EFFICACY OF SELECTED CHICKEN MANURE CHARGED BIOCHAR ON MANAGEMENT OF BACTERIAL WILT (*Ralstonia solanacearum*), GROWTH AND YIELD OF POTATO (*Solanum tuberosum* L.)

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A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements for Award of Master of Science Degree in Crop Protection of Egerton University

EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented for examination in this university or any other for the award of a degree.

Signature Date 1st October 2025

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Recommendation

This thesis has been submitted for the degree of Master of Science in Crop Protection with our approval as university supervisors.

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DEDICATION

I dedicate this thesis to my beloved Mom Euphrasie Mukeshimana, whose unwavering love, support, and sacrifices have laid the foundation of my life and learning. To my husband Bandetse Emmanuel, thank you for your constant encouragement, patience, and belief in me through every step of this journey. To my daughter Niyo Julia and my Son Niyo Ryan, you are my greatest inspiration and motivation. May this achievement show you the value of perseverance and dreams. With all my heart, this is for you.

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ABSTRACT

Potato (*Solanum tuberosum* L.) is a vital staple crop globally but its production is highly restricted by bacterial wilt caused by *Ralstonia solanacearum* responsible for significant losses of yield. The aim of this study was to investigate the efficacy of chicken manure charged biochar in management of bacterial wilt and performance of potato. *In vitro* experiment was carried out at Biotechnology laboratory of Egerton University to determine the antibacterial activity of selected chicken manure charged biochar extracts on growth of *Ralstonia solanacearum in vitro.* Pot and field experiments aimed to determine the effect of selected chicken manure charged biochar on bacterial wilt incidence and severity, growth and yield of *Shangi* potato variety. Field experiment was conducted in Mau-Narok and Egerton, arranged in randomized complete block design (RCBD) with three replicates. The feed stocks started include: maize cobs (MC), Maize straw (MS), rice husk (RH), bean waste (BW) and eucalyptus branches (EU). Data collected for *in vitro* experiment was number of colony forming units from each plate while for pot and field experiments data was on disease incidence, severity, growth and yield. Data was analyzed using R software version 4.4.3, general linear model (GLM) technique was used to conduct ANOVA and means separated using Fisher's least significant difference (LSD) test at 5%. Results revealed that all charged biochar were alkaline with maize straw charged (MSCB) highest at 10.4 while plain rice husk was acidic at 5.4. Charged biochar exhibited higher nitrogen content with bean waste charged (BWCB) highest at 1.5% N and plain eucalyptus (EUPB) lowest at 0.4% N. Maize cobs charged biochar (MCCB) had the highest available phosphorus at 1846 mg/kg while the lowest was 671.5 mg/kg for rice husk charged biochar (RHPB). MCCB had the highest organic carbon at 74.2% while EUPB was lowest at 23.5%. Biochar extracts inhibited the growth of the pathogen by 100% for eucalyptus followed by 98% for bean waste while the least was 2.3% exhibited by plain maize stalk. The treatments reduced disease incidence and severity and the highest were EUCB followed by BWCB at 91.8% and 75.3%, respectively. EUCB increased plant height by 63.5% which was highest in pot experiment while BWCB was highest at 34.5% for in field experiment. Treatments increased yield by 64.6%, 56.7% and 51.4% for bean waste, eucalyptus and rice husk charged biochar, respectively. This study demonstrated that charged biochar has potential in control of bacterial wilt and improving potato yield.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS AND ACRONYMS

AMF	Arbuscular Mycorrhizae Fungi
AUDPC	Area Under Disease Progress Curve
CAN	Calcium Ammonium Nitrate
CaO	Calcium Oxide
CEC	Cation Exchange Capacity
CFU	Colony Forming Unity
CRD	Completely Randomized Design
DAP	Diammonium phosphate
DNA	Deoxyribonucleic Acid
DSI	Disease Severity Index
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agriculture Organization of the United Nation
GLM	General Linear Model
KALRO	Kenya Agricultural and Livestock Research Organization
Mb	Mass of biochar
NA	Nutrient Agar
NPK	Nitrogen, Phosphorus, Potassium
OC	Organic Carbon
PCR	Polymerase Chain Reaction
PDI	Percent Disease Index
PH	Potential of Hydrogen
RCBD	Randomized Complete Block Design
SAR	Systemic Acquired Resistance
SDS	Sodium Dodecyl Sulphate
SMSA	Semi Selective Media South Africa
TN	Total Nitrogen
TZC	Triphenyl Tetrazolium Chloride

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Potato (*Solanum tuberosum*), is a starchy edible tuber belonging to the solanaceae family and one of the most important food crops. The nutritious tuber ranks third after wheat and rice as human staple food and about one billion of people consume it in their daily life worldwide (FAOSTAT, 2023). In Kenya, potato plays an important role in the country's food security as it is staple food for more than a half of Kenyan population (Kochanek *et al.*,2022). The tuber crop is highly nutritious and provide several important nutrients that are essential for overall health. It is a source of carbohydrate which is major ingredient for body's energy. It also contains significant amounts of dietary fiber, especially if consumed with the skin (Navarre *et al.*, 2019). Additionally, potatoes are also good source of several minerals which perform important roles in human health. Potato production in Kenya, is mainly done on highlands area and on the slopes of Mount Kenya. The actual average yield in Kenya is about 10 tonnes ha^{-1} which is 75% lower than the expected yield which can increase up 40 tonnes ha^{-1} under optimal conditions. This gap per unit area is credited to several factors including biotic and abiotic among them we have unavailability of certified seeds to small scale farmers, non-following up of the crops at every growing stage, low skilled farmers, pests and diseases (Kwambai *et al.*, 2023).

Bacterial wilt is one of the main diseases that contribute to yield reduction of solanaceous crops such as potato, tomato, eggplants and chilies. The disease is prevalent in wet tropics, sub-tropics and also in some temperate regions in different parts of the world. The disease is caused by the bacterium pathogen *Ralstonia solanacearum* that lead the infested plant to wilt while remaining green that make the disease to be called "green wilt" disease (Imazaki, 2022). Plant infection can happen through stem injuries caused by cultural practice or insect damage. Disease transmission can also be between plant to plant when bacterial spread from diseased plants the neighboring undiseased plants often via roots through irrigation methods (Fu *et al*., 2020). Under favourable conditions, bacterial wilt is able to cause up to 100% loss of potato yield if uncontrolled. The bacterium typically enters potato plants by infecting the roots, either through wounds caused by mechanical injury or at the points where lateral roots emerge. Soil dwelling organisms like root-knot nematodes can also damage the roots and facilitate the entry of bacterium (Khan *et al.*, 2020).

Several approaches have been implemented to control and manage bacterial wilt including: bio-fumigation and cultivating crop varieties that are resistant to the disease. However, these efforts have yielded limited results due to the organism's ability to withstand harsh environmental conditions, the ability to cause diseases in a diverse variety of hosts within the Solanaceae family, along with its significant genetic variability (Mohammed *et al*., 2020). One of the methods that showed effectiveness in controlling the disease is soil solarization but its effectiveness is short lived, preventing its use throughout the year (Kanyua *et al*., 2020). Environmental friendly and sustainable strategies such as biocontrol organic additives like compost, manure and plant residues have gained popularity as techniques for suppressing soil-borne diseases (Liu *et al*., 2015). The adoption of organic amendments can be alternative sustainable methods of controlling the soil-borne disease reduces dependence on synthetic inorganic compounds which are source of environmental pollution (Elsayed *et al*., 2020; Gao *et al*., 2019). The organic amendments could enhance microbial activity and boost populations of beneficial microorganisms or groups of microorganisms to prevent the invasion of pathogens (Imazaki, 2022).

Soil organic additives are usually adopted in agriculture to raise or buffer soil pH and they beneficially influence soil health well as plant growth (Ninh *et al*., 2015). Research reports have revealed that soil amendments have the potential of reducing the severity of this disease in different crops and impact the yield quantity and quality of those crops by improving the biological, chemical and physical properties of the soil and this improves the overall health of the crop (Ansari & Mahmood, 2017). The degraded organic matter by the action of microorganisms release antibacidal materials in the soil, restricting the nutrients availability for the pathogen thus influence the endurance of the pathogen (Lu *et al*., 2016). Soil amendments also comprise bioactive particles including growth regulators, toxins and vitamins which regulate the micro-organisms. Additionally, soil amendments with farm yard manure have shown potential to improve tomato yield and reduce the bacterial wilt (Ansari & Mahmood, 2017). Addition of biochar for the purpose of amending soil controlling bacterial wilt contribute to potato yield increment.

