BINDURA UNIVERSITY OF SCIENCE EDUCATION

FACULTY OF AGRICULTURE AND ENVIRONMENTAL SCIENCE

DEPARTMENT OF CROP SCIENCE

EFFECTIVENESS *OF Bradyrhizobium japonicum* (MAR 1305) INOCULATION IN DIFFERENT SOIL pH LEVELS ON GERMINATION PERCENTAGE AND YIELD PERFOMANCE OF SOYABEAN

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BINDURA UNIVERSITY OF SCIENCE EDUCATION

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RESEARCH PROJECT

DECLARATION

I, Melissa Chimhuka do hereby declare to the Senate of Bindura University of Science Education that this research project is a result of my original research work. It is being submitted for the fulfillment of the Bachelor of Agricultural Honours Degree in (Crop Science) and that to the best of my knowledge, the findings has neither been submitted nor being concurrently submitted in any other institution before.

Name: Melissa Chimhuka

Signature ……………………………… Date …25/05/2023………………

I have supervised the research project for the above mentioned and I am convinced that the research project:

a) Can be submitted
b) Project Supervisor Signature …

Date: 25 May 2023

ii) …………………………………………. Signature …………………………………

I certify that I have checked the Research project and I am satisfied that it confirms to the Department of Crop Science guidelines for project preparation and presentation. I therefore authorize the student to submit this dissertation for marking.

Quality

Controller …………………………………..Signature ……………………………………..

DEDICATION

I dedicate this project to Mr. Choto.

ACKNOWLEDGEMENTS

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ABSTRACT

Zimbabwe is experiencing an increase in Soyabean (*Glycine max*) production as a result of its contributions to poverty alleviation, food and nutrition security, and as a major foreign currency earner. However, productivity is still declining due to decreased soil fertility and expensive inorganic fertilizer prices. As a result, a study was conducted in the 2022/2023 cropping season at Blackforby Agricultural College to investigate the effectiveness of *Bradyrhizobium japonicum* MAR 1503 inoculation in different soil pH levels on soya beans germination % and yield performance. All the treatments were tested for germination %, and yield performance. The experiment was designed using a randomized complete block design (RCBD), with six treatments replicated three times, (*Bradyrhizobium japonicum* inoculation in Neutral soils pH) (*B. japonicum* inoculation in Acidic soils pH 5.5), (*B. japonicum* inoculation in Alkali soils pH 8) and (without inoculation in Neutral soils pH), (without inoculation in Acidic soils pH 5.5) and (without inoculation in Alkali soils pH 8) were the treatments. The data was analyzed using ANOVA with GENSTAT 18th edition, and LSD ($P<0.05$) was used to distinguish between significantly distinct means. There was no significant different ($p < 001$) on germination % which shows no interaction between *B. japonicum* and soil pH, as soybean inoculated with *B.japonicum* in neutral pH performed better than in acidic and alkali pH treatments. *Bradyrhizobium japonicum* in Neutral pH and treatment without *B. japonicum* in Neutral pH germinated first before other treatments. There were also significantly differences in final yield components between inoculated treatment in different pH levels and the uninnoculated treatments in different pH levels ($P<0.05$). Results showed that combination of *Bradyrhizobium japonicum MAR* 1503 and Neutral pH produced high quality and quantity soyabean.

LIST OF ABBREVIATIONS AND ACRONYMS

ADP:	Adenosine Diphosphate
ANOVA:	Analysis of Variance
ATP:	Adenosine Triphosphate
BNF:	Biological Nitrogen Fixation
Ca:	Calcium
CEC:	Cation Exchange Capacity
DR&SS:	Department of Research and Specialist Services
FAO:	Food and Agriculture Organization of United Nations
Fe:	Iron
HCL:	Hydrochloric Acid
ha:	Hectare
K:	Potassium
LSD:	Least Significant Difference
m:	Meter
Mg:	Magnesium
N:	Nitrogen
N_2:	Dinitrogen
NAD:	Nicotinamide Adenine Dinucleotide
NADH:	Reduced Nicotinamide Adenine Dinucleotide
NO_3:	Nitrate
RCBD:	Randomized Complete Block Design

SPRL: Soil Reproductive Research Laboratory

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 Background

Soyabean (*Glycine max*) is an important grain legume for enhancing food and nutritional security in Sub-Saharan Africa due to its high protein and oil content (Mpepereki et al., 2021; Giller, 2008). It is becoming an important cash crop or economic crop for most farmers in Zimbabwe because of the readily available market (Kainga and Mpepereki, 2021). Soyabean can be grown as a green manure and cover crop because it grows quickly and contributes nitrogen to the soil. Nitrogen availability is probably the second most limiting factor in agricultural production, second only to water availability (Date, 2000).

The decrease in the soya bean production have been caused by soil fertility decline which is considered as one of the major limitations in growth and yield improvement of soya bean (Buresh *et al.*, 2004). The other practices that limit the increase in yields are the absence of effective rhizobia in the soil and changes in soil pH due to high rainfall which causes leaching of soil nutrients and the flow of water during erosion which contains $CaCO_3$ or $MgCO_3$ from illegal miners in Mashonaland Central of Zimbabwe (Mpepereki et al, 2021).

Keeping the pH of soil between 5.5 and 7.0 enhances nutrients' availability such as nitrogen and phosphorus, the ability of microbes to breakdown crop residues and symbiotic nitrogen fixation (Ferguson et al., 2006). Rienke and Joke (2005) reported high yields in loamy textured soil, and that if the seeds are able to germinate, they grow better in clayey soil.

However, soyabean improves soil fertility by fixing nitrogen (N) through a symbiotic association with soil rhizobia through a process known as Biological Nitrogen Fixation (BNF). The most common for soyabean is Rhizobium inoculation which is an agronomic practice that improves some soil health parameters and enhances overall grain legume productivity (Gopalakrishnan et al., 2015).

The use of Rhizobium inoculation is a means of improving the growth and yield of soyabean since studies have shown that rhizobium inoculation can lead to increase in the nitrogen content in the soyabean plant, which increases in the growth and yield of soyabean. (Kumar et al, 2019).

A study conducted by Kumar et al. (2019) compared the growth and yield of soyabean that has been inoculated with rhizobia to those that had not in slightly acidic soils, they found that the plants that had been inoculated had significantly higher yields than those that had not been inoculated. Another study by Chaudhury et al. (2021) found that rhizobium tropics inoculation significantly increase the growth and yield of soya beans grown in low nitrogen soils in acidic soil, suggesting that the Rhizobia tropic strain was successfully fixing nitrogen from the atmosphere.

Another study by Braulio R et al (2020) on the effectiveness of *Bradyrhizobium elkanii* inoculation in soyabean in different pH levels shows that the strain has higher yields in acidic soils. The aim of the study is to investigate the effectiveness of *Bradyrhizobium japonicum* (MAR 1305) inoculation in different soil pH levels on germination percentage and yield performance of soyabean.

1.2 Problem Statement

Farmers have been realizing low crop stains because of changes in soil pH due to high rainfall which causes leaching of soil nutrients and the flow of water during erosion which contains $CaCO_3$ or $MgCO_3$ from illegal Miners in Mazowe area, however the effectiveness of *Bradyrhizobium japonicum* can be influenced by soil pH thus the symbiotic relationship between the roots and the bacteria requires an optimum pH for improved nitrogen fixation and plant growth. However, there is an information gap on the optimum soil pH required for the performance of *Bradyrhizobium japonicum* MAR 1305 inoculation in soyabean (*Glycine max* L. Merill) and the aim this study is to found out the ideal soil pH for the performance of this strain in Mazowe area Mashonaland Central where the soyabean production is increasing.

