan soils. The effectiveness of the native rhizobia isolates was tested its effects on nodulation growth. There experiments setup had two controls the positive and the negative controls. The results which came out showed that the presence of native rhizobia strains will then overcome the problem of high cost in synthetic Nitrogen fertilizers.

CHAPTER 3

Materials and Methods

3.1 Area of the study

The experiment was carried in the greenhouse at soil Productivity Research Laboratory in Marondera Zimbabwe in Mashonaland East Province. Soil Productivity Research Laboratory (SPRL) in Zimbabwe is a renowned institution dedicated to enhance soil fertility and Agricultural Productivity. It is located in Marondera, it conducts research on sustainable management. Their primary objective includes improving soil productivity, promote efficient nutrient management, and provide expert analytical services. By collaborating with local farmers, extension workers, and stakeholders, SPRL aims to develop and disseminate innovative solutions for optimal crop health. Research station aims and objectives are to provide knowledge, techniques and skills to the agriculture sector as well as performing regulatory and specialist services. The Soil Productivity Research Laboratories constitutes of three sections which are the microbiology laboratory, legume inoculant factory and the chemistry section. The microbiology maintains purification, isolation, characterization of *Rhizobium* strains, *Rhizobia* strain bank, quality control and quality assurance of inoculated packs. Legume biological nitrogen fixation (BNF) is produced in mass to provide famers. The BNF is made of rhizobia which is nitrogen fixing bacteria called rhizobia. With the aids of the microbiology laboratory maintains the largest culture collections of Tropical Rhizobia in the world which accommodates more than 500 strains. The chemistry department is involved with pH test, nitrogen tests, determination of micronutrients, exchangeable cations, and soil analysis and fertilizer recommendations to the farmers. Moreover, the Research officers at the station partake activities such conducting greenhouse, field trials each year to improve and maintain the standard of the strains and legume plants. The activities are performed to attain the mandate of the organization which is to be highly productive and competitive agricultural sector supported by demand driven innovative research and development.

3.2 Arrangement of pots in the greenhouse table

The experiment was completely randomized completely randomized. 6 isolates were be replicated 4 times and two controls (negative control and positive control) which will give 32 pots. Each treatment has for pots meaning that each treatment was replicated four times. The accuracy of results is improved by increasing the number of replications.M1 to M6 are indigenous isolates which have been collected in different places of Zimbabwe on cowpea nodules.

TREATMENT	**POTS NUMBER**
MI	5 10 20 25
M2	6 13 17 27
M3	4 16 22 26
M4	7 9 21 30
M5	3 15 18 28
M6	2 11 23 32
COM276	1 12 24 29
NAGATIVE (-VE)	8 14 19 30

Table 3.1*: The table above shows the arrangement of the pots.*

3.3 **Collection of the soil**

The soil was collected from a field at Soil Productivity Laboratory field and the history of the field which was traced and it showed no interaction with rhizobium innoculants. It is not recommended to use the soil which was inoculated because it increases the amount of rhizobium hence affecting the outcome. The soil was sieved through a 2mm sieve and this was done to remove residues for example grass and leaves. The soil was tested for its pH and this was done to make sure that the soil was in the range which most soils in Zimbabwe. It was good to make the soil was in acidic range because it going to help many farmers since most of the Zimbabwean soil is acidic. Soil was put into clean sacs which were then 121℃ C for 45 minutes to make sure that there was no interaction of the plants with other microbes.

3.4 Preparation of isolates

The isolates were kept in a freezer in the laboratory. They were streaked on the plates using streak plate techniques. After seven days of incubation, they transferred into another plates. Yeast mannitol agar with Congo red was used. Yeast mannitol agar is used because its composition favours the growth of rhizobium isolates and it is also easy to prepare. There is a rule which says anything that absorbs Congo red is not rhizobium. A health culture was taken from plate. A loop was used to transfer under sterile condition in the lamina flow cabinet. The subculturing process was done in the light room to reduce chances of contamination. Yeast Mannitol Agar without Congo red was prepared and poured in slant bottles and lastly allowed to cool. Subculturing was done using a loop and the culture was transferred from the plate to the slant bottle. It can only transfer to the slant bottle after it appears to be clean. It will be then streaked into a slant bottle using a zigzag method. Bottles were labelled according to the isolate which they had.