Biochar enriches soil structure and boosts the retention of nutrient where it makes nutrients more accessible to plants, this lead to healthier potato plants that are better able to resist disease. Biochar can help in balancing soil pH, creating environment that increase favor of potato performance and reducing the stress that otherwise makes plants more susceptible to bacterial wilt (Haider *et al*., 2022). Previous studies have indicated that a soil amendment consisting of urea and calcium oxide (CaO) effectively controlled bacterial wilt in potato crop by impacting pH levels and buildup of nitrite in the field (Liu *et al*., 2015). Addition of rock dust controlled bacterial wilt in tomatoes in experiment conducted in greenhouse by increasing the pH of the soil and calcium content (Kochanek *et al*., 2022; Wang *et al*., 2020). A recent study conducted in China under greenhouse conditions revealed that the amending soil with biochar made from wheat straw and peanut shell biochar lessened the occurrence and severity of tomato bacterial wilt at 65.7% and 28.6% respectively and promoted the growth of tomatoes as well as the yield (Li *et al*., 2022). Fertilizer based on corn cob biochar had been used in management of Downy mildew caused by *Peronosclerospora spp* in corn. This has improved the growth of corn crops by increasing the chlorophyll content index hence influenced the nutrient synthesis and crop health (Rahim *et al*., 2024). Rice husk biochar had performed well in management of root rot caused by *Fusarium solani* in ginseng (a perennial medicinal plant belonging to the Araliaceae family) by promoting the growth of beneficial organisms and suppressing the pathogenic fungi hence reduced the disease (Eo *et al*., 2018). Bean waste biochar has reduced the incidence and severity of bacterial wilt in tomatoes at 13% when applied as soil amendment in sandy soil (de Medeiros *et al*., 2022). Field experiment conducted in North Central Nigeria has shown that eucalyptus biochar mixed with saw dust performed well in management of parasitic nematodes associated with Beniseed (*Sesamum indicum* L) where it has reduced the population of nematodes at 29.7% and improved the yield (Singh *et al*., 2017).

Chicken manure is known to have capacity to suppress soil-borne pathogens by creating environment which is less conducive to pathogens and boost crop immunity by providing nutrients that are essential for crop growth and development. Chicken manure reduce plant stress which could expose plants to infection by providing balanced nutrition as it is rich in nitrogen, phosphorus, potassium and micronutrients that improve plant vigor and disease resistance (Zhang *et al.*, 2021a). Chicken manure can add organic carbon to the soil and thus stimulate soil microorganisms that are beneficial to plants which out-compete pathogens through competition for food, parasitism and antibiosis. Research was conducted in China to assess the impacts of chicken manure substrate on microbial characteristics of the rhizosphere and pathogenic fungi in rice seedling substrates. The study revealed that chicken manure promoted the recruitment of plant growth-promoting rhizobacteria and fungi by enhancing microbial activity and affected negatively the abundance of pathogens and thus reduced the related diseases (Zeng *et al.*, 2022). Chicken manure reduce pathogen's population in the soil by releasing ammonia, organic acids, phenols and volatile fatty acids compounds that are toxic to several soil-borne pathogens. Plant resistance hormones like ethylene, salicylic acid and jasmonic acid, their production is stimulated by microbes that are enriched by chicken manure and thus increase the ability of crop to resist infections by triggering its defense mechanisms (Minkina *et al*., 2023).

Charged biochar is biochar treated or infused with nutrients materials or other beneficial substances to enhance its properties for agricultural applications (Asif *et al*., 2023). Charging biochar is way of improving its nutrient-holding capacity and to create a nutrient-rich soil amendment. Charged biochar acts as storage compartment for microorganisms and nutrients (Hou *et al*., 2022). Charging biochar is essential because if it is used without being charged or activated it will absorb nutrients and microorganisms from the soil, where this can result in plant growth reduction for the first growing season as it plain biochar is like a sponge (Rozie, 2022). Several studies have been conducted to investigate biochar activated by different nutrient rich substances in crop disease control. A study conducted in China by Bolan *et al*. (2023) in pot experiment to investigate the effects of biochar combined with compost revealed that incidence and severity of *Ralstonia solanacearum* in tomato seedlings was significantly reduced by application of that combination. The combination of biochar and compost also improved the microbial shift, organic carbon and soil pH by changing soil chemical properties, roots exudates retention and shifting of rhizosphere bacterial communities that antagonize *Ralstonia* (Longwe *et al.,* 2023). Biochar, when charged with microbial inoculants performed well as beneficial microbes' carrier and improved their establishment and disease suppression in different crops such as potato, tomato and sorghum. Soaking biochar in microbial inoculants resulted in better survival and efficacy of the inoculants compared to inoculants alone. This is due to high porosity of biochar that help in protection of microbes in biochar pores and provide a better habitat for beneficial microbes (Yang *et al*., 2022). Chicken manure, a nutrient-rich organic waste, when used to charge biochar, enhances its beneficial properties which benefit agriculture, providing a dual benefit of waste management and soil amendment. The integration of these renewable resources aligns with the principles of the bio-circular economy and promotion of sustainable agricultural practices (Jaroenkietkajorn *et al*., 2024)

1.2 Statement of the Problem

Bacterial wilt caused by *Ralstonia solanacearum* which is a soil-borne bacterium, is a significant hazard to cultivation of potato in the world, resulting in substantial reduction of yield, with potato farmers potentially experiencing losses of up to 100%, under environmental conditions that favor the pathogen and on susceptible potato varieties. This pathogen is likely to be found in soils where pH is neutral to slightly acidic. These soil conditions arise due to continuous cultivation, extreme application of synthetic fertilizers leading to soil degra dation. Most smallholder farmers have limited information about this disease and this makes it a big challenge in potato production, where some choose to abandon their land due to this disastrous pathogen. In Kenya, the disease causes 50-100% loss and is rampant in Nakuru, Kisii, Nyeri, Meru, Embu, Bomet and Nyandarua that are major potato-growing areas in the country. Some farmers use agrochemicals such as copper oxychloride to control bacterial wilt, which raises environmental and human health concerns. Besides, these chemicals are not selective and they do not only affect pathogens in the soil but also beneficial soil organisms. Crop rotation and intercropping are cultural practices that some farmers apply to control the disease but do not give the sustainable solution due to pathogen's capability of infecting multiple hosts and its ability to remain in the soil for a long time without host. The investigation of the potential of other alternative strategies to control this disease is crucial. Using selected biochar charged with chicken manure as soil amendment can help in suppressing bacterial wilt by improving soil health and making nutrients available to plants and this help to improve plant health and thus plant is able to resist the pathogen's attack.

1.3 Objectives

1.3.1 General Objective

To contribute to enhanced food security by use of selected biochar charged with chicken manure for managing bacterial wilt and enhancing potato production.

1.3.2 Specific Objectives

i. To determine the effect of selected charged biochar extracts on growth of *Ralstonia solanacearum in vitro*.
ii. To determine the effect of selected charged biochar on bacterial wilt incidence and severity in potatoes.
iii. To determine the effect of selected charged biochar on potato growth and yield.

1.4 Hypotheses

i. Selected charged biochar extracts have no significant effect on growth of *Ralstonia solanacearum in vitro.*
ii. Selected charged biochar have no significant effect on bacterial wilt incidence and severity in potato.
iii. Selected charged biochar have no significant effect on potato growth and yield.

1.5 Justification

Bacterial wilt, caused by *Ralstonia solanacearum* is one the most significant threat to potato cultivation globally, leading to substantial economic losses. In Kenya it is a second among major central hazard to potato production following late blight of potato (Kirigo, 2019). Addressing this challenge is indispensable for ensuring food security and maintainable agriculture practices. Conventional control measures for bacterial wilt often involve the use of chemicals in process which raise health and environmental problems. Hence, there is a pressing need for alternative, environmental friendly strategies in controlling the disease. Biochar, is known for its capacity to suppress soil borne pathogens and enhance plant growth through modulation of soil microbial communities. When biochar is applied as soil amendment especially after being charged, it provides habitats for beneficial microorganisms such as mycorrhizal fungi and rhizobia that form symbiotic relationships with plants, enhancing nutrient uptake and improving plant health and plants are able to resist diseases (Joseph *et al.*, 2021). Biochar has the potential to persist in the soil for long periods, providing long-term benefits for disease management and yield enhancement.

Charged biochar plays a valuable role in enhancing soil-borne disease management by promoting a healthy soil environment, enhancing availability of nutrient and stimulating plant defense mechanisms (Murtaza *et al.*, 2021). Integrating charged biochar into agricultural practices offers a sustainable and effective approach to reducing the incidence and severity of soil-borne diseases (Asif *et al*., 2023). This is beneficial to potato farmers because it helps to reduce the cost of production as chemicals are very expensive and farmers need to buy seasonally. Integration of biochar enhances the welfare of the farmers as well as the whole community and also contribute to climate change mitigation by involvement in waste management especially biological wastes from plants and animals.

CHAPTER TWO

LITERATURE REVIEW

2.1 Global Potato Production

Potato is an essential crop globally cultivated on an area of about 19 million hectares of farmland worldwide, its yearly production is around 378 million tons globally (de Haan & Rodriguez, 2024). China is the first country that produce potato in the world where it produces about 95.5 million tons, which is 25% of the world's potato production. In some areas, it is largely cultivated as a food crop but nowadays it is source of revenue for farmers (Ortiz & Mares, 2017). Potato is presently fourth most central source of food considering human utilization worldwide, after wheat, rice and maize. Around 1.3 billion individuals consume potatoes by way of a principal food (greater than 50 kg per individual per year) counting locales of India and China (Beals, 2019). Due to population growth and demand, potato production has shifted from North America and Europe towards Africa and Asia where some countries are expanding production because they consider potato as an urban food security crop (Mishra *et al.*, 2024).