1.3 Justification

Nitrogen is a significant limiting nutrient element in most soils in terms of soyabean growth and productivity due to the high cost of N fertilizers, however farmers can resort to bio fertilizers like the use of *Bradyrhizobium japonicum*, which is both cost-effective and environmentally friendly (Kumar et al, 2019). This necessitates the availability of information on soil conditions which favors the symbiotic relationship between the bacteria and the soyabean roots to improve N fixation. The results of this experiment will provide more information on the Biological Nitrogen Fixation performance of the *Bradyrhizobium japonicum* strain, in different soil pH levels in order improve germination % and yield.

1.4 Objective

The overall objective of this study is to assess the inoculation response of soyabean to a *Bradyrhizobium japonicum* strain in different soil pH levels.

1.4.1 Specific Objectives

1. To determine the effects of *Bradyrhizobium japonicum* inoculation in different soil pH on germination percentage of soyabean.
2. To determine the effects of *Bradyrhizobium japonicum* inoculation in different soil pH on yield components of soyabean.

1.5 Hypothesis

1. H_O Different soil pH levels have a positive effect on the germination percentage inoculated soyabean.

   H_1 Different soil pH levels do not have a positive effect on the germination percentage inoculated soyabean.

2. H_O Different soil pH levels have a positive effect on the growth performance and final yield of inoculated soyabean.

   H_1 Different soil pH levels do not have a positive effect on the growth performance and final yield of inoculated soyabean.

CHAPTER 2

LITERATURE REVIEW

2.1 Importance of soyabean

Soybean (*Glycine max (L.)* is an important legume grown world-wide and accounts for nearly 50% of the world's area under legume cultivation (Herridge et al., 2008). It is also one of the most traded legumes in the world accounting for over 84.5% of the traded grain legumes (Murage et al., 2019) due to its nutritional importance as a key source of vegetable oil and protein for human food and supplementary animal feeds (Abou-Shanab et al., 2017 ;Da Silva Júnior et al., 2018). Thus, soybean has contributed greatly to human health and socio-economic well-being of low-income rural populations across the globe (Maphosa & Jideani, 2017; Masciarelli et al., 2014). It can also be used for industrial use in terms of paints and cleaning products, as biodiesel and as high performing engine oil (Murage et al., 2019). Soya bean conserve soil moisture due to the early maturity of soyabean and the canopy cover (SeedCo, 2023).

2.2 Soil fertility challenges and their solutions

Hartemink (2017) defined soil fertility as the quality of a soil that enables it to provide nutrients in adequate amounts and in proper balance for the growth of specified plants or crops. A fertile soil is characterized by its ability to supply essential plant nutrients and water in adequate amounts and proportions for plant growth and reproduction; and the absence of toxic substances which may inhibit plant growth (Ullah et al., 2008). Soil degradation is one of the main challenges affecting agricultural sectors in many countries in Africa, with an estimated 65% of the arable land in Sub-Saharan Africa noted to be damaged, leading to low productivity (FAO, 2020). The soil degradation is characterized by nutrient depletion, soil compaction, salinization, soil erosion and even organic matter reduction (Lal, 2015). On average, the Sub-Saharan lands lose about 22kg N, 2.5kg P_2O_5 and 15kg K_2O per hectare annually (Stoorvogel and Smaling, 2019). The loss of nutrients has been found to be due to under-replenishment of nutrients and reduced fallows. The degradation of soils and poor soil health lead to food and nutrition insecurity (Singh and Ryan, 2015) and inefficiencies in terms of the input returns such as inorganic fertilizers, labour and seeds in smallholder farmers (Mekuria, 2020).

Many solutions have been proposed to tackle the challenges of low soil fertility for improved crop productivity. These include the use of inorganic fertilizers and organic fertilizer, and integration of BNF in legumes. Most smallholder farmers do not have the finances to purchase the fertilizers (Alliance for Green Revolution in Africa, 2014). Due to population increase and environmental degradation, there has been researches for sustainable agriculture intensification technologies that enhance agricultural productivity, protect ecosystem and upgrade their flexibility (Lampkin *et al.*, 2015).Vanlauwe *et al.*, (2015) suggested the use of integrated soil fertility management as a respectable measure of attaining sustainable agriculture intensification whereby agronomic efficiency is also considered for soil fertility improvement.

According to Sulieman (2015), legumes possess great potential for use in soil stabilization, reforestation, and agricultural practices. Legume crops have important agronomical and environmental advantages due to their capacity to reduce molecular atmospheric nitrogen ($N_{2)}$ to ammonium via symbiotic N_2 fixation (Edgerton *et al.*, 2008). Nitrogen fixation comprises the decline of the inert dinitrogen gas (N_2) into ammonia (NH_3) in the presence of the enzyme nitrogenase (Brady and Weil, 2008). The BNF process contributes to increase of nitrogen in the soil and improves soil fertility. Benefits of legume N_2 fixation include soil fertility upgrading (mostly through legume plant residues left after harvesting), reserves the fertilizer costs and improved protein nutrition (Mpepereki,2003). Nitrogen fixation technologies acceptance in crop production is significant because decline in soil fertility is a huge problem in parts of Sub-Saharan Africa. It is an extensive limitation in growth and yield development in maize based-cropping and farming systems throughout East and Southern Africa (Mekuria et al., 2004).

Inoculants are required to enhance yield and quality of the produce and maximizing the economic returns for soyabean. Even though the need of using rhizobia in every year is still a question, in many studies.

2.3 Summary of series which occurs during biological nitrogen fixation

According to Giller (2018) the equation (i) summarizes series that occurs during biological nitrogen fixation mainly the biochemical process:

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16\ ADP + 16\ Pi$$

ATP generation and coupling are possible targets for improvement as it is used as the source of energy for reduction of N_2 into NH_3 (Brady and Weil, 2008). Nitrogenase in the *Bradyrhizobium japonicum* acts as a complex which has two protein components (Nelson and Cox, 2018). One of the components has the active site where N_2 is reduced; this active site is called iron-molybdenum cofactor or the dinitrogenase (Cheng, 2008). The other component which allows the reinsertion of electrons to the first component for N_2 reduction to occur is called the Fe protein or dinitrogenase reductase (Sylvia *et al.,* 2005). The presence of ATP molecules in the process contributes the energy for breaking the triple bond of N_2 and allows the hydrogen atoms to combine with the N atoms.

According to Kenston Oliver Willard Njira's research (2016), biological reduction of atmospheric N_2 to ammonia (NH_3) (N_2 fixation) provides about 65% of the biosphere available N. Large amount of ammonia is produced by legume-rhizobia symbioses, which is enhanced by the infection of the legume, this may results in the formation of root nodules which is the site where the nitrogen fixation process take place (Logwig *et al*., 2003). (C Masclaux-Daubresse 2010) research also states that, it should be noted that nitrogen from other sources such as fertilizers also dissociate into NH_4^+ and NO_3^-, which eventually are also taken by plants. NH_3 is mostly contained in the nodules of the legumes where it is easily assimilated by the plants (Serraj, 2004). Plants use nitrogen for biosynthesis, mostly when it is in a reduced state (McCashin, 2000). Nitrate reductase are the enzymes which mainly catalyze or initiate the conversion NO_3^- to NH_4^+ and NO_3^- to NO_2^- , this occurs in the cytosol of the leaves, and enzymes nitrite reductase involve in the conversion of NO_2^- to NH_4^+ mainly translocated to the chloroplasts.

2.4 Importance of legumes in improving soil fertility

Nyemba and Dakora (2010) and Ojiem *et al*., (2007) suggested a range of 33 to 124kg N ha^{-1} of BNF by groundnuts in their report, and Egbe *et al*.,(2007) and Njira *et al*.,(2012) suggested a range of 20 to 124kg N $ha^{-1}$by pigeon pea in their report from the studies which were researched within Sub-Saharan Africa. These studies have shown that the process of BNF contributes to the increase of N in the soil and enhances soil fertility improvement. Practicing of legume N_2 fixation technologies in the production of crops is significant because a decline in soil fertility has been a major problem in parts of Sub-Saharan Africa. Generally it has been reflected as a common drawback in growth and yield improvement in many farming systems in the Southern Africa (Buresh *et al*., 1997). Soil fertility can be amended by the use of mineral fertilizers, however their usage by poor resources farmers in particular the smallholder farmers is restricted by the high prices which are beyond their capabilities to purchase them, resulting in the lowest yields of production (Druilhe and Barreiro-Hurle, 2012). Palm *et al*., (2003) reported the clarifications to smallholder farmers' soil fertility complications that they may be attained through tactical combination of organic means, mainly from N_2 fixing legumes, with small quantity of mineral fertilizers, which is the integrated soil fertility management.