Fig 3.1 *subculturing of rhizobial isolates in the lamina flow cabinet*

3.5 Preparation of pots

It is good to make sure that the pots are sterilised using 70% alcohol because the contamination of pots can also contaminate the soil that has been autoclaved. The pots were washed with distilled water and then sterilized using 70% alcohol. Pieces of sacs were also cut to and placed in pots to make sure that there was no loss of weighed soil. There pots that have been used on this study have holes beneath. The sacs were washed with distilled water and sterilized. Pots were labelled. Each pot had 7kg of soil.7Kgs of soil will allow root development for six weeks without limit was good enough to allow the root development at six weeks. Single super phosphate was applied to 28 pots except for the pots with negative control because most of the farmers in Zimbabwe apply Single super phosphate. Four pots with negative controls were left without single super phosphate because a negative control was representing a field without rhizobium and fertilizer. Single super phosphate was applied because according to research which was carried out by National Farmers Union (2016) most cowpea farmers use SSP.

3.6 Preparation of the seeds

The seeds were bought from Crop Breeding institute (CBI). Seeds of high quality were treated with 70% alcohol and 3% sodium hypochlorite and rinsed seven times with distilled water. A big glassy petri -dish was spread with cotton and water was poured inside to provide moisture for the seeds. Seeds were spread on the wet cotton and the Petri dish was covered with an aluminium foil and labelled the name of the seed. Planting was done five days after pregermination test. The pregermination test was done and the advantage of using pregermination test it allows one to plant seeds which he or she is sure that they will germinate. Cotton was used to provide the germination area The pots were watered to make sure that the seeds were are not plated on dry soil. Farmers plant cowpea on wet fields because moisture is one of the factors which affects seed germination so in this study it was provided. Watering of pots was done with distilled water to avoid contamination from tap water or any other sources. The inoculum was applied soon after the emergency of the crop. 5ml of the broth which contained a specific strain was injected into the soil near each plant. Each pot has got its own syringe so as to avoid mixing of isolates.

Fig 3:2 A table in the greenhouse shows labelled pots and the plant tags which are in the pot with plant tags which made data collection easy

3.7 Maintenance and management of the pots in the greenhouse

Weeding was done on weekly basis using hands on weekly basis. Farmers remove weeds using hoes or chemicals. Weeds compete with plants for nutrient so if weeds were allowed to grow with, they were going to use the nutrients which were in the soil thereby affecting the growth of cowpea. In this study weeds were removed as they were seen. Inoculation was done after a week of planting and 100ml of inoculant was injected on the plant in the soil a week after germination.10ml of the isolate broth was injected on each plant in the soil and this was done soon after germination.100ml of Nutrient solution was also used to water plants every week. Watering with distilled will done 4 times a week which are Sunday, Tuesday, Thursday and Friday days a week so as to try to provide conditions in the field and 200ml was poured per each pot. Watering with Nutrient solution will be done once a week on Wednesday and 200ml was poured. For security purposes there were two locks on greenhouse gate then the other one was on. When the temperature got too hot water was poured on the floor to cool the greenhouse temperature. There was also a thorough check of the plant tags so as to avoid confusion in recording.

3.8 Harvesting and data collection

After six weeks pots were shaken so as to separate the soil from the pot and also make sure that there was no destruction of the rhizosphere. Spotlight were also watered to make removing easy and the soil around the roots was loosened. The soil that was removed from the plant carefully to avoid destruction of roots. The plant roots were washed carefully. And also avoiding removing nodules. According to Singh, Chamblis and Sharma (2020), the plant parts should be removed so as do measurents easily. Root length was measure using a 30cm ruler and nodule count was also done by counting nodules per plant and biomass fresh weight were taken using a balance (top loa digital balance 0.01 precision). Biomass dry weight was taken after oven drying for 72 hours at 60°C. According to FAO (2015) for dry weight also avoid drying less than 48 hours since there could be water left in the plants or roots. Dry weight was taken on an analytical balance and this was done following a protocol from FAO (2015) which encourages the use of 0.001g balance since weights reduce after drying. This was done in a room since the balance could be affected by outside conditions. All the parameters were recorded in the excel sheet.

3.9 Data analysis

For data analysis one way Analysis of variance (ANOVA) was done using Genstat 18th edition. One way ANOVA was used because it is used when one to know if there is any significant difference between the means of two or more independent groups (Hardason,2018). Also, Fisher's Least Significant Difference is used after performing Analysis of variance (ANOVA). If ANOVA is significant, Fisher's LSD can be used for pairwise comparison between treatments means. Separation of values was done using Fisher's protected LSD at 5 % level of significance.