2.2 Potato Production in Kenya

Potatoes are the most important food and cash crops for farmers in Kenya where it is cultivated under the areas of 123,000 hectares. Around 83% of potato production takes place in Kenya's highland regions (1,200-3,000m above sea level). The average potato yield in Kenya ranges from 10–15 tons per hectare (ha^{-1}) which is 2–3 times less than the achievable yield (20-40 tons per hectare) due to different factors such as the lack of certified seed availability, increased pests and diseases, poor soil fertility and unpredictable rainfall. The per capital consumption of potatoes in Kenya is estimated to be around 30-40 kilograms per year which is equivalent to two million tons per year as total national consumption whereas the annual production is dropping to 1.7million tons per year which cause the significant threat to food security (Kwambai *et al*., 2023). Different strategies are needed to fill this gap to ensure food security as potato is main staple food for Kenyans, Africans and worldwide in general. Potatoes in Kenya, are mainly grown in Eastern, Central and Lift valley regions (Safiorganic, 2023) (Table 1).

Table 1: Major Potato-Growing Regions in Kenya

Region	**County**
Central	Nyandarua, Kiambu, Nyeri, Kirinyaga, Murang'a
Eastern	Meru, Makueni, Embu, Tharaka Nithi
Upper part of Rift Valley	Nakuru, Narok, Bomet, Elgeyo Marakwet, Kericho, UasinGishu, Baringo

Source: (Safiorganic, 2023)

2.3 Importance of Potato

Potatoes are the third most important staple food worldwide after rice and wheat and the annual consumption is about 350–370 million tonnes globally (FAOSTAT, 2023).They are also of paramount importance due to their nutritional value, agricultural significance, economic impact, culinary versatility, and contributions to food security as they are rich in carbohydrates and provide high calories, proteins and vitamins such as: C and B6. The supply and accessibility of both micronutrients and macronutrients like potassium (K), magnesium (Mg), nitrogen (N), calcium (Ca), phosphorous (P), and sulfur (S) in plant physiology, impact the dietary and other quality characteristics of potato tubers (Wijesinha-Bettoni & Mouillé, 2019). Potatoes have a place in agriculture sector, considered as a valuable crop for smallholder farmers and large-scale agricultural enterprises alike. Due to the agronomic characteristics of potatoes, their adaptability to diverse climates and their contribution to crop rotation, soil health and biodiversity, potatoes are important in sustainable agriculture (Navarre *et al*., 2019). Potato contributes to job creation along the value chain as it involved several people with different knowledge from production, storage, processing to marketing (Górska-Warsewicz *et al*., 2021).

2.4 Major Diseases of Potato

2.4.1 Late blight and Bacterial Wilt

Late blight is ranked as first major constraints for potato production globally and in Kenya. The disease is caused by *Phytophthora infestans* an air-borne fungal pathogen and the disease can cause the yield loss of up to 100% if uncontrolled. The disease is favored by cool, humid conditions with temperature range of 10-20^{0}C, rain that can result in prolonged leaf wetness also favor the pathogen to grow and spread. The disease is mainly spread by wind and rainfall splash by carrying the sporangia and zoospores produced by the pathogen that can spread the disease from leaf to leaf and field to field rapidly. The first symptoms of disease pale green leaves that rapidly enlarge to brown, underside of the leaves a white, downy or fuzzy growth can be seen in humid conditions. The disease starts and develops on foliage and tubers, contaminated seed potato can carry the pathogen into the soil during planting but the pathogen cannot persist long in soil. Highland area with frequent rainfall are prone to disease and this is can be explosive epidemics if it is not controlled properly and on time (Bradshaw, 2025).

Bacterial wilt is placed second disease of potato in world in Kenya. This is caused by *Ralstonia solanacearum*, a soil-borne pathogen that grows well in environment with warm temperatures range of 25–35 °C, soil with high moisture and poor drainage. The disease is favored by soil acidity and continuous cultivation of potato in the same land can increase the buildup of pathogen inoculum. Bacterial wilt can cause the yield loss of up to 100% in conditions favoring the pathogen and with susceptible potato variety (Musah *et al*., 2025)**.**

2.4.2 Early Blight and Potato Viruses

Early blight is caused by an air-borne fungus *Alternation solani*, it can cause 20–50% yield loss. Warm temperature ranges between 24–29 °C, low soil fertility and drought stress can favor the disease increase. The disease is mainly feast by wind and rain splash that carry conidia produced by the pathogen on infected plant debris and infect leaves and stems. The infection begins on older leaves and spread through the canopy. Pathogen (fungus) can remain in soil and crop residues as conidia and mycelium but the spread and epidemics are motivated by air-borne spores (Babarinde *et al*., 2025). PVY, PLRV, PVX, are the major potato viruses that contribute to yield loss especially in quality. Viruses are driven by vector insects mostly aphids and leafhoppers and can cause the progressive yield reduction over season if farmers use their saved tubers that can be main source of inoculum. Most potato viruses are favored by warm conditions that increase virus multiplication and activities of vector (Nduwayezu *et al.,* 2024).

2.5. Bacterial Wilt and Its Significance

Bacterial wilt, caused by *Ralstonia solanacearum* is a significant obstacle in the cultivation of economically important crops. It leads to substantial yield reduction in solanaceous crops and other crops grown in tropical, subtropical, and temperate regions worldwide (Wang *et al.,* 2019). The yield losses can be as high as 91% for tomatoes, 10%-30% for tobacco, 33%-100% for potatoes, 80%-100% for bananas, and up to 20% for groundnuts (Maureira *et al*., 2022). The yield loss due to bacterial wilt poses a significant threat to income and food security for farmers and country in general. When the yield is lost all the inputs invested in potato farming is also wasted that can lead the farmer to the poverty. Bacterial wilt in kenya can cause the loss of 7-15 billion ksh due to waste of inputs (seed potato, fertilizer, labours and other disease management strategies that can be expensive (Stanley *et al.*, 2024). Bacterial wilt is typically favored by the range of temperatures between 25°C to 37°C. In regions with the mean soil temperature below 15°C, it normally cannot pose problems. Infection is promoted by wetness soil at room temperature environments (Andre *et al*., 2015).

2.6. Description of *Ralstonia solanacearum*

Ralstonia solanacearum is a soil borne, aerobic, gram-negative, motile bacterium with a polar flagella tuft, non-spore-forming, rod-shaped, and belongs to the β-proteobacteria group, posing a serious threat to the production of numerous crop and plants globally (Wang *et al*., 2019). It is currently considered as complex species consisting of three species, five races (as it can affect numerous crop species), six biovars because it is able to oxidize hexose, alcohol, sorbitol and disaccharides as well. it also consists four phylotypes based on geographical origin of strain: phylotypes I, II, II and IV originated from Asia, America, Africa and Indonesia respectively (García *et al*., 2019). The bacterium can be detected using sensitive methods such as vascular flow testing, ELISA **(**enzyme-linked immunosorbent assay**),** and Polymerase chain reaction (PCR) (Choudhary *et al*., 2018; Délices *et al.,* 2019)**.** Due to its global scientific and economic importance, this pathogen is included in the "Top 10" list of plant pathogenic bacteria in the field of molecular plant pathology (Abhilash *et al*., 2016)**.**

2.7. Interaction of *Ralstonia solanacearum* with Crop

This soil borne bacterium, can cause infection to over 200 types of plants, counting important crops. It can survive for extended periods in the harsh environment and becomes more resistant when stressed by environmental factors, such as cold temperatures. This makes it easily spread through surface irrigation or contaminated soils (Elsayed *et al*., 2020). Infection begins the time bacterium get in the primary root tissue through wounds or natural tuber eyes such as secondary root emergence spots (Bindal & Srivastava, 2019; Panth *et al.,* 2020). The pathogen then aggressively colonizes the host plant's root system before spreading systemically, leading to the appearance of typical symptoms in the shoot (Mohammed *et al*., 2020). The bacteria enter plants through xylem vessels, colonizes them, by producing sticky exopolysaccharides (EPS) and vast bacterial populations and blocks water movement, leading to wilt. The primary cause of wilt is the blocking of pit membranes in petioles and leaves by extracellular polysaccharide. The bacteria can quickly spread into the crown and stem through the plant's vascular system (Im *et al*., 2020) (Figure 1).