2.5 Factors affecting the response of legumes to inoculation and biological nitrogen fixation.

2.5.1. Temperature

Elevated temperature has a direct effect on nodule production and function, as well as an indirect effect on host plant metabolism (Hungria and Vargas, 2000; Aranjuelo et al., 2008). Kabauma (2013) asserts that temperature plays a very important role in nodulation survival and persistence of rhizobial strains in the soil. Temperatures above 35^0C kills the rhizobia and temperature around 8^0C inhibit effective nodulation and nitrogenase activities. One of the indirect impacts of high temperature is the reduction of photosynthetic activity (Islam, 2015) caused by stomatal pores closure (Aranjuelo et al., 2014) under increasing carbon dioxide (CO_2) and temperature Under high temperatures and high CO_2 levels, plant leaves' photosynthetic efficiency decreases, resulting in the inhibition of Rubisco activity, which causes a reduction in N assimilation and reduced carbohydrate concentration in the nodules, resulting in inefficient N fixation (Aranjuelo et al., 2008, 2009). Furthermore, high temperatures increase respiration rate, resulting in less substrate availability for the nodules, leading to nodule senescence (Mohammadi et al., 2012). Optimum temperature must around 20-25^0C (Bordeleau and Prevest, 1994). Temperature also affects all enzymatic reactions.

2.5.2. Moisture soil status

Soil moisture stress reduces nodule weight and nitrogenase activity, affecting the N2 fixation process (Siczek and Lipiec, 2011; Dabessa et al., 2018; Santachiara et al., 2019). Drought is one of the most significant environmental factors affecting the survival of N2-fixing bacteria and the nodule initiation stage because plants require more appropriate moisture during this stage than other phases (Monica et al., 2013). Despite the fact that some rhizobial populations exist in desert soils and are effective at nodulation, viable strains cannot survive or function in high moisture environments (Lucrecia et al., 2003; Bashan et al., 2014; Dabessa et al., 2018).Too much moisture content and water logging prevent the development of root hairs and sites of nodulation and interfere with a normal diffusion of oxygen in the root system.

2.5.3. Mineral Nutrition

The symbolic relationships of the legume and the rhizobia are also negatively affected by nutrient deficiencies and toxicities (Chalket al. 2010). Miguez-Montero et al, (2020) assert that low levels of phosphorus in the soil reduce Biological Nitrogen Fixation, delays nodulation and infection of the primary roots. Aluminum and manganese toxicity and low levels of calcium inhabit growth of rhizobia and nodulation. The aluminum and manganese toxicity is caused by soil acidity. Manganese toxicity results in the inhibition of calcium (Jaiswal et al., 2018).

2.6 Effects of *Bradyrhizobium japonicum* on soyabean

Bradyrhizobium japonicum is a common strain of nitrogen-fixing bacteria that forms a symbiotic relationship with the soya bean plant. The bacteria infect the soyabean plant's root nodules and convert atmospheric nitrogen into a form the plant can use for growth and development. Several studies have shown that inoculating soyabean seeds with *B.japonicum* increases plant growth and yield significantly.

According to Li et al. (2019), *B.japonicum* inoculation increased yield by 26.3% when compared to non-inoculated plants. *B.japonicum* inoculation increased soyabean biomass by 37% and nitrogen fixation by 50% when compared to control plants (Serrano-Serrano et al. (2018). Overall, these findings indicate that *B.japonicum* inoculation can be an effective method of increasing soyabean growth and yield.

2.7 Effects of pH levels on the growth of soyabean

The pH level of the soil can have a significant impact on soyabean growth and yield. Soybean plants may experience stunted growth, significantly reduced nodulation, and decreased yield. Prevost and Bordeleau (1994), when the pH of the soil is too high, in alkaline. Soyabean plant may experience reduced growth rate and yield, as well as increased susceptibility to diseases and pests. Suleiman and Hago (2009).

Microbial processes such as breaking down of organic matter and also death of soil microbes which improves soil fertility is caused by acidity in soils and can potentially cause the death of the plants (S Matsumoto., 2017). Acidic soil affects roots, reducing water and nutrient intake and reducing the life of soil microbes (Kidd and Proctor, 2001).

Furthermore, soil acidity has been shown to have a negative effect on the BNF process by modifying cell-membrane permeability induced by excess H^+ which increases cation efflux and hence reduces nutrient availability to plants (Ferreira et al., 2016). Soil alkalinity lowers rhizobia abundance, activity, and diversity (Li et al., 2011; Zhang et al., 2017, 2018). Extreme soil acidity and alkalinity both hinder the growth and process of nodule initiation of rhizobia in the soil (Slattery et al., 2001; Azcon and Barea, 2010; Gómez Padilla et al., 2016).

2.8 Causes of soil Acidity

Leaching of cation by heavy rainfalls as CO_2 dissolved in water to produce H_2CO_3 which is carbonic acid which removes the basic cations that is Mg^+, Na^+ , Ca^{2+} , K^+ from alkaline soil and form bi-carbonate which are soluble in water. These bi-carbonate are leach down due to heavy rainfall and causes soil acidity. (Md Mosaddekur., ND) Also decomposition of organic matter in soil produce different types of organic acid for example Carbonic acid. These organic acid produce H^+ which can create soil acidity (Graham et al, 1994).

2.9 Causes of Soil Alkalinity

Rich rocks with Calcium Carbonate form alkali soil after weathering and breaking down. (Master Class, 2021).Higher amounts of exchangeable Sodium (Na^+) ions increases pH (Lajos et al, 2014). The natural cause is the availability of Sodium Carbonate and Potassium bicarbonate during weathering (Kavipriya, 2021).

2.10 Effects of Dolomotic lime on *Bradyrhizobium japonicum*

Dolomitic lime, which contains both calcium carbonate and magnesium carbonate, can tentially affect the activity of *Bradyrhizobium* bacteria in the soil. Application of dolomitic lime can reduce the survival and activity of *Bradyrhizobium* whilst moderate amounts may actually enhance their activity on nodulation of legume plants.

2.11 Effects of Dolomotic lime on soyabean (*Glycine max*)

Calcium carbonate and magnesium carbonate, can have a varying effects on the nodulation of soyabean plant by rhizobium depending on the application rate and soil pH thus addition of dolomitic lime can increase nodulation and nitrogen fixation thereby increasing the growth rate and yield, Mahmud (2022).

CHAPTER 3

METHODOLOGY

3.1 Study site

The experiment was carried out at Blackforby Agricultural College situated approximately 60km north west of Harare on Klein Kopjes Farm in Concession, Mazowe District, Zimbabwe. Its geographical coordinates are 17°22'59"S 30°56'59"E (Online google maps, 2022), which is located in Natural Region IIA of the Agro-ecological zones of Zimbabwe. The minimum and maximum temperatures for the area are 23^{o}C and 30^{0}C respectively and an average rainfall of 750 mm-1000 mm per year. The soil fertility analysis results Soils are reddish brown loams with moderate to low water holding capacity with a pH ranging of 5.5 on the Calcium Chloride scale.

3.2 Experimental design and field layout

The research was a 3 X 2 factorial experiment which was laid out as a Randomized Complete Block Design (RCBD). The trial was replicated three times with a total of 18 treatments. The first factor was soil pH which had three levels; acidic, alkaline and pH modification. The second factor was inoculation which was at two levels; inoculated and un-inoculated treatments. Slope was the blocking factor.