CHAPTER 4

4.0 Results

Treatment	Nodule number	Root length (cm)	Biomass Fresh weight (g)	Biomass Dry weight (g)
COM 276	26.67^{c}	18.47	2.48	2.37
M1	31.67^{cd}	**20.98**	2.75	**2.83**
M2	**39.67^{e}**	20.42	2.75	2.55
M3	19.00^{b}	20.71	2.8	2.6
M4	36.33^{de}	20.62	**2.92**	2.58
M5	30.67^{cd}	14.57	2.15	1.82
M6	35.00^{de}	20.27	2.26	2.43
ZERO (-ve)	0.00^{a}	18.53	1.71	1.82
P-value	**<0.001**	**0.653**	**0.577**	**0.854**
G.M	**27.38**	**19.36**	**2.48**	**2.37**
LSD	**5.891**	**7.328**	**1.36**	**1.642**
SE	**3.403**	**5.021**	**0.786**	**0.949**
CV %	**12.4**	**25.9**	**31.7**	**39.9**

Table 4.1: *Summary statical table for cowpea data*

Treatments include

The numbers in bold are the best performers

COM276: Positive control which is a known effective commercial rhizobial strain

M1-M6: They are indigenous rhizobial isolates

ZERO (ve): Negative control

4.1Nodule number

The nodulation of plants is the key for nitrogen fixation.M2 had the highest nodule number (39.67), showing a strong symbiosis. The negative control had no nodules. There is significant difference among the treatment for nodule number since (P<**0.001).**

4.2 Root length

M1, M3 and M4 had both the highest root length (20.98,20.65cm and 20.62cm) and it also showed good root system.M5 had the lowest root system.0.653 is the p value greater than 0.05 and this indicates that there is no significant difference among the treatments in terms of root length.

4.3 Biomass Fresh weight /Dry weight

The parameters are there to indicate plant productivity. The isolate M4 had the highest fresh weight (2.92g) and relatively high dry weight (2.58g) thereby suggesting good overall growth. The uninoculated control had the lowest this showed the importance of inoculation. In biomass dry the p value is 0.577 and its greater than 0.05. This shows that there is no significant difference among the treatments in terms of biomass fresh weight. In biomass dry is also greater than 0.05 and this data indicates that there is also no significant variation among the treatment among the treatment for biomass dry weight.

4.4 Overall observations

In biomass fresh weight and biomass dry weight was obtained and in general higher CV percentages indicates higher variability among replicates but there are also other factors which can interfere. Higher CV percentages are common in greenhouse due to uncontrollable environmental factors such as temperature gradient, light heterogeneity, and moisture variation which can lead to increased temperature variability in plant response (Pooter et al.,2015) In this case drying was supposed to be done using an oven but the plant was left in the oven over the weekend and there was poor electricity and could create difference in drying. Higher CVs are common in biological experiments, especially under greenhouse conditions where microenvironmental conditions are not perfect. In this case the greenhouse that was used had open glasses which were tried to be closed but failed to do so. There was interference of outside temperature which were not from the greenhouse. The parameter which shows significance difference is the number of nodules. Numbers which have the same letter are not significantly different and those which have same letters are not significantly different.

Nodule number

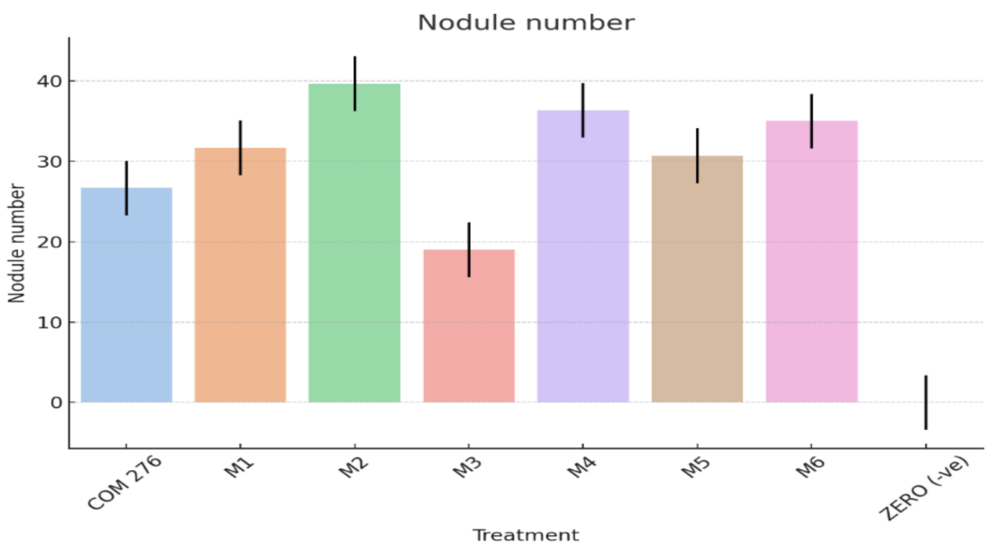

Fig 4.1*: Distribution of number of nodules under different treatments for cowpea*