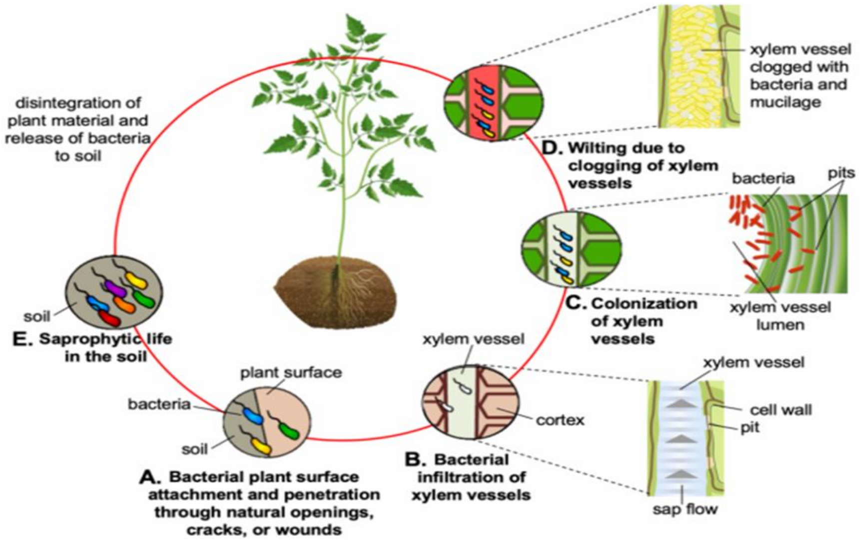

Figure 1: Different Phases of *Ralstonia solanacearum* Interaction with Host Plant

Source: (Singh *et al*., 2022)

2.8. Symptoms of Bacterial Wilt

The disease causes the entire plant to wilt and die within a short period of time. The first parts to be affected are young leaves that become wilt especially at day time when the temperature is high. The symptoms of wilting may disappear at evening time but this cannot livelong (Liu *et al*., 2023) . There is no yellowing of the leaves and there is no spotting of fruits. All the branches of the plant will wilt at a similar time (Plate 1 B). When you cut across the stem of the wilted plant the pith will look dark and watery (Mutimawurugo *et al*., 2019). There will be a greyish, slimy liquid on pressing on the stem. Later in the disease the pith will decay and the stem will be hollowed out. The affected roots will decay and turn dark brown or black. If the soil is wet, the diseased roots will become soft and slimy (Tafesse *et al*., 2021). The affected tuber will show bacterial streaming when cut (Plate 1 A).

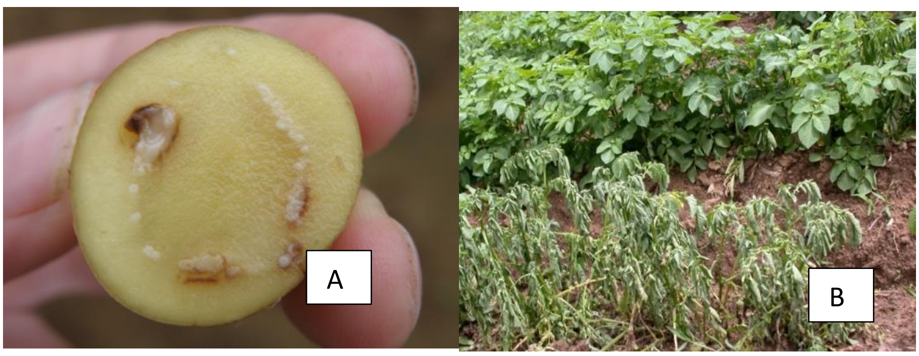

Plate 1: Symptoms of bacterial wilt infested potato (A) on tuber and (B) on foliage)Source**:** (Kilonzi et al., 2024)

2.9. Chemical Control of Bacterial Wilt

Chemical fumigants and bactericides, such as metam sodium, chloropicrin, 1,3-dichloropropene, and streptomycin sulfate, as well as resistance activators like acibenzolar-s-methyl, have been found to reduce bacterial wilt occurrence and increase yields, their use is discouraged due to environmental concerns and the development of high levels of resistance (Ochilo *et al*., 2019). Copper compounds like (copper hydroxide, copper oxychloride), have shown the potential in controlling bacterial wilt but they also have limited effectiveness in soil because bacteria are well-protected in soil microhabitats. Chemicals can lead to resistance development when used the same continuously without interchanging with other of different active ingredients. They can also lead to soil toxicity and environmental pollution due to their accumulation especially copper based compounds (Katan, 2017; Panth *et al*., 2020).

2.10. Host Plant Resistance

Host plant resistance is the ability of certain crop varieties to tolerate or resist infection by pathogen. Resistance can be qualitative when it is controlled by major resistance genes that make it complete but not durable or quantitative when it is controlled by many genes which is partial but more durable. This resistance acts in different mechanisms: structural barriers that reduce bacterial spread by formation of thick cell wall, tyloses and lignin. Through physiological defense, plant produce antimicrobial compounds (Mansfield *et al*., 2012). Through breeding, some potato varieties that show moderate to strong resistance to bacterial wilt have been developed. However, even the most resistant varieties still carry hidden infection and some of these resistant types have lower tuber quality. Conversely, many of the resistant varieties with good quality are hybrids, that are too costly for most small-scale farmers (Qi *et al*., 2021).

2.11. Cultural Methods in Bacterial Wilt Management

Farmers practice crop rotation by growing different crops in the same area over a series of growing season. Rotating with non-host crops (e.g., cereals, maize, beans) reduces *Ralstonia* inocula in the soil but the pathogen's aptitude to stay long in the soil and its wide range of host plants make crop rotation less effective. This technique can be effective long times (3–5 years), which may not be practical for smallholder farmers (Jeger *et al.*, 2021). Farmers are mostly advised to use the certified seeds to prevent introduction of the pathogen into clean fields. Certified seeds are expensive and not readily available to the market especially in developing countries, this leads farmers to recycle their own seeds that can reintroduce the pathogen into their fields (Venbrux *et al*., 2023). Field sanitation such as uprooting and destroying diseased plants reduces inoculum spread and removing weeds that host the pathogen can also diminish survival. This technique is limited due to labor-intensive, it requires high men power and it can spread infection quickly if it is not done early and thoroughly (Hoang *et al*., 2022).

Adjusting planting time and location can be adopted as a way of controlling soil borne pathogens, planting in cooler seasons and non-infected fields minimize disease pressure as bacterial wilt is favored by warm, moist conditions. Farmers find it difficult to shift to clean fields due to land scarcity and this is limited because farmers rely only on seasonal rain (Bock *et al*., 2022)**.** Intercropping system, an agricultural practice where farmers grow two or more crops together in the same field at one growing season. The capacity of the pathogen to thrive in different environments, and its varied hosts, its high variability makes this method less effective in controlling the bacterial wilt (Fu *et al.*, 2020). The pathogen can also remain in inactive state inside local weeds making different adopted management strategies inefficient for the diseases associated with this pathogen. This technique can also limit the yield of main crop due to competition of nutrients and space (Manda *et al*., 2020).

2.12. Physical Control of Bacterial Wilt

Since the bacterium causing bacterial wilt can persist in soil and plant debris, proper sanitation practices can help reduce its spread. This includes removing and destroying infected plants, as well as cleaning tools, equipment, and containers to prevent contamination. In the process of soil solarization which involves covering the soil with clear plastic during the hottest part of the year to heat it up and kill pathogens, including the bacteria causing bacterial wilt. This method requires careful timing and can be effective in reducing soil borne pathogens (Sarkar & Chaudhuri, 2016). Steam sterilization system that involves applying steam to soil or planting substrates to kill soil borne pathogens show more effectiveness in controlling bacterial wilt but requires specialized equipment and careful application to ensure thorough sterilization (Murtaza *et al*., 2021). Physical barriers, such as root barriers or mulches, can be used to prevent the spread of bacterial wilt in the soil. These barriers can help restrict the movement of the pathogen and reduce its ability to infect susceptible plants (Rizwan *et al*., 2016).

2.13. Biological Control

This method involves the use antagonistic microorganisms associated with plants and has produced promising results. Microbial biological control products offer a promising alternative for controlling bacterial wilt. They are safer, greener, more productive and diminish the risk of resistance development (Anith *et al*., 2019)**.** Kenya has recently reported great success in controlling bacterial wilt of field tomato using locally isolated microorganisms. Although local microbial resources have been shown to be effective against this disease under field conditions the potential to biocontrol is still not fully exploited (Imazaki, 2022). Subsequently, some study was conducted to investigate the biological control efficacy of locally isolated antagonistic bacteria and fungi against bacterial wilt in field-grown tomatoes in Kenya. That study reported effectiveness of *bacillus* spp against *Ralstonia* in the laboratory and greenhouse through the production of antibiotics and lytic enzymes that stimulate plant defenses (Andati *et al.,* 2023).

Antagonistic microorganisms such as: *Bacillus subtilis, Bacillus amyloliquefaciens, Pseudomonas fluorescens, Pseudomonas putida, Paenibacillus polymyxa* produce toxins and lytic enzymes and compete with *Ralstonia* for nutrients and habitation that cause pathogen suppression. Some beneficial organisms like *Trichoderma harzianum, Trichoderma viride, Aspergillus niger,* directly attack on pathogen structure through mycoparasitism process. They improve root growth and plant vigor reducing wilt incidence indirectly (Liu *et al*., 2015). Endophytic microorganisms such as: *Burkholderia*, *Enterobacter, Ralstonia pickettii,* suppress *Ralstonia* by blocking its entry and inducing systemic resistance. *Ralstonia* can be inhibited by antibiotics and secondary metabolites produced by actinomycetes **(***Streptomyces spp*) (Fu *et al.,* 2020)**.** The techniques stated above are limited due to different factors. Field performance can be inconsistent; pathogen may survive in deeper soil layers. Many of beneficial organisms are sensitive to soil environment and may require formulation improvements for field use (Kariuki *et al*., 2020).