Table 3.2 Summary of treatments of the experiment

	Inoculation with *Bradyrhizobium japonicum* MAR 1503	
Soil pH	**Inoculated**	**Uninnoculated**
5.5	A1	B1
7	A2	B2
8	A3	B3

3.3 Agronomic Procedures

3.3.1 Land Preparation

The land was ploughed first up to a depth of 40cm, and also a disc harrow was used to break the soil clods providing a fine tilth, which is good seed soil contact to promote germination and root penetration for nutrient exploitation. A rake was used to remove all the debris. After that 18 experimental plots measuring (2x1) m were established, a path way of 0.5m between the plots was left to carter for operations.

Fig 3.3 shows the Experimental plot

3.3.2 Soil Test and Analysis

Soil samples were taken from each field using a cylindrical auger in a zig zag way and the samples were taken to ZFC for soil testing and Analysis. It was done using Calcium Chloride Test. The results obtained shows that the soils were clay loamy soils with a pH of 5.5 which was slightly acidic, and after liming the samples were taken again to ZFC for analysis and the results obtains shows 3 different pH levels 5.5 , 7 and 8.

3.3.3 Lime Application

The required amount of lime added in the soils with neutral pH was 3 kg per bed and in the soils with Alkali pH was 6kg per bed, lime was broadcasted on the soil surface and incorporated up to 20 cm depth by using a Tongue hoe in treatment three month before planting of soybean in order to allow lime to react with the soil correcting the acidity and raising the pH to alkaline before planting. The lime required to raise the pH was calculated as follows:

Clay loam soil has a pH of 5.5

However, the soil pH of the upper 30 cm has to be raised to 7. Calculate the lime requirement [2.5 cmol of lime/kg soil is required to change soil pH by 1.0; the CaCO3has an equivalence value of 100%; Bulk density = 1600 kg/m3 {Clay or humus} (H+, Al^{3+}) +2$CaCO_3$ + H_2O ⇋ {Clay or humus} (Ca^{2+}) + Al (OH) $_3$ + 2CO_2 A change of 1 pH unit requires 2.5cmol of lime/kg soil

∴ 1.5 pH units require 1.5 x 2.5 = 3.75 cmol of lime/kg soil

Mass of 3.75 cmol pure $CaCO_3$:

RMM of $CaCO_3$ = 100g

1 mol = 100 cmol = 100g

∴ 3.75 cmol = 3.75 cmol/100cmol x 100g = 3.75 g CaCO3

Since ratio of reacting moles is 1:2 (1Ca: $2H^+$)

∴ Mass of $CaCO_3$ required = 3.75g/2 = 1.875g $CaCO_3$/kg soil

Mass of a 25 cm layer of soil per ha = Bulk density x volume of soil slice

= 1600 kg/m3 x 100m x 100m x 0.25 m = 4 000 000 Kg

Amount of pure CaCO3 required = 4 000 000 kg x 1.875g/1000g = 7 500 kg

= 7.5 t/ha

Since CaCO3 equivalence value is 100%

∴ Amount of lime → 7.5 x 100/100 = 7.5 t/ha

Since not all CaCO3 in limestone will completely react in soil amount calculated in

Lab buffer curve is usually increased by a factor of 2

i.e. 7.5 t/ha x 2 = 15 t CaCO3/ha

∴ 1ha = 10 000m^2 = 15t lime

2m^2 = 15t X 2 / 10 000

=0.003t = 3kg lime per bed.

3.3.4 Fertilizer Application

Basal dressing with compound L (5% N: 18%$P_2O_{5:}$10% K_2 O: 0.25 B: 8% S) fertilizer at 150kg/ha was applied by banding method, 0,03kg per each $2m^2$ plot was applied. Ammonium nitrate fertilizer (34.5% N) was split applied at weeks 3, 6, and flowering stage at rates of 20, 35, 45 kg/ha. Ammonium nitrate was applied immediately ahead of irrigation cycles to avoid crop burn.

3.3.5 Seed Inoculation

Seeds were inoculated on treatments A1(*Bradyrhizobium* inoculation in acidic pH 5.5), A2 (*Bradyrhizobium japonicum* inoculation in neutral pH 7) and A3 (*Bradyrhizobium japonicum* inoculation in alkali pH 8) by coating with slurry of 1g *Bradyrhizobium japonicum* MAR 1305 strain and a sticker (10% sugar solution) per bed with inoculated seeds. A premeasured amount of sticker solution and inoculant were thoroughly mixed to make smooth liquid slurry. This was added to the weighed amount of seed and the mixture was stirred constantly till the seed was evenly coated. The inoculation of seeds with the rhizobia was done just before planting.

3.3.6 Planting and Irrigation

Planting was done on the 3rd of December, at a seed rate of 100kg/ha which results in 0.4kg of seed being used. Three lines where made in each bed with a depth of 5cm, inter row was 45cm whilst intra row was 5cm. soon after planting the seeds were watered up to a field capacity and also during dry spells the seed beds were constantly irrigated to avoid a soil crust since soyabean is a poor germinator and easily succumb to soil crusts, when necessary irrigation was applied.

3.3.7 Weed and Pest management

Scouting was done before and after pesticide applications almost twice a week for regular checking of pest and diseases from emergence stage up to harvesting. Weed control was done by physically removing the weeds using small hoes and hand pulling. Dimethoate 40EC at 0.2 liters per hectare was used to control the pest like semilopper and aphids.

3.4 Data Collection

3.4.1 Germination Percentage

Germination percentage data was collected through physical counting in the fields 2 weeks after planting and was calculated using the formulae;

$$\frac{\text{no. of seeds germinated}}{\text{No. of seeds sown}} \times 100$$

3.4.2 Final Yield

Final yield parameters are plant height, number of nodules, pod size and the final yield.

- **Plant Height**

  Plant height was measured at 2 weeks intervals from 2 weeks after emergence up to 8 weeks. The measurements were taken using a string and a ruler, from the ground to the tip of the upper leaf and the height of the seedlings was expressed in centimeters. The plant height of randomly tagged plants was used to deduce the growth rate of the plants. A sample of 5 plants from each plot was measured and then averaged.

- **Number of Nodules**

  At eight weeks, Five plants where randomly selected from each treatment and uprooted and the nodules were physically counted, averaged and recorded for analysis

- **Number of pods**

  5 plants were randomly selected from all the treatments at 8 weeks and physically counted, averaged and recorded for analysis

- **Shelling percentage**

  This was calculated by dividing the seed weight over weight of pods multiplied by 100.

  $$\frac{\text{Seed weight}}{\text{Weight of pods}} \times 100$$

- **Final Yield**

  The final yield weight was measured using electrical scale in (kg).

3.5 Data Analysis

Data collected was subjected to analysis of variance (ANOVA) at $P \leq 0.05$ level of significance, using GenStat 18th edition statistical package. Means were separated using Least Significant Difference test (LSD) at 0.05 level of significance to compare the significant means of treatments effect.