The number of nodules formed per pot varied significantly across treatments The commercial inoculant (COM276), used as a positive control .M2 recorded the highest nodule count averaging approximately 40 nodules per pot. This was followed closely by M4 and M6, which also exhibited high nodulation. In contrast, the uninoculated control (UNINOC) had the lowest nodule count, confirming the importance of inoculation for effective nodulation. M3 showed relatively low nodulation despite of being an inoculated treatment

Root length (cm)

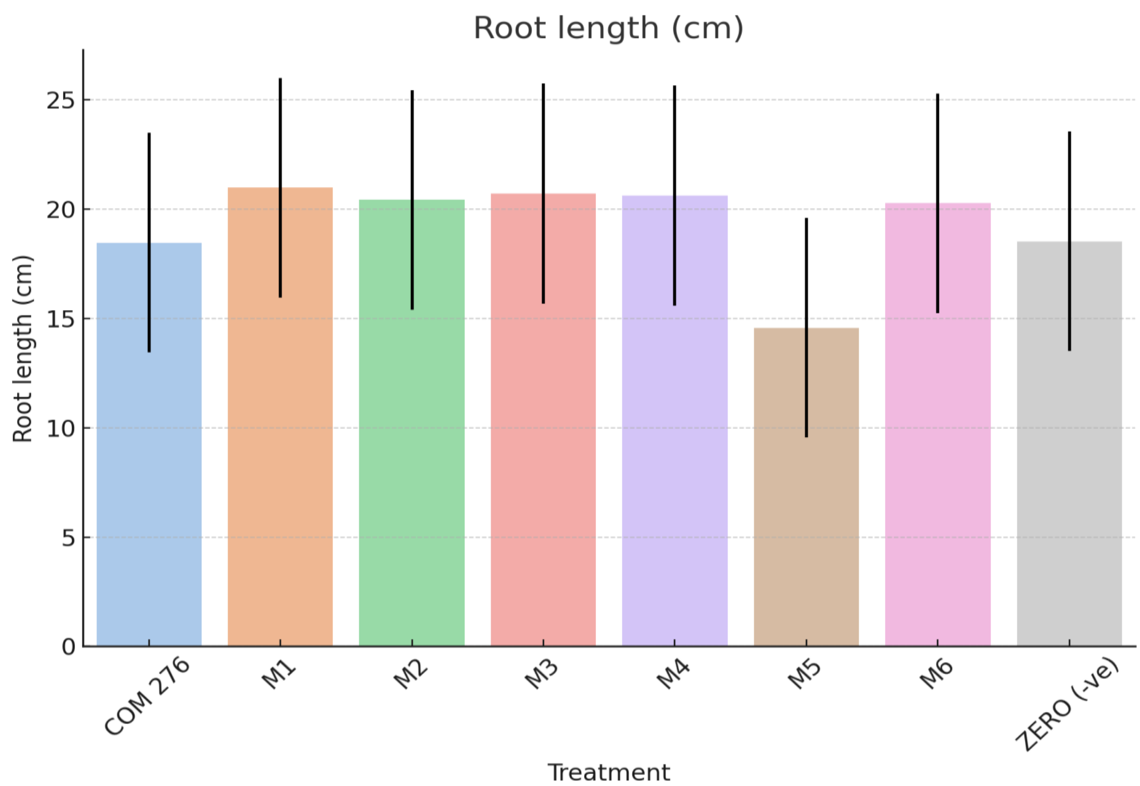

Fig 5.2: *Distribution of root length under different treatments for cowpea*

M1(20.98cm), M4(20.65), M3(20.61) had the longest roots. Long roots can access deeper moisture and nutrient, important under stress conditions. This suggest that these isolates do not improve only nodulation but they also improve root architecture.M5 had the shortest roots (14.57cm) and this shows weak growth promoting capability.