2.14. Use of Biochar in Soil-Borne Disease Management

Biochar has been shown to alter soil microbial communities in ways that can suppress soil-borne pathogens through creating conditions unfavorable for the survival and proliferation of pathogens, reducing their population densities in the soil (Longwe *et al.,* 2023). When biochar is applied as soil amendment especially after being charged with manure, it provides habitats for beneficial microorganisms such as mycorrhizal fungi and rhizobia that form symbiotic relationships with plants, enhancing nutrient uptake and improving plant health. In turn, healthier plants are better able to resist diseases (Joseph *et al*., 2021). Some plant diseases are favored by acidic soil conditions, biochar can help create an environment less conducive to certain disease pathogens by maintaining a more stable pH as it has a liming effect (Koch *et al*., 2020).

Biochar has the potential to persist in the soil for long periods due to its stable organic carbon and acts as a substrate for beneficial microorganisms, including biocontrol agents (e.g., *Trichoderma*, *Bacillus*). Organic compounds from biochar can have inhibitory effects on Pathogens when they are dissolved (Asif *et al*., 2023). Biochar acts like microbial hotel by providing habitats and shielding niches for beneficial microorganisms. Biochar has many micro and macro-pores that increase surface area and can adsorb pathogen toxins and enzymes that results in reduction of their virulence. Biochar also foster microbial diversity and competition that limits pathogen proliferation (de Medeiros *et al*., 2021).

Low particle density of biochar makes soils more aerated and lighter, improves drainage. It helps biochar to enhance root growth and plant's ability to resist infection by balancing soil moisture which reduce stress that would otherwise make plant vulnerable to diseases (Manasa *et al.,* 2024). Biochar being rich in macro and micro nutrients needed by a crop, enhance plant nutrition, make it stronger and plant is healthier with more robust defense systems. Biochar can make it harder for pathogens to penetrate due to some nutrients in it (Calcium and silcon) that play specific roles in strengthening plant cell walls (Elangovan & Mudgil, 2023).

2.15. Charged Biochar on Potato Growth and Yield

Biochar is a type of organic material rich in carbon (C), nitrogen (N), oxygen (OH), and hydrocarbons. The Carbon content in biochar ranges from 380 to 800 g/kg-1, and is characterized by aromatic structure as well as alkyl structure. Biochar is also rich in other inorganic elements such as Si , K Al , Ca , and P (Agong *et al*., 2021). Biochar acts as a soil conditioner and nutrients retainer, when charged with chicken manure which is rich in Nitrogen, Phosphorus and Potassium that are essential for plant growth, it can enhance soil fertility and provide a sustained release of nutrients to potato plants. Potato require consistent moisture throughout their growing season, improved soil structure and water holding capacity due to biochar's high surface area and porosity is beneficial for potato growth and yield (Hou *et al*., 2022). Because of to biochar's high water and nutrients retention, charged biochar can reduce nutrients loss from the soil through leaching or runoff (Rozie, 2022). Charged biochar can potentially lead to increased root mass and improved nutrient uptake in potato plants through improvement of soil structure and providing a favorable environment for root growth (Poveda *et al*., 2021). When charged biochar is used as a soil amendment ,their combined effects of improving the fertility the soil, its capacity to retain as well as nutrient availability, root development can ultimately lead to increased potato yields (Tian *et al*., 2021).

2.16. Chicken Manure in Management of Soil-Borne Disease

Composted chicken manure used as soil amendment enhance structure of the soil, water holding capacity, availability of nutrients to plant. Fresh chicken manure suppresses soil borne pathogens by releasing ammonium, which is toxic to some pathogens. It can also stimulate beneficial microorganisms in the soil that out-compete or suppress pathogens (Kwak *et al*., 2018). Application of composted chicken manure helps in breakdown of organic matter in soil and create a more stable and soil amendment which is rich in nutrients. Fresh chicken manure contributes to reduction of environmental impact and improve farmers' safety when applied as alternative to chemical fumigants (Ding *et al*., 2013). Chicken manure biofumagation effect lowers the pathogen populations in the soil by producing organic acids, ammonia and other volatile compound during its decomposition. Some studies show that induced systemic resistance (ISR) is linked to manure application where beneficial microbes stimulated by manure helps in activation of plant's natural defense systems and plant is able to respond quickly and strongly when attacked by pathogens (Iacomino *et al*., 2022; Zhang *et al.*, 2021a).

CHAPTER THREE

EFFECTS OF SELECTED CHICKEN MANURE CHARGED BIOCHAR ON GROWTH OF *Ralstonia solanacearum in vitro*

Abstract

Ralstonia solanacearum which causes bacterial wilt is a overwhelming pathogen that cause yield declines for crops in Solanaceae family. Its management is challenging due to its genetic variability and environmental adaptability. The objective of this study was to determine the antibacterial effect of different chicken-manure charged biochar on growth of *Ralstonia solanacearum in vitro*. A study was conducted *in vitro* to assess the antibacterial effect of specific chicken manure-charged biochar extracts against *Ralstonia solanacearum* at the microbiology laboratory of Egerton University. The study tested charged biochar extracts from various feed stocks: maize cobs (MC), maize straw (MS), rice husks (RH), bean wastes (BW) and eucalyptus branches (EU). The physical and chemical properties of biochar were assessed according to established standard procedures. The antibacterial efficacy of charged biochar extract was evaluated by mixing nutrient agar (NA) in petri dishes with 0.5 ml of the extracts prior to solidification. 0.1 ml of bacterial suspension was spread on each plate. Sterile distilled water and copper oxychloride was used as negative and positive control respectively. The plates were incubated at 28°C in growth chamber for 48 hours. The experiment was arranged in a completely randomized design (CRD) with three replicates. Charged biochar exhibited higher nitrogen content than that of plain, with bean waste charged biochar having the highest at 1.5% and plain eucalyptus (EUPB) biochar lowest at 0.4%. For available phosphorus, maize cob charged biochar (MCCB) exhibited the highest with 1846 mg/kg while the lowest was plain rice husk biochar at 671.5 mg/kg. Organic carbon at 74.2% for MCCB was the highest and eucalyptus plain exhibited the lowest at 23.5%. The antibacterial assay indicated that all biochar extracts significantly suppressed the growth of the pathogen in comparison to the negative control. The application charged eucalyptus biochar extracts reduced pathogen growth by 100% which was highest inhibition followed by 98% inhibition for bean waste charged while plain maize straw was lowest at 2.3 %. Charged biochar especially that derived from bean wastes and eucalyptus is best way of controlling bacterial wilt.

3.1 Introduction

Ralstonia solanacearum, a soil-borne pathogen which is accountable for bacterial wilt in numerous species, is one of the soil-borne diseases that contributes to significant crop losses in vital plants globally (Shitiavai *et* a*l*., 2021). It is an aerobic, gram-negative, motile bacterium characterized by a tuft of polar flagella, non-spore-forming, rod-shaped, and classified within the β-proteobacteria group, presenting a significant threat to the cultivation of many crop plants worldwide (Maji & Chakrabartty, 2014). The pathogen is classified as a complex species comprising three species, five races (due to its ability to harm various crop species), and six biovars, as it may oxidize hexose, alcohol, sorbitol, and disaccharides. It comprises four phylotypes based on the geographical origin of the strains: phylotypes I, II, III, and IV originated from Asia, America, Africa, and Indonesia, respectively (Wamani *et al*., 2023).

This pathogen is placed second among plant pathogenic bacteria in molecular plant pathology due to its global scientific and economic significance (Abhilash *et al*., 2016). The *Ralstonia* population can attain 10^3–10^6 cfu/gram in soil and plant tissue during peak infection (Wang *et al*., 2018). The pathogen can cause yield losses in different crops; 91%, 90%, 30%, and 100% in tomato, potato, tobacco, and banana respectively (Chuang & Ko, 2019). The pathogen's capacity to infect plant from diverse families and its resilience in various environments including soil and water, results in significant diseases affecting agricultural crops globally, rendering various management and control methods less effective (García *et al.*, 2019).

Numerous studies have established techniques for controlling the pathogen including biological, cultural, physical, chemical, and integrated biocontrol strategies. Crop rotation and intercropping reduced the disease severity but can’t terminate it from the soil due the capacity of the pathogen to infect a widespread of hosts in the solanaceae family and remain in soil for long time without a host (Ding *et al.*, 2013; Marian *et al*., 2019; Wei *et al*., 2015). Soil solarization has been documented as an effective method for controlling bacterial wilt, but its effectiveness is short lived, preventing its use throughout the year (Balestra *et al.*, 2019).