Fig 3.9 Skeletal ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2				
Replication.*Units* stratum					
Inoculation	1				
Soil_pH	2				
Inoculation.Soil_pH	2				
Residual	10				
Total	17				

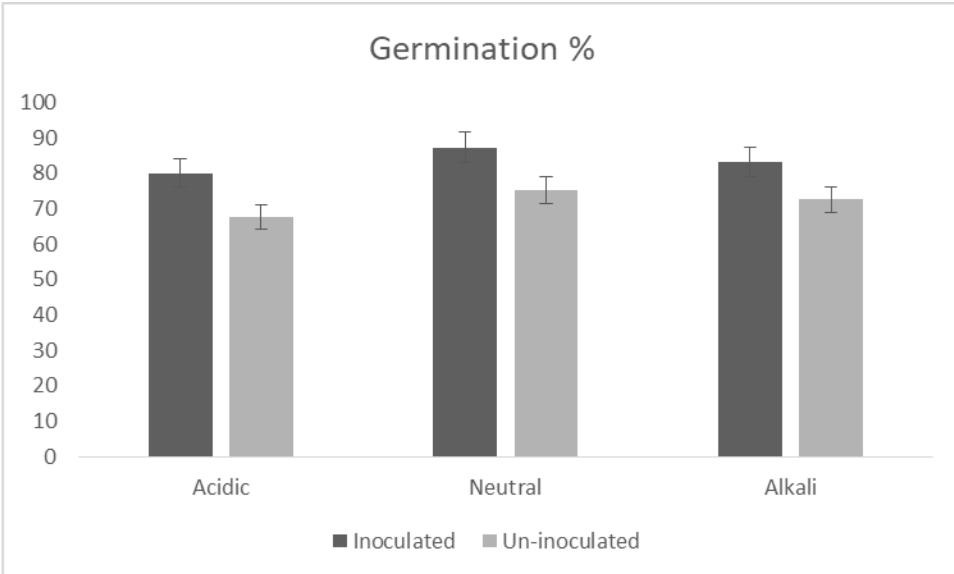
CHAPTER 4

RESULTS

4.1 Germination Percentage

The effect of *Bradyrhizobium japonicum* inoculation in different soil pH levels on the germination percentage of soya beans is presented in Figure 4.1. This shows that the different treatments had a no significant difference between the treatments with B.japonicum inoculation and uninnoculated as the ($p > 0.05$). There was a significant difference between different pH levels as the ($p < 0.01$). This shows that there was no interaction between B.japonicum inoculation and Soil pH. High germination % was recorded in treatment with *Bradyrhizobium japonicum* in neutral pH for both inoculated and uninnoculated treatments. It was observed that those treatments in neutral soil pH germinated first before those in other treatments.

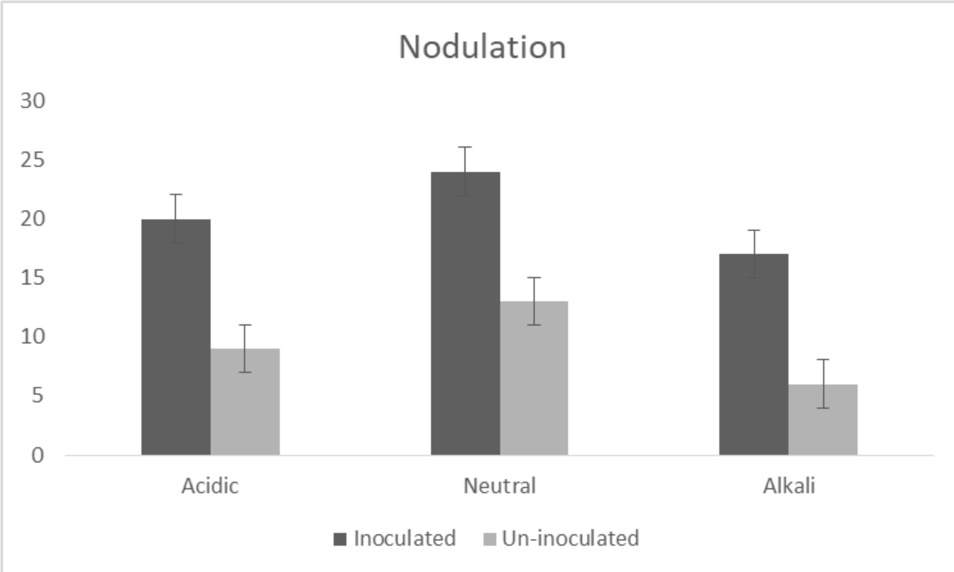
Figure 4.1: Effects of Bradyrhizobium japonicum Inoculation on different pH levels on the germination percentage of the soyabean plants.



4.2 Nodulation of soyabean

The effect of *Bradyrhizobium japonicum* inoculation and different soil pH levels on the number of nodules per plant is presented in Figure 4.2. Analysis shows that different treatments had a significant difference ($p<0.001$) on nodulation. Both inoculation and soil pH had an effect on the nodulation of soya beans. Highest nodule number was recorded in treatment that was inoculated in neutral pH whereas lowest nodulation was recorded in treatment with uninnoculated soyabean in alkali soil pH. It was observed that the nodules produced in inoculated treatments were big in size with a red colour within them.

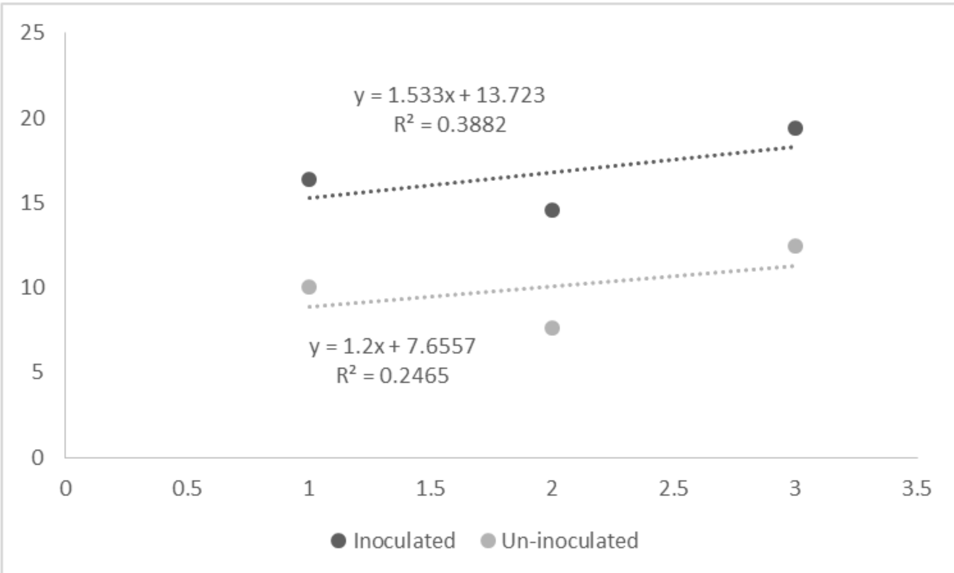
Figure 4.2: Effects of Bradyrhizobium japonicum Inoculation on different pH levels on the number of nodules per plant



4.3 Number of pods per plant

There was a significant difference between the pH and inoculation on the number of pods per plant (p<.001). Number of pods Y can be explained by independent variable whilst, soil pH can be explained by dependent. R^2= 0.3882 of the variation in the dependent variable that is soil pH can be explained by the independent variable number of pods included in the model. R^2 = 0.2465% of the variation in the dependent variable can be explained by the independent variable included in the model. There is a positive relationship between the independent and dependent variable a unit increase will increase the dependent variable by the magnitude of 1.533. Highest pod number was recorded in neutral soils with inoculation whereas lowest nodulation was recorded in the alkaline soil. The number of pods was inversely proportional to the increase or decrease in pH.