Biomass fresh weight (g)

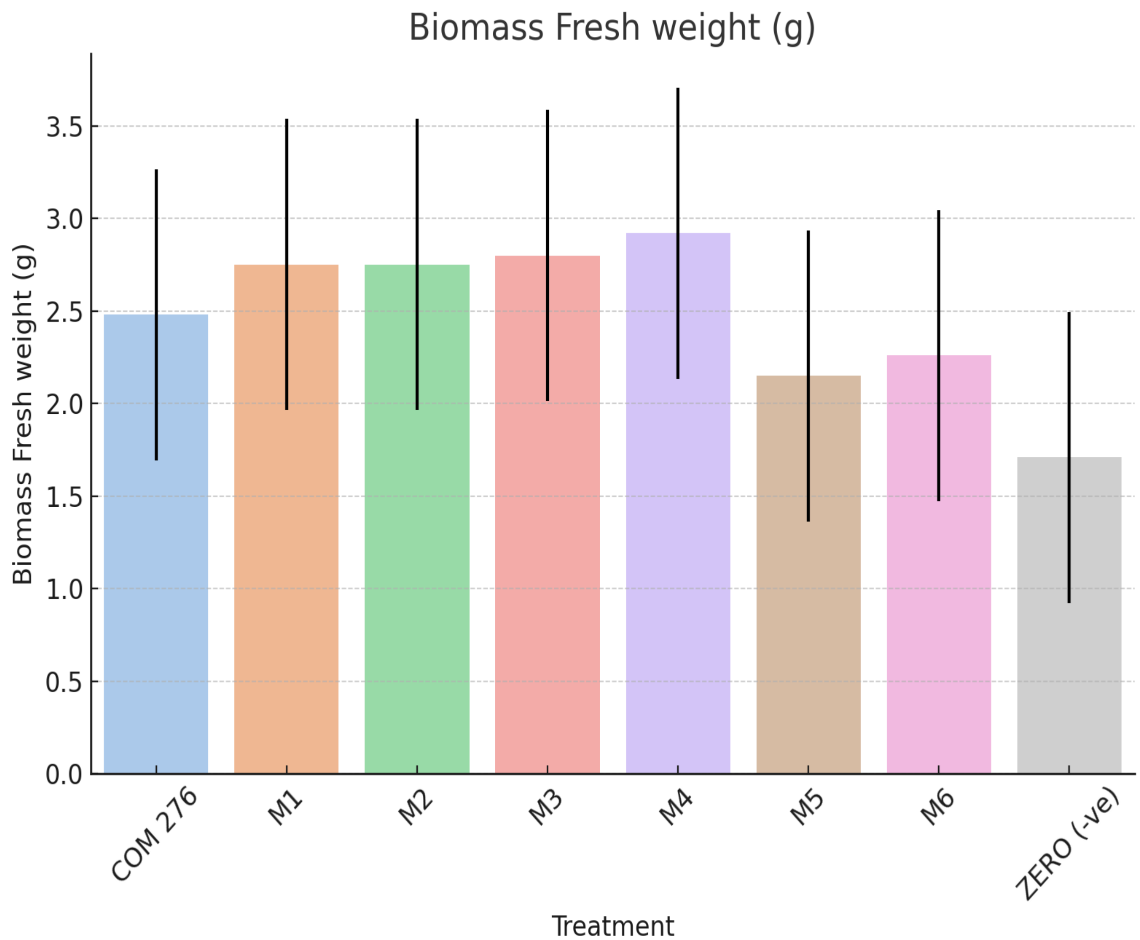

Fig 4.3: *Distribution of biomass fresh weight of cowpeas under different treatments*

Fresh biomass weight followed a trend that, in part, mirrored the nodulation data. M4 resulted in the highest biomass accumulation (3.0g) thereby indicating superior plant growth. Treatment M1, M2 and M3 also produced relatively low biomass weights. Surprisingly despite its low nodule count, M3 still supported high biomass production ZERO which the negative recorded the lowest biomass, thereby reaffirming the beneficial role of rhizobial inoculation.