The application of organic amendments as a sustainable alternative for mitigating soil-borne diseases is significant due to its environmental sustainability (Elsayed *et al*., 2020; Gao *et al*., 2019). Organic additives are recognized to enhance microbial activity and increasing populations of particular microorganisms or groups of microorganisms to prevent disease invasion (Imazaki, 2022). Soil supplements are frequently used in agriculture to raise or stabilize soil pH and are thought to positively influence soil health and plant growth (Ninh *et al.*, 2015). Biochar is one of the soil additives applied to mitigate soil-borne diseases by improving soil and crop health. Different studies have been conducted to investigate the effect of biochar from different feed stock in management of bacterial wilt and other significant soil borne diseases in different crops (Poveda *et al*., 2021).

3.2 Materials and Methods

3.2.1 Preparation of Biochar and its Extracts

Biochar was prepared at Egerton University, Engineering Department using a modified kiln. The raw materials used were sourced from various places; Bean waste from KALRO Njoro, Eucalyptus (*Eucalyptus saligna*) branches, maize cobs and maize straw were gathered from Field 7, Egerton University. Materials were dried until the moisture content is at minimum to facilitate the burning of materials, then put in a kiln. They were heated at a temperature of 400°C in a pyrolysis process 2 hours for bean waste and maize straw while maize cobs and eucalyptus branches were pyrolysed for 4 hours. All the openings of a kiln were closed to minimize the oxygen, at the end of the process biochar was harvested and allowed to cool before store it. Rice husk biochar was sourced from Mwea, supplied by Safi Organics Company Ltd, while chicken manure was sourced from a commercial battery cage poultry farm in Njoro. Fresh chicken manure was piled under shade for four weeks to allow decomposition; it was turned weekly for aeration and uniform decomposition. This composting was to reduce foul odor, destroy weed seeds and minimize microorganisms which might include pathogens. When manure turned dark brown and produced an earthy smell it was a sign of it being wholly decomposed (Pernet & Ribi Forclaz, 2019).

Biochar was activated by mixing with chicken droppings in a 1:1 ratio and watering it consistently for four weeks before use to promote nutrient transfer from the manure to the biochar (Azargohar & Dalai, 2018). A charged biochar extract was made by grinding the biochar to fine powder then mixed with sterile distilled water in a ratio of 1:4. The mixture was shaken for 30 minutes with a magnetic stirrer, left to settle for 72 hours, and subsequently filtered using Whatman filter paper of 125 mm to remove solid residues and obtain extracts. The extracts were sterilized using autoclave to avoid any contamination that can be caused by microorganisms from the charged biochar. Afterwards biochar extracts were stored in sealed glass bottle in refrigerator.

3.2.2 Physical Properties

a) Density

Density was calculated by putting water in graduated cylinder to 500 ml; initial volume (V1) where 250 g of oven dry biochar was submerged in water marking it as final volume (V2). Volume of biochar was obtained by subtracting the initial volume of water from final volume. Biochar volume (Vb) =V2-V1. Density of biochar was calculated by applying the formula: Mb/Vb .Where Mb is mass of dry biochar and Vb is volume of biochar (de Jesus Duarte *et al.*, 2019).

b) Porosity

Porosity was calculated by applying the formula:

P=$Vw \div Vt \times 100\%$………………………………………………. formula 1

P: porosity of biochar

Vw: volume of water

Vt: volume of mixture of water and biochar

Chemical Properties

a) Determination of Nitrogen

Total nitrogen was determined by use of the Kjeldahl Digestion Method where 0.3 g of charged biochar was weighed and put into kjeldahl tubes, 2.5 ml of digestion mixture composed by concentrated sulphuric acid (H_2SO_4) that helps to break down of organic matter, Potassium sulfate (K_2SO_4) that increase the boiling point of sulfuric acid for more efficiency digestion and Copper sulfate ($CuSO_4 \cdot 5H_2O$) responsible in speeding up the oxidation of organic nitrogen to ammonium were added. The mixture was placed into the digestion block with the temperature set at 150^oC for 1 hour after that the solution was cooled for 3-5 min. Three (3 ml) of hydrogen peroxide (H_2O_2) was added in sequence and the mixture was returned back into the digestion block and the temperature was raised to 250^oC for a period of two hours until the solution became clear then removed and cooled (Singh *et al.,* 2017). After cooling, 25 ml of distilled water was added and the content of digestion tube was transferred into 50 ml of volumetric flask and filled to the mark using distilled water. An aliquot of 10 ml was taken and put into distillation tube, add 10 ml of 40% of NaOH to neutralize solution. The solution was steam distilled into a conical flask containing 5 ml of boric acid and mixed indicator and the developed color became green. The distillate received was titrated using N/140 (0.1N) HCl till the end point where the colour changed from green to pink. To obtain the amount of total nitrogen present in charged biochar the formula below was applied:

$$\%TN = \frac{Vol\ x\ 0.1\ x\ vol\ of\ digest}{1000\ x\ sw\ x\ al} \text{x100}$$ ……………………………formula 2

Where, Vol: volume used in titration, 0.1: concentration of the acid used for titration, sw: sample weight

b) Determination of Phosphorus

Mehlich III method was used to determine available phosphorus where 0.5 g of air-dried sieved charged biochar was weighed and extracted with (0.01M HCl+0.025N H_2SO_4). The concentrations in mg kg^{-1} against the absorbance of the y-axis plotted on the graph. The quantity of available phosphorus in the charged biochar extract was calculated as indicated in formula 3:

$$AP = \frac{C\ x\ vol\ x\ DF}{sw}$$ ……………………..formula 3

AP: Available phosphorus, C: The concentration of phosphorus in sample, Vol: Total Volume after topping to the mark, DF: Dilution factor.

c) Determination of Organic Carbon in Charged Biochar

Wet digestion method was used to determine organic carbon in charged biochar where 0.3 g of charged biochar was weighed and 10 ml of potassium dichromate and 20 ml of concentrated sulfuric acid added to the solution then left to for 30 minutes. The mixture was diluted by adding 200 ml of distilled water,10 ml of phosphoric acid was added and five drops of diphenylamine to indicate colour change and then titrate with ferrous ammonium sulphate till the end point (Poveda *et al*., 2021).

The percentage organic carbon was determined using the formula:

$$\%OC = \frac{10(BT - ST)x\ 0.03\ x\ 100}{BT}$$ ………………….formula 4

%OC: Percent organic carbon, BT: blank titre, ST: Sample titre

d) Determination of pH

The potential of hydrogen ions in charged biochar was determined by electrometric method and measured by pH meter with buffers of pH 7 and pH 4.The ratio of charged biochar to water was 1:2.5 (Kalra, 1995).

e) Determination of Cation Exchange Capacity (CEC)

Ammonium Acetate Exchange was used where 1 g of fine dry charged biochar was weighed into 60 ml of plastic bottle. 40 ml of ammonium acetate was added, measure the pH of the solution using pH meter. The mixture was set for 15 hrs. Centrifuge the mixture to separate the biochar from the solution and transfer the supernatant to another container. The final pH of the solution after adsorption was measured using the pH meter. The change in pH (ΔpH) as a measure of the cations adsorbed by the biochar was calculated using the formula:

$CEC = \frac{Va\ x\ N\ x\ 100\ x\ Vb}{sw\ x\ al}$……………………………….formula 5

Va: volume of the acid used, Vb: Volume of extracts, N: concentration of acid used, SW: sample weight, al: aliquot

3.2.4 *Ralstonia* solanacearum Isolation and Identification

Potato (Shangi variety) showing bacterial wilt symptoms was collected from Egerton University teaching and research field 7, washed thoroughly in tap water for 10-15 minutes to remove adhering soil debris then the branches removed leaving only the stem and main roots. It was surface sterilized using 4% sodium hypochlorite followed by dipping into 70% ethanol for 30 seconds (Prior *et al*., 2008). Using sterile scalpel, it was cut into small pieces (15 cm) then submerged into sterile distilled water for 30 minutes under laminar flow (hood), to allow the bacterial ooze from the cut end. The inoculation loop was streaked on nutrient agar media after being dipped in the ooze. The streaked plates were incubated in growth chamber for 48 hours at 30°C to obtain the bacterial culture (Ito *et al*., 2018). Single spore technique was done from the 48 hours old bacteria to obtain pure culture. Pathogen was identified by looking at size, colour, shape and gram staining reaction was performed and further polymerase chain reaction (PCR) molecular identification was done.