***Figure 4.3: Effects of Bradyrhizobium japonicum inoculation and different soil pH levels on number of pods per plant*.**



4.4 Plant Height

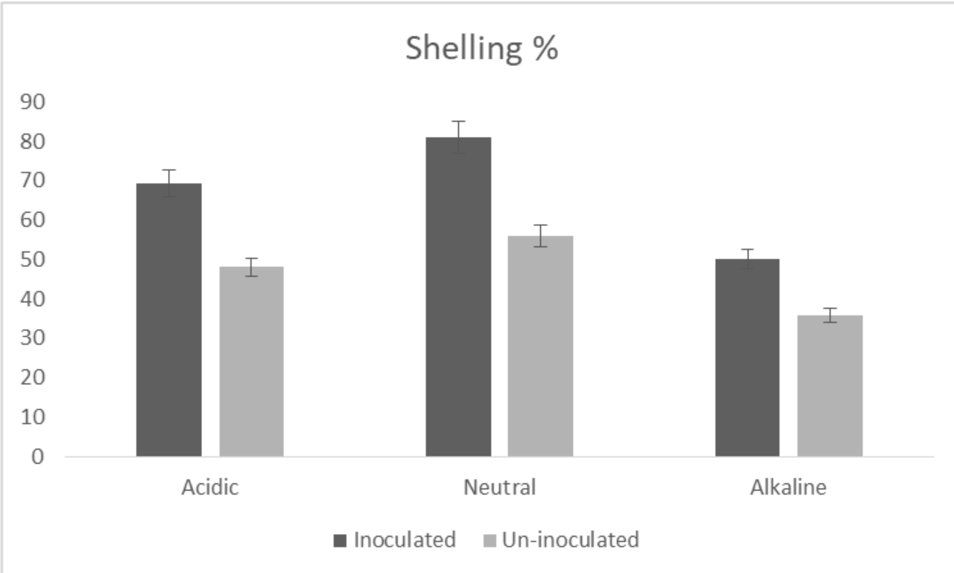
There was a significant difference between B.japonicum inoculation and soil pH on the height of soya bean plants ($p<.001$). Figure 4.4 shows that all treatments were significantly different ($p<0.05$) from each other in their plant heights inoculated in neutral pH had the highest significant plant height while uninnoculated in alkali pH had the lowest. Both soil pH and inoculation had an effect on the height of soya beans.

Figure 4.4: Effects of Bradyrhizobium japonicum inoculation and different soil pH levels on plant height

4.5 Shelling %

There was a significant difference between soil pH and inoculation on shelling % of soya bean plants ($p<.001$). Both pH and inoculation had an effect on shelling % of soya beans. Figure 4.5 shows that all treatments were significantly different ($p<0.05$) from each other in their shelling %, highest shelling % was observed in neutral soils with inoculation whereas lowest shelling % was recorded in the alkaline soil without inoculation. An increase or decrease in soil pH from the neutral resulted in the decrease in shelling %.

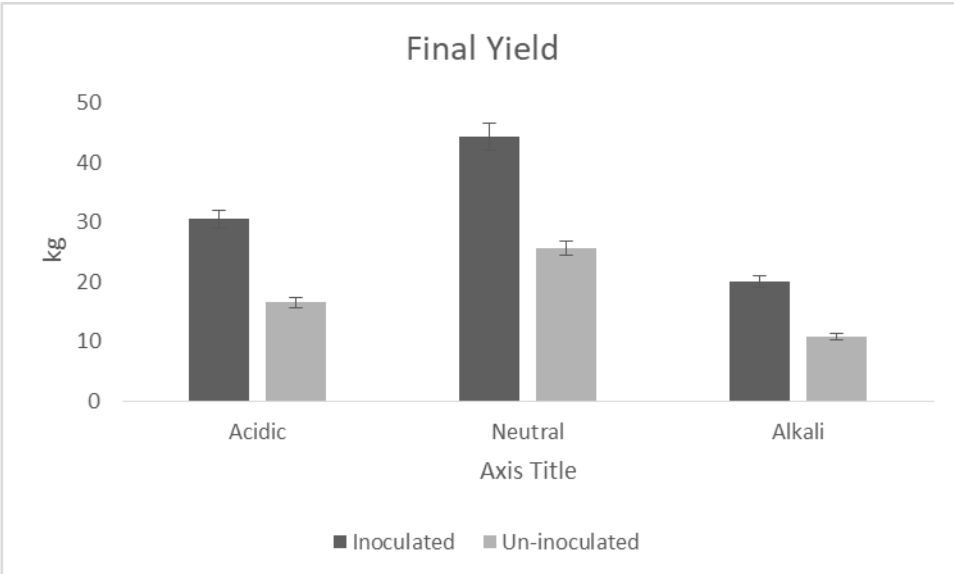
Figure 4.5: Effects of Bradyrhizobium japonicum inoculation and different soil pH levels on shelling %.



4.6 The Final Yield

There was a significant difference between Bradyrhizobium inoculation and soil pH on the Final yield of soya bean plants ($p<.001$). Figure 46 shows that all treatments were significantly different ($p<0.05$) from each other in their yields in (kg) Highest yield was observed in treatment with inoculation in neutral soil pH whereas lowest final yield was recorded without inoculation in alkali soil. An increase or decrease in soil pH from the neutral resulted in the decrease in soya bean yield.

Figure 4.6: Effects of Bradyrhizobium japonicum inoculation and different soil pH levels on Final yield



CHAPTER 5

Discussion

5.1 Germination percentage

The use of effective *Bradyrhizobium japonicum* strain MAR 1305 in neutral pH had no effects on germination percentage of soya beans. Higher germination % is in treatment with *Bradyrhizobium japonicum* inoculation in neutral pH and in treatment without inoculation in Neutral pH than in Treatment with *Bradyrhizobium japonicum* inoculation in Acidic soils and Treatment without inoculation in Acidic soils whilst Treatment with *Bradyrhizobium japonicum* inoculation in Alkali soils and treatment without inoculation in Alkali soils have the least germination %.

Soil pH influences germination as it is a key variable due to its influence on other soil properties, microbial activities, nutrient solubility and availability necessary for germination (Chiara et al, 2018). For instance, in lower pH acidic soils most macronutrients are more available than in neutral and alkali pH but they became toxic when their concentration is too high (Loncaric et al, 2008). Therefore germination in neutral pH is higher as it does not contain toxic elements which cannot be absorbed by the seed.

Soil pH decreases the availability of Phosphorus in the soil which is very important nutrient in the symbiotic association process as it is involved in plants' energy (ATP) acquisition, storage, and utilization (Mmbaga et al., 2014). Phosphorus is required for seed germination and root growth. This Macronutrient is required mostly by new developing plants, developing new tissues and also performs a number of functions which increase the early growth (Rao, 2010). This root system support is especially important for root development (Mmbaga et al., 2014). Hence, its availability in sufficient quantities increases the germination rate (Jarecki et al. 2020).

Soil pH also determining the reaction of plant growth hormones and their quantities available for seed absorption in the soil for germination to occur (Penn., 2019). Not only that soil pH affects the microbial activities in the soil, thus the soy root symbiosis with *B.japonicum* is affected. Micro-organisms are affected by iron (Fe^{3+}) and aluminum (Al^{3+}) abundance in acidic soil, as well as calcium (Ca^{2+}) and magnesium (Mg^{2+}) abundance in alkaline soil. These elements fix soil P in the form of oxides and hydroxides, rendering P unavailable for seed uptake (Dabessa et al., 2018).

5.2 Yield parameters

The results of this study indicates that the inoculation in Neutral soil pH with an effective B.japonicum MAR 1305 strain produced better final yield, also contributed and influenced growth rate in many yield parameters which include nodulation, plant heights, number of pod weights, and shelling%. Treatments with *Bradyrhizobium japonicum* inoculation in Neutral pH, treatment with *Bradyrhizobium japonicum* inoculation in Acidic pH and treatment with *Bradyrhizobium japonicum* inoculation in Alkaline pH indicates the highest number of nodules shows that *Bradyrhizobium japonicum* forms a symbiosis relationship to form root nodules. *Bradyrhizobium japonicum* migrates to the roots and connect to the root hairs. The plant then produces flavonoids, which stimulate the expression of nod genes in bacteria. The expression of these genes causes the creation of enzymes known as Nod factors, which cause root hair curling. The rhizobia are curled up with the root hair during this phase (Małek et al., 2010).

Bradyrhizobium japonicum infect the root hair cells through an infection thread that develops through the root hair and into the main root. As a result, the infected cells divide and form a nodule, within which the *Bradyrhizobium japonicum* bacteria can convert atmospheric nitrogen into ammonia (NH_3) or ammonium (NH_4^+) that can be used by the plant as stated by (Małek et al., 2010) in his study. A research conducted by McKenzie et al. (2001), which suggested that inoculation may increase nodulation, and that the rise was bigger (15%) on fields without a history of legumes than it was (5%) on fields with a history of legumes. On fields with no history of legumes, as opposed to those with a history, the inoculant was more likely to increase the number of nodules and total N. Due of soyabean preference for symbiosis with the nodule bacteria, the research site's low N soil content (0.05%) led to an increase in nodulation and biological nitrogen fixation.