Plant biomass dry

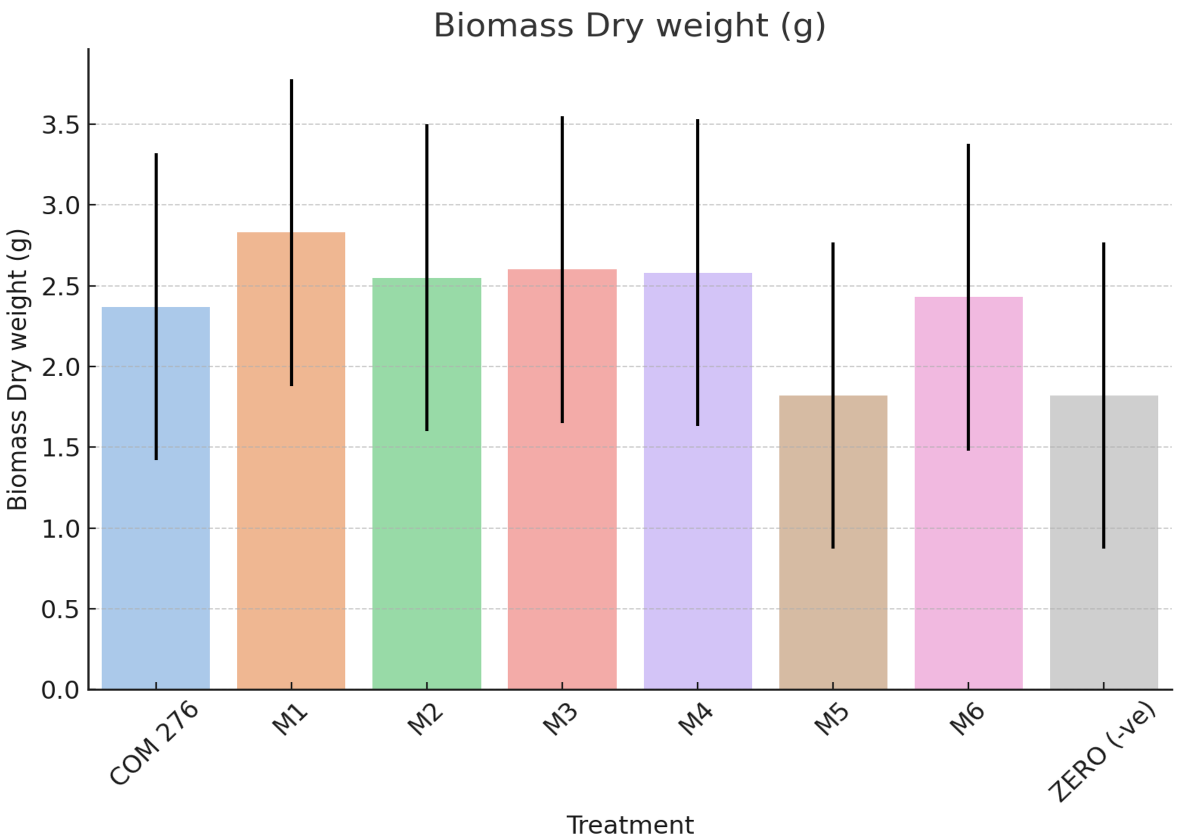

Fig 4.4: *Distribution of biomass dry weight under different treatments*

Biomass dry weight data (Figure 4.2) reaffirmed the trends observed in fresh weight .M1, M2, and M4 has the highest dry biomass accumulation while M5 and M6 were less effective. The uninoculated control consistently showed the lowest dry weight, indicating lowest growth in the absence of rhizobial symbiosis.

CHAPTER 5

5.1: Discussion

The results of this clearly demonstrate the influence of indigenous rhizobial isolates on cowpea (Vigna unguiculata) growth and biomass accumulation under acidic greenhouse conditions (pH 4.5). Treatments M1, M2, and M4 showed consistently superior performance across multiple parameters, including shoot biomass, nodule number, and root length, suggesting that these rhizobial isolates possess strong plant growth-promoting traits and effective symbiotic nitrogen fixation capabilities. According to the research which was carried by Nyaga and Njeru (2020) the application of native rhizobium strain also showed better results this also give emphasis on the use of indigenous rhizobium isolates. Again, Nyaga and Njeru (2020) mentioned that in the study which they carried, the native rhizobium strains significantly increase nodule number, nodule dry weight, plant dry weight and root length compared to the negative control

Among all treatments, M2 recorded the highest nodule number (39.67), root fresh weight and dry root weight (0.64 g), showing its high compatibility and effectiveness in promoting rhizosphere activity and nutrient acquisition.M2 has shown highest nodule number but has also did not lead in plant fresh weight and plant dry weight. This shows nodule number also shows that nodule number alone may not reflect the plant yield (Gyogluu et al.,2018). According to Abdyl and Keyser (2010) some plant form large number of nodules bus shows poor Nitrogen fixation ability M1 and M4 also significantly improved biomass production, root length, and nodulation, outperforming the commercial strain COM 276 in several parameters. This highlights the potential of indigenous strains to outperform commercial inoculants when adapted to local environmental conditions.