3.2.5 DNA Extraction

DNA was extracted by organic extraction method (phenol-chloroform extraction) (Gupta, 2019). Bacterial cells were harvested by centrifugation of 24 hours old bacteria at 10000 revolutions per minute (rpm) for 5 minutes at room temperature using high speed refrigerated centrifuge (HC-3018R). 1ml of bacterial suspension was mixed with 30µl of Sodium dodecyl sulfate (SDS) and 3µl (microliter) of proteinase K incubate for 1 hour at 37 ^{o} C. 100µl of 5M (mole) Sodium chloride (Nacl) and mixed. 80µl of cetyl trim ethyl ammonium bromide (CTAB) was added, mix and incubated for 10 minutes at 65^{o}c. 80µl of chloroform lisomyl was added, centrifuge for 10 minutes at 14000 rpm at room temperature. Viscous aqueous supernatant was carefully collected into fresh test tube. 80µl of Isopropanol added to precipitate the DNA, centrifuge at 14000 rpm at room temperature for 10 minutes. Mix from bottom to top, centrifuged for 10 minutes at 14000 rpm at room temperature. DNA precipitate was washed with 500 ml of 70% ethanol centrifuge for 10 minutes at 14000 rpm at room temperature. The supernatant was carefully removed with tip to remain with DNA pellet. DNA pellet was redisolved in Tris-EDTA (TE) buffer and stored at 4 ^{o} C for the next day. 1000bp DNA ladder was used to confirm the isolate by Polymerase chain reaction (PCR) technique. The pair of specific-species primers used are as shown below;

760F 5’GTC GCC GTC AGC AAT GCG GAA TCG -3’

759R 5’-GTC GCC GTC AAC TCA CTT TCC-3’.

A total volume of 25µl contained 12µl of PCR Master Mix, 1µl of forward primer (759), 1µl of reverse primer (760), 5µl of DNA template, 6 µl Milli Q water (nuclease-free water) were used to carry out PCR amplification. In a thermal cycler, the cycling program is used as follow: A primary denaturation phase at 95℃ for 5 minutes, followed by 35 cycles of 95℃ for 30 s, 57℃ for 45 s and 72℃ for 45 s, followed by a last extension step of 72℃ for 5 minutes. When cycling program was complete, reaction was stopped at 4℃. The suspensions of *R. solanacearum* bacteria of about 10^8 CFU/ml was considered positive control while sterile distilled water was utilized as negative control (Salanoubat *et al*., 2002). Agarose Gel Electrophoresis was done to see the DNA fragment from the bacterial sample. The expected PCR product of 280 base pair was visualized under UV light and photographed.

3.2.6 Inhibitory Effect of Selected Chicken Manure Charged Biochar on *Ralstonia solanacearum in vitro*

Experiment was conducted at Egerton University microbiology laboratory. Bacterial suspension was prepared by pouring sterile distilled water over the stored 48 hour old bacterial growths on nutrient agar (Pradhanang *et al*., 2000). The effects of charged biochar extracts was determined by mixing 0.5 ml of sterile biochar extract with the media (nutrient agar) on plates before the media solidified. Bacterial suspension of 0.1 ml with a concentration of 6 $x10^5$ Cfu / ml adjusted by the hemocytometer under light microscope was spread on each plate and incubated for 72 hours at 28^0C to allow bacterial growth. The experiment was laid out in completely randomized design (CRD) with three replications.

Experimental model

$Y_{ij}=\mu+B_i+\varepsilon_{ij}$

Where:

Y_{ij}: Overall observations

µ: Overall mean

B_i: Effects due biochar extract

ε_{ij}: Random error

3.2.7 Data Collection and Analyses

Data on bacterial growth was collected after 72 hours of incubation; colonies were counted on each plate (solid media) using a colony counter. The inhibition percentage (%I) for each biochar exytract was calculated by applying the formula below:

$\%I = \frac{Cc - CT}{Cc} \times 100$……………………………………………………formula 6

Where %I is inhibition percentage, Cc is number of colonies counted on negative control plates, CT is the colony counted from plates treated with biochar extracts. The colony counts from each plate were subjected to analysis of variance (ANOVA) and analyzed with R software version 4.4.3. The treatments mean separation was done by using Least Significant Difference (LSD) test at a significance level of 0.05.

3.3 Results

3.3.1 Physical Properties of Biochar

Plain biochar exhibited greater porosity than charged biochar with plain maize cob highest at 76.50% and charged rice husks lowest at 70.50%. The density of plain maize cobs was lowest 0.6 g/ml while most charged biochar had densities of 1 g/ml (Table 2).

Table 2: Physical Properties of Biochar

Treatments	Porosity in %	Density in g/ml
BW PB	72.7[b]	0.8[b]
BW CB	70.5[c]	1.0[a]
EU PB	70.5[c]	1.0[a]
EU CB	70.5[c]	1.0[a]
MC PB	76.9[a]	0.6[c]
MC CB	72.7[b]	0.8[b]
MS PB	72.7[b]	0.8[b]
MS CB	70.5[c]	1.0[a]
RH PB	72.7[b]	0.8[b]
RH CB	70.5[c]	1.0[a]

Means followed by the same letter along the column are not significantly different at $p \leq 0.05$ according to Fischer's least significant difference (LSD) test.

Key

BW: Bean waste, EU: Eucalyptus, MC: Maize cobs, MS: Maize straw, RH: Rice husk, PB: plain Biochar, and CB: Charged biochar.

3.3.2 Chemical Properties of Selected Charged Biochar

Chemical analyses revealed that percent organic carbon (% OC) content in all charged biochar was nearly double that of plain biochar with charged maize cob biochar highest at 74.2% and plain eucalyptus biochar lowest at 23.5%. Charged biochar exhibited elevated levels of available phosphorus (AP) with the highest for charged maize cobs at 1846 mg/kg and plain rice husk with lowest at 671.5 mg/kg. For total nitrogen (TN) the highest content was seen in charged bean waste biochar with 1.51% while the lowest was 0.4% for plain eucalyptus biochar, for cation exchange capacity (CEC), charged maize straw biochar exhibited the highest value of 68.47 meq/100 g while maize cobs with 24.47 meq/100 gram is the lowest. Apart from plain rice husk biochar which was slightly acidic at pH of 5.3, all selected biochar exhibited alkaline properties with the highest pH values of 10.35 for charged maize straw biochar and the lowest of 7.2 for charged rice husk biochar (Table 3).

Table 3: Chemical Properties of Selected Charged Biochar

Treatments	pH	%OC	AP(mg/kg)	TN (%)	CEC in meq/100gg
BW PB	10.2^{b}	51.8^{d}	825.0^{g}	0.9^{d}	41.3^{e}
BW CB	10.3^{a}	70.4^{b}	1815.8^{b}	1.5^{a}	44.3^{d}
EU PB	9.6^{e}	23.5^{h}	1053.0^{g}	0.4^{g}	48.3^{c}
EU CB	9.7^{d}	71.1^{b}	1587.3^{d}	1.3^{a}	54.3^{b}
MC PB	10.2^{b}	44.3^{e}	714.5^{h}	0.9^{d}	24.5^{i}
MC CB	10.2^{b}	74.2^{a}	1846.0^{a}	1.2^{b}	40.6^{f}
MS PB	9.9^{c}	41.4^{f}	1352.3^{e}	0.5^{e}	54.2^{b}
MS CB	10.4^{a}	70.0^{b}	1765.2^{c}	1.2^{b}	68.4^{a}
RH PB	5.4^{g}	31.3^{g}	671.5^{i}	0.5^{f}	29.8^{h}
RH CB	7.2^{f}	60.3^{c}	864.5^{f}	0.6^{e}	36.1^{g}

Means followed by the same letter along the same column are not significantly different according to Fischer's Least Significant Difference (LSD) test at $p \leq 0.05$.

Key

BW: Bean waste, EU: Eucalyptus, MC: Maize cobs, MS: Maize straw, RH: Rice husk, PB: plain Biochar, and CB: Charged biochar, pH: Potential of hydrogen, %OC: percent organic carbon, AP: available phosphorus, TN: total nitrogen, CEC: cation exchange capacity.

Means followed by the same letter in the same column are not significant at $p \leq 0.05$

3.3.3 Identification and Confirmation of *Ralstonia solanacearum*

The pathogen appeared as white creamy on plate with nutrient agar (NA) media, small size 0.6 micrometer (µm) in width and 1.8 µm length, rod-shaped and appeared pink under microscope after gram staining which confirmed the bacteria to be gram negative (Chuang & Ko, 2019; Nishat *et al*., 2015; Razia *et al*., 2021). Further confirmation was done and PCR showed the positive result of *R. solanacearum* (Plate 2).