A study by Chaudhury et al. (2021) found that rhizobium inoculation significantly increase the growth and yield of soya beans grown in low nitrogen soils. Another study that was conducted by Jarecki et al. (2020) shows that inoculated soyabean had a higher nodulation in slightly acid soils. Also the presence of native symbiotic bacteria also cause nodulation in uninnoculated soils (Abou-Shanab et al.2017).

However treatment with *Bradyrhizobium japonicum* inoculation in Neutral pH was the highest because the inoculum is active in Neutral pH, which does not contains toxic nutrients elements such as Al^{2+} and Fe^{3+} which reduces Nodulation, in addition Aluminum has been discovered as the major limiting factor in the growth and development of roots in acid soils a study by (Sujatha and Mehar, 2015) suggest that. Acidic soils and Alkali soils contains Fe^{2+} and Mo^{3+} ions respectively causing poor nodules formation and functioning (Rukia et al., 2020).

A study that was conducted by Jarecki et al. (2020) shows that inoculated soyabean had a higher nodulation in slightly acidic soils. Also the presence of native symbiotic bacteria also cause nodulation in uninnoculated soils (Abou-Shanab et al.2017).

Soyabean plants inoculated in neutral soil pH was effective in the plant height of the soya beans as the Neutral pH contain higher quantities of essential nutrients which are necessary for plant growth like Nitrogen, phosphorus and Potassium. Phosphorus is one of the key macronutrients that plants require, according to (Rukia et al., 2020)., as it is involved in a wide range of plant processes, from cell division to the establishment of a strong root system that promotes proper and uniform ripening of the crop. P is especially important for newly growing plants and developing tissues, as well as for a number of other processes that speed up early growth (Rukia et al., 2020).

All the 3 essential nutrients NPK are available in large quantities in the Neutral soil pH Compared to the other treatments, the treatment that is inoculated in neutral pH had a faster rate of growth because its roots enabled the plants to obtain nutrients from deeper levels and also the presence of *B.japonicum* in the soil increases the nitrogen content in the soil thereby allowing luxurious growth and formation of healthy pods and many quality seeds. According to Ahmad (2007), the supplied nitrogen by BNF might have the same function to the crop which is as good as that is provided by compound D. B.japonicum in its optimum pH according to this research it pH 7 (neutral pH) alone can produced nitrogen which is sufficient for plant use and was significantly higher than other treatments.

This was due to the correct soil pH an effective rhizobium strain which was used; the BNF increased the nitrogen amount for plant use. According to (Kumar, 2019) they stated that when legume seeds are well inoculated with an effective Rhizobium inoculant, soya bean could acquire approximately 80% or more plant useable nitrogen through biological nitrogen fixation. A study by Pannecoucque et al (2018) shows that inoculating soyabean seed with rhizobium bacteria increases the yield component yield.

Another study by Braulio R et al (2020) on the effectivess of *Bradyrhizobium elkanii* inoculation in soyabean in different pH levels shows that the strain has higher yields in acidic soils. But according to this research *Bradyrhizobium japonicum* shows higher yields in Neutral pH.

CHAPTER 6

Conclusion and Recommendations

6.1 Conclusion

From this study, it can be noted that there was no interaction between *Bradyrhizobium japonicum MAR* 1305 inoculation and Neutral soil pH. However, there was significant effect on germination due to soil pH, with a significant effect on the germination percentage and yield performance as compared to using *Bradyrhizobium japonicum* inoculation in Acidic and in Alkali soils in soya bean production.

The study shows *Bradyrhizobium japonicum* inoculation alone can be important in BNF but its production can be more pronounced by doing a soil pH test and Analysis first in order to know the soil pH levels of the soil, liming using Dolomotic lime or Calcitic lime which can be in form of Magnesium Carbonate ($MgCO_3$) and Calcium Carbonate ($CaCO_3$) respectively can be done to amend the soil pH level from acidic to a Neutral pH as the rate of BNF is increased in Neutral soil pH thereby improving the soil fertility which increases growth and yield performances of soya beans.

Furthermore, Neutral pH promotes the rate of germination .This was observed by synergetic effect of *Bradyrhizobium japonicum MAR 1305* strain in Neutral soil pH on germination and yield performance.

6.2 Recommendations

Since the country is pushing towards the production of organic products and higher yields, farmers should embrace inoculation of *Bradyrhizobium japonicum* MAR 1305 strain in Neutral soil pH which has desirable effects on germination percentage and yield parameters of soyabean.

From the results in this research it is recommended that farmers should inoculate *Bradyrhizobium japonicum* MAR 1305 strain in Neutral soil pH for higher germination % and higher yields in soya beans production. Moreover, farmers should do soil Analysis test before inoculation in order to amend to the correct soil pH favorable for the action of *Bradyrhizobium japonicum* in BNF. Farmers are also encouraged to use Dolomotic lime to change soil pH from acidic to Neutral and also addition of manure or organic matter can be used to reduce soil pH from Alkali to Neutral. However further studies are still required to assess the response of other *Bradyrhizobium japonicum* strains in different soil pH levels and in Zimbabwe’s different environmental conditions.

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APPENDICES

Analysis of variance

Variate: Germination_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	68.1111	34.0556	36.93	
Replication.*Units* stratum					
Inoculation	1	612.5000	612.5000	664.16	<.001
Soil_pH	2	169.4444	84.7222	91.87	<.001
Inoculation.Soil_pH	2	2.3333	1.1667	1.27	0.324
Residual	10	9.2222	0.9222		
Total	17	861.6111			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Replication 1 *units* 1	-1.78	s.e.	0.72
Replication 3 *units* 1	1.72	s.e.	0.72

Tables of means

Variate: Germination_%

Grand mean 77.72

Inoculation	Inoculated	Un-inoculated
	83.56	71.89

Soil_pH	Acidic	Alkaline	Neutral
	73.83	78.00	81.33

Inoculation	Soil_pH	Acidic	Alkaline	Neutral

Inoculated	80.00	83.33	87.33
Un-inoculated	67.67	72.67	75.33

Standard errors of means

Table	Inoculation	Soil_pH	Inoculation Soil_pH
rep.	9	6	3
d.f.	10	10	10
e.s.e.	0.320	0.392	0.554

Standard errors of differences of means

Table	Inoculation	Soil_pH	Inoculation Soil_pH
rep.	9	6	3
d.f.	10	10	10
s.e.d.	0.453	0.554	0.784

Least significant differences of means (5% level)

Table	Inoculation	Soil_pH	Inoculation Soil_pH
rep.	9	6	3
d.f.	10	10	10
l.s.d.	1.009	1.235	1.747

Stratum standard errors and coefficients of variation

Variate: Germination_%

Stratum	d.f.	s.e.	cv%
Replication	2	2.382	3.1
Replication.*Units*	10	0.960	1.2

Analysis of variance

Variate: Number_of_Nodules_per_Plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	10.3333	5.1667	31.00	
Replication.*Units* stratum					
Inoculation	1	544.5000	544.5000	3267.00	<.001
Soil_pH	2	148.0000	74.0000	444.00	<.001
Inoculation.Soil_pH	2	0.0000	0.0000	0.00	1.000
Residual	10	1.6667	0.1667		
Total	17	704.5000			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Replication 1 *units* 1	0.833	s.e.	0.304
Replication 3 *units* 1	-0.833	s.e.	0.304