The highest biomass was obtained in indigenous rhizobia isolates because the use of indigenous isolates enhances plant growth leading to increased biomass agreeing with (Jalloh at el, 2020, Matse at el., 2020). The high biomass in indigenous isolates is due to the production of plant - growth promoting hormones (Mabrouk et al.,2018). According to Stajkovic (2011), the symbiotic of rhizobium innoculants in the root nodules is improved by the effectiveness of the isolates to fix Nitrogen into ammonia.

In contrast, the uninoculated control (ZERO) frequently recorded the lowest values in nearly all measured parameters. The complete absence of nodules in this treatment confirms the absence of native rhizobia activity under the acidic pH and supports the need for inoculation in such soils. M5 performed poorly across several parameters, particularly in root weight and length, suggesting either a weak strain or poor plant-microbe compatibility. According to the research which was carried out by Nyaga and Jeru (2020) nodules were obtained in the uninoculated but in this research project there was no nodulation on the uninoculated this shows that there are some factors which could have affected nodulation in the uninoculated pots.

The high coefficients of variation (CV%), reflect details variability in plant responses. This could be attributed to biological variability among rhizobia strains or subtle differences in greenhouse microenvironments (Gerrano, Rensburg and Adebola,2018). Zhalnina et al (2018) mentioned that the variability of microbiota contributes significantly to data variation and also in the inoculation studies that involve symbiotic organisms like rhizobia. However, the significant treatment effects, as indicated by the low p-values (<0.001) for key traits such as nodulation, root weight, and length, confirm that the differences observed are due to the inoculants rather than random variation. Higher CV percentages were also obtained from a thesis which was done by Esnart Nyirenda (2016) titled assessment of abundance and effectiveness of cowpea (*Vigna Unguiculata*) rhizobia in soils from different field in Chiwosya extension planning area, Mchinji district and this shows that higher CV percentages are common biological experiments.Overally, the data suggest that specific indigenous rhizobial isolates, particularly M2, M1, and M4, have the potential to improve cowpea productivity under acidic conditions. These isolates should be evaluated under field conditions so as to validate their effectiveness and possibly be developed into locally adapted biofertilizers.

5.2 Summary of the results

The greenhouse experiment was conducted to assess the effectiveness of indigenous rhizobial isolates on the growth and biomass of cowpea under acidic) conditions showed variations across treatments for cowpea. The variation across the parameters is shown by the difference between the minimum and maximum values (Ngata and Mwangwela,2019). The results indicate that the application of selected native rhizobia significantly enhanced cowpea growth parameters when compared to the negative control which was uninoculated control and even to the commercial inoculant, COM 276.Nkomo (2020) mentioned that the use of native rhizobium isolates in Buhera District gave a better harvest compared to the use of innoculants from other countries and this supports the results of this project although high CV percentages were obtained. According to Buhera District Food and Nutrition security (2022), the soils in Buhera are also acidic which makes these results useful in areas like Buhera, Bocha and Marange.

Among all the treatments, M2, M1, and M4 consistently produced superior results across multiple traits. M2 exhibited the highest number of nodules (39.67), the heaviest root fresh weight (0.943 g), and a high root dry weight (0.64 g), suggesting excellent symbiotic efficiency and robust root development. M1 showed the highest root length (20.98 cm) and one of the highest biomass dry weights (2.83 g), while M4 led in biomass fresh weight (2.92 g) and maintained high nodulation and root development values. These three isolates demonstrated strong growth-promoting potential and adaptability to acidic conditions (pH 4.5), indicating their promise as effective bio-inoculants.

In contrast, ZERO (-ve), the uninoculated control, frequently had the lowest performance in all growth and yield-related traits. The absence of nodules in this treatment further validates the importance of inoculation under low-pH soil conditions. M5 also showed suboptimal performance, particularly in root length, indicating either poor adaptation or a weak symbiotic relationship with the host plant. There are also other factors which should be taken into consideration for M5 to have the shortest route. Kamara et al (2020), mentioned that soil compaction, inadequate watering, nutrient defencies and soil potential Hydrogen imbalance can also least to poor root development. During harvesting the pot showed one of the pots showed less moisture content and the reason was that the pot was aligned with a hole in the greenhouse therefore there was interference of outside temperatures. In terms of adequate watering, it could be also the reason since sometimes the greenhouse received high heat and it was noticed later. The remedy of high temperature was to pour cold water in the greenhouse floors and pH imbalance could be due to ununiform watering of the pots and it’s a common human error. Omoigui, Kamara and Ishiyaku (2017) mentioned that also variety genetic predisposition could affect root length and it could be another factor which affected indigenous rhizobial isolate M5.

Statistical analysis verified the differences among treatments were highly significant ($p < 0.001$) for a key parameter such as nodule number of nodules. Again Omoigui, Kamara and Ishiyaku (2017), said that when carrying out cowpea projects nodule number and size should also be taken into consideration since nodules can indicate the effectiveness of symbysiosis.In. In this research since nodule numbers showed significant difference it then shows the effectiveness of rhizobial isolates. This underlines the effectiveness of specific isolates in improving cowpea growth. Zaharan (2001), mentioned that they are many factors to be considered which affect the CV percentage for example host plant genotype. Legumes which are of the same species may vary their responds to specific rhizobial strain and this could have cause high CV percentage. Abiotic stresses and soil heterogeneity greatly affect nodulation and biological nitrogen fixation to inconsistent experimental results (Hungria and Vargas,2010) and according to Afonin et al (2022), manual errors in biomass weighing, nodule count, and plant handling can introduce significant variability across replicates. However, high coefficients of variation (CV%), particularly in plant fresh biomass and dry biomass, suggest notable variability in plant responses, which may be attributed to biological variability among isolates or microenvironmental differences within the greenhouse.

Overall, the results demonstrate that specific indigenous rhizobia isolates possess considerable potential to enhance cowpea growth and productivity in acidic soils. Their performance surpassed that of the commercial strain in several aspects, emphasizing the importance of locally adapted microbial solutions for sustainable agriculture.

5.4 Recommendations

1.Field Trials: The top-performing isolates (M2, M1, and M4) should be tested under field conditions to validate their performance beyond controlled greenhouse environments.

2. Soil pH Management: As soil acidity negatively affects nodulation, future research should explore liming or other soil amendment strategies in combination with inoculation to boost effectiveness.

3. Strain Characterization: Further biochemical and molecular analyses are recommended to understand the genetic diversity and functional mechanisms of the most promising rhizobial isolates.

4. Formulation Development: Develop user-friendly and stable biofertilizer formulations based on the top indigenous isolates for agricultural use.

5. Farmer Awareness: Promote farmer education and outreach programs to increase awareness of the benefits of native inoculants and proper application methods.

6. Policy Integration: Encourage the inclusion of native microbial inoculants in agricultural policy frameworks as part of integrated soil fertility and sustainable farming strategies.

5:3 Conclusion

This study has demonstrated that specific indigenous rhizobial isolates—particularly M2, M1, and M4—significantly enhance the growth and biomass production of cowpea (Vigna unguiculata) under acidic greenhouse conditions. These isolates outperformed the commercial strain (COM 276) and the uninoculated control, indicating their strong potential as effective biofertilizers. Their ability to promote nodulation, increase biomass, and improve root development suggests they are well-adapted to the local soil environment. The absence of nodulation in the uninoculated control further emphasizes the importance of rhizobial inoculation in low-pH soils. Overall, these findings support the use of locally sourced microbial resources as sustainable and eco-friendly tools to enhance legume productivity.

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APPENDICES

Appendix 1: Preparation of Yeast Extract Mannitol Agar with Congo red indicator.

Procedure

The following chemical were added

- Solution P 10ml
- Solution Q 10ml
- Solution D 1ml
- Mannitol 10ml
- $CaCO_3$(AR) 0.18g
- Agar (Oxoid) 15g
- Dist. H2O 879ml
- Yeast Water 100ml

Media was boiled for 1 hour. After 1hour 10ml of Congo red indicator was added. Dispensing 12.5ml into tubes and sterilize for 30 minutes

Preparation of Congo red solution.

- 1g of Congo red was dissolved into 400ml Distilled water.

1. Preparation of Congo red plates.
2. Subculture of strains.

Autoclaving of discards at 120^0C for 15 mins.

2.Preparation of stock solutions

- 50g K_2HPO_4
- 20g $MgSO_4$ and 10g Nacl
- 1g Fecl

They were mixed with 500ml distilled water into separate Duran bottles

Appendix 2: Preparation of stock solutions

- 50g K_2HPO_4
- 20g $MgSO_4$ and 10g Nacl
- 1g Fecl

They were mixed with 500ml distilled water into separate Duran bottles

- 5.Autoclaving Discards
- Cleaning the laboratory

Appendix 3: Agar plates pouring plates pouring techniques under sterile conditions