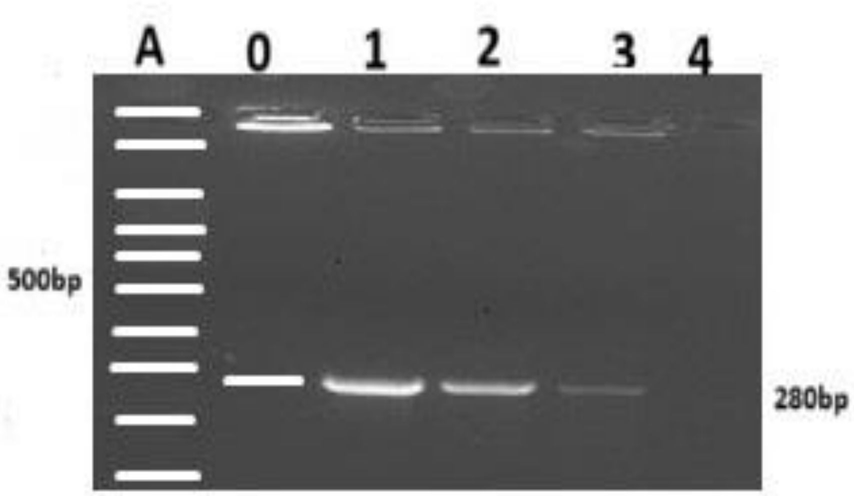

Plate 2: Amplified 280 bp DNA fragments from *Ralstonia solanacearum* with specie - specific primers. A = 1000 bp DNA ladder, 0= Positive control, 1 to 3 is the replicate of the isolates, 4= Negative control

3.3.4 Anti-Bacterial Assay of Selected Charged Biochar on *Ralstonia solanacearum in vitro*

All the treatments (both charged and plain biochar) significantly inhibited *R. solanacearum in vitro c*ompared to distilled water used as negative control whereas eucalyptus registered 100% inhibition of bacterial growth same as copper oxychloride used as a positive control (Table 4 and Plate 3).

Table 4: Anti-Bacterial Assay of Selected Charged Biochar on *Ralstonia solanacearum in vitro*

Treatment	Colony Count (CFU)	Percent Inhibition (%)
Distilled Water	168[a]	0[k]
MS PB	164[b]	2.3[j]
MS CP	133[c]	18[e]
MC PB	90[d]	45[h]
MC CP	71[e]	57[g]
RH PB	63[f]	70[f]
BW PB	49[g]	87[d]
RH CP	21[h]	92[c]
DAP	18[i]	98[b]
BW CB	3[j]	100[a]
COPPER OXY	0[k]	100[a]
EU BP	0[k]	100[a]
EU CB	0[k]	100[a]

Means followed by the same letter in the same row are not significantly different at $p \leq 0.05$

Key

BWCB: Bean waste charged biochar, EUCB: Eucalyptus charged biochar, MCCB: Maize cobs charged biochar, MSCB: Maize straw charged biochar, RHCB: Rice husk charged biochar, EUPB: Eucalyptus plain Biochar, MCPB: Maize cobs plain biochar, MSPB: Maize straw plain biochar, RHPB: Rice husk plain biochar, BWPB: Bean waste plain biochar, and DAP: Diammonium phosphate

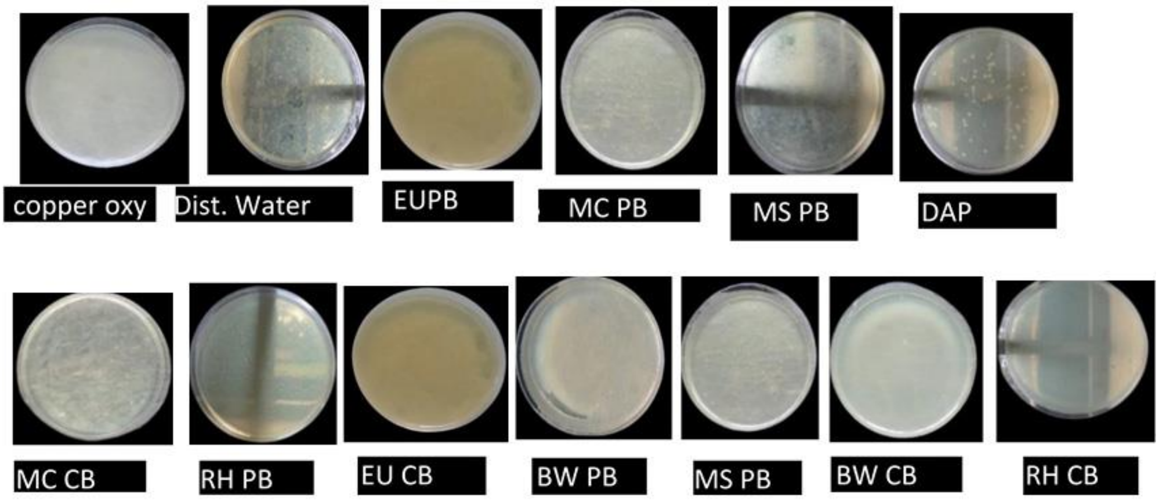

Plate 3: Inhibitory effect of Selected Charged Biochar on Growth of *Ralstonia solanacearum in vitro*

Key

BWCB: Bean waste charged biochar, EUCB: Eucalyptus charged biochar, MCCB: Maize cobs charged biochar, MSCB: Maize straw charged biochar, RHCB: Rice husk charged biochar, EUPB: Eucalyptus plain Biochar, MCPB: Maize cobs plain biochar, MSPB: Maize straw plain biochar, RHPB: Rice husk plain biochar, BWPB: Bean waste plain biochar, and DAP: Diammonium phosphate

3.4. Discussions

The physical and chemical properties of the selected chicken manure-charged biochar indicate its good and high quality. The charged biochar showed alkaline property by exhibiting higher pH than plain biochar. The assimilation of organic acids and ammonium forms when charging biochar with manure contributed to pH adjustment obtained (Upadhyay *et al*., 2024). Alkalinity of charged biochar makes it more effective in controlling soil borne diseases by making environment not conducive to pathogens. The results from the study are in line with the report of Nair *et al*. (2014) whose research reported that biochar from various feed stocks is alkaline in nature though it may differ depending on materials used and pyrolysis temperature. A notable improvement observed in the cation exchange capacity (CEC), reflect an increased ability of biochar to retain and exchange essential cations such as K^{+}, Ca^{2+}, and Mg^{2+}. This aligns with findings by de Medeiros *et al*. (2022)who reported that manure-charged biochar significantly improve CEC due to both surface modification and added organic matter.

High content of nutrients in charged biochar compared to plain biochar is due to high holding capacity of biochar that allows it to absorb and retain nutrient from chicken manure.

Biochar is like sponge; it will absorb and retain the nutrients from the soil once it is used as soil amendment without mixing it with nutrient rich substance and this may result in stunted growth of crops for the first season. This has been proved by this study where charged biochar is high in nutrient than plain biochar (El-Naggar *et al*., 2019; Khadem *et al*., 2021). Even though the carbon content in biochar remained high ensuring the long-term carbon sequestration potential of the biochar, charging introduced more labile carbon fractions. These are readily decomposable and can stimulate microbial activity, which is beneficial for soil health and may enhance the suppression of pathogens from soil such as *Ralstonia solanacearum* (El-Naggar *et al*., 2019).

The inhibitory effects exhibited by the tested biochar extracts are supported by numerous studies on biochar's efficacy in managing crop diseases, despite the absence of study specifically on biochar extracts. Marian *et al***. (**2019) stated that biochar and vinegar produced by pyrolysis contain phenolic compounds and organic acids, which are also found in biochar extracts and are recognized for their antibacterial properties, damaging the cell membranes of pathogens. Biochar extracts have concentration of soluble salts such as: K^+, Na^+, Ca^{2+}, Cl^- that can raise osmotic pressure and acts as bactericidal if at high concentration. During pyrolysis, biochar some plant-derived feedstock produces soluble antimicrobial compounds and phenolic and organic acids that destroy the cell wall of pathogen and thus prevent its growth. Terpens phenolic volatiles and other low molecular weight organics that can be desorbed from can have antimicrobial properties. This could be the reason of inhibitory effect of biochar extract tested in this study (Khadem *et al*., 2021).

The low porosity and high density in charged biochar compared to plain biochar is due to the filling of biochar pores with organic and inorganic compounds introduced by chicken manure used to charge biochar. The higher pH in biochar extracts is the primary characteristic that generates adverse conditions for pathogens, hence resulting in their suppression by making environment not favorable to disease that result in pathogen growth reduction (Azargohar & Dalai, 2018). The findings related to the selected biochar extracts in the study are consistent with those of prior researchers. Eucalyptus which exhibited 100% inhibition of pathogen growth is due to its antibacterial properties, attributed to its abundance of essential oils and terpenes that effectively suppress bacterial proliferation. Low molecular weight of essential in eucalyptus biochar extract and its lipophilic nature helps it to penetrate the pathogen’s cell membrane damage cell structure and cause the leakage of cellular components that results in cell death.

Additionally, tannins and polyphenols present in the biochar extracts possess potent antibacterial characteristics, disrupting microbial protein synthesis as confirmed by Garcia *et al*. (2019). Volatile Organic Compounds (VOCs) included in eucalyptus-derived biochar exhibit inhibitory effects on pathogens due to their volatility and capacity to disrupt cellular processes. Eucalyptus biochar extracts have a strong antibacterial activity given by essential oils and phenolic that can survive through pyrolysis as condensable organics. The reported findings of bean waste inhibiting the growth of *Ralstonia solanacearum* are attributed to the alkaloids present in bean waste biochar, recognized for their antibacterial and antifungal effects. Previous researchers demonstrated that phenolic compounds, organic acid and flavonoids found in maize cobs, maize straw, and rice husks contributed to their antibacterial efficacy (Hossain *et al*., 2020; Khan *et al*., 2014). Bean waste antimicrobial properties is due to their amount of protein, flavonoids