Tables of means

Variate: Number_of_Nodules_per_Plant

Grand mean 14.833

Inoculation	Inoculated	Un-inoculated
	20.333	9.333

Soil_pH	Acidic	Alkaline	Neutral
	14.500	11.500	18.500

Inoculation	Soil_pH	Acidic	Alkaline	Neutral
Inoculated		20.000	17.000	24.000
Un-inoculated		9.000	6.000	13.000

Standard errors of means

Table	Inoculation	Soil_pH	Inoculation Soil_pH
rep.	9	6	3
d.f.	10	10	10
e.s.e.	0.1361	0.1667	0.2357

Standard errors of differences of means

Table	Inoculation	Soil_pH	Inoculation Soil_pH
rep.	9	6	3
d.f.	10	10	10
s.e.d.	0.1925	0.2357	0.3333

Least significant differences of means (5% level)

Table	Inoculation	Soil_pH	Inoculation Soil_pH
rep.	9	6	3
d.f.	10	10	10
l.s.d.	0.4288	0.5252	0.7427

Stratum standard errors and coefficients of variation

Variate: Number_of_Nodules_per_Plant

Stratum	d.f.	s.e.	cv%
Replication	2	0.9280	6.3
Replication.*Units*	10	0.4082	2.8

Analysis of variance

Variate: Number_of_Pods_per_Plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	2.78111	1.39056	24.88	
Replication.*Units* stratum					
Inoculation	1	204.02000	204.02000	3650.46	<.001
Soil_pH	2	70.94778	35.47389	634.72	<.001
Inoculation.Soil_pH	2	0.42333	0.21167	3.79	0.060
Residual	10	0.55889	0.05589		
Total	17	278.73111			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Replication 1 *units* 1	0.472	s.e.	0.176
Replication 3 *units* 1	-0.394	s.e.	0.176

Tables of means

Variate: Number_of_Pods_per_Plant

Grand mean 13.422

Inoculation	Inoculated	Un-inoculated
	16.789	10.056

Soil_pH	Acidic	Alkaline	Neutral
	13.217	11.100	15.950

Inoculation	Soil_pH	Acidic	Alkaline	Neutral
Inoculated		16.367	14.567	19.433
Un-inoculated		10.067	7.633	12.467

Standard errors of means

Table	Inoculation	Soil_pH	Inoculation Soil_pH
rep.	9	6	3
d.f.	10	10	10
e.s.e.	0.0788	0.0965	0.1365

Standard errors of differences of means

Table	Inoculation	Soil_pH	Inoculation Soil_pH
rep.	9	6	3
d.f.	10	10	10
s.e.d.	0.1114	0.1365	0.1930

Least significant differences of means (5% level)

Table	Inoculation	Soil_pH	Inoculation Soil_pH
rep.	9	6	3
d.f.	10	10	10
l.s.d.	0.2483	0.3041	0.4301

Stratum standard errors and coefficients of variation

Variate: Number_of_Pods_per_Plant

Stratum	d.f.	s.e.	cv%
Replication	2	0.4814	3.6
Replication.*Units*	10	0.2364	1.8

Analysis of variance

Variate: plant_height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	1.11632	0.55816	10.32	
Replication.*Units* stratum					
Inoculation	1	755.63281	755.63281	13973.43	<.001
Soil_pH	2	182.33861	91.16931	1685.94	<.001
Inoculation.Soil_pH	2	1.03083	0.51542	9.53	0.005
Residual	10	0.54076	0.05408		
Total	17	940.65934			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Replication 2 *units* 6	0.349	s.e.	0.173

Tables of means

Variate: plant_height

Grand mean 42.424

Inoculation	Inoculated	Un-inoculated
	48.903	35.944

Soil_pH	Acidic	Alkaline	Neutral
	41.379	39.154	46.738

Inoculation	Soil_pH	Acidic	Alkaline	Neutral
Inoculated		47.892	45.908	52.908
Un-inoculated		34.867	32.400	40.567

Standard errors of means

Table	Inoculation	Soil_pH	Inoculation Soil_pH
rep.	9	6	3
d.f.	10	10	10
e.s.e.	0.0775	0.0949	0.1343

Standard errors of differences of means

Table	Inoculation	Soil_pH	Inoculation Soil_pH
rep.	9	6	3
d.f.	10	10	10
s.e.d.	0.1096	0.1343	0.1899

Least significant differences of means (5% level)

Table	Inoculation	Soil_pH	Inoculation Soil_pH
rep.	9	6	3
d.f.	10	10	10
l.s.d.	0.2443	0.2991	0.4231

Stratum standard errors and coefficients of variation

Variate: plant_height

Stratum	d.f.	s.e.	cv%
Replication	2	0.3050	0.7
Replication.*Units*	10	0.2325	0.5

Analysis of variance

Variate: Shelling_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	0.5700	0.2850	0.44	
Replication.*Units* stratum					
Inoculation	1	2264.6450	2264.6450	3529.32	<.001
Soil_pH	2	689.1300	344.5650	536.98	<.001
Inoculation.Soil_pH	2	27.3033	13.6517	21.28	<.001
Residual	10	6.4167	0.6417		
Total	17	2988.0650			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Replication 1 *units* 1	1.33	s.e.	0.60

Tables of means

Variate: Shelling_%

Grand mean 63.72

Inoculation	Inoculated	Un-inoculated
	74.93	52.50

Soil_pH	Acidic	Alkaline	Neutral
	63.97	56.02	71.17

Inoculation	Soil_pH	Acidic	Alkaline	Neutral
Inoculated		75.20	68.73	80.87
Un-inoculated		52.73	43.30	61.47

Standard errors of means

Table	Inoculation	Soil_pH	Inoculation Soil_pH
rep.	9	6	3
d.f.	10	10	10
e.s.e.	0.267	0.327	0.462

Standard errors of differences of means

drf

Table	Inoculation	Soil_pH	Inoculation Soil_pH
rep.	9	6	3
d.f.	10	10	10
s.e.d.	0.378	0.462	0.654

Least significant differences of means (5% level)

Table	Inoculation	Soil_pH	Inoculation Soil_pH
rep.	9	6	3
d.f.	10	10	10
l.s.d.	0.841	1.030	1.457

Stratum standard errors and coefficients of variation

Variate: Shelling_%

Stratum	d.f.	s.e.	cv%
Replication	2	0.218	0.3
Replication.*Units*	10	0.801	1.3

Analysis of variance

Variate: Final_Yield_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	0.2744	0.1372	0.33	
Replication.*Units* stratum					
Inoculation	1	870.8356	870.8356	2083.89	<.001
Soil_pH	2	224.0311	112.0156	268.05	<.001
Inoculation.Soil_pH	2	0.4978	0.2489	0.60	0.570
Residual	10	4.1789	0.4179		
Total	17	1099.8178			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Replication 1 *units* 2   1.04   s.e. 0.48

Tables of means

Variate: Final_Yield_kg

Grand mean 21.99

Inoculation	Inoculated	Un-inoculated
	28.94	15.03

Soil_pH	Acidic	Alkaline	Neutral
	21.50	17.93	26.53

Inoculation	Soil_pH	Acidic	Alkaline	Neutral
Inoculated		28.50	25.07	33.27
Un-inoculated		14.50	10.80	19.80

Standard errors of means

Table	Inoculation	Soil_pH	Inoculation Soil_pH
rep.	9	6	3
d.f.	10	10	10
e.s.e.	0.215	0.264	0.373

Standard errors of differences of means

Table	Inocculation	Soil_pH	Inoculation Soil_pH
rep.	9	6	3
d.f.	10	10	10
s.e.d.	0.305	0.373	0.528

Least significant differences of means (5% level)

Table	Inoculation	Soil_pH	Inoculation Soil_pH
rep.	9	6	3
d.f.	10	10	10
l.s.d.	0.679	0.832	1.176

Stratum standard errors and coefficients of variation

Variate: Final_Yield_kg

Stratum	d.f.	s.e.	cv%
Replication	2	0.151	0.7
Replication.*Units*	10	0.646	2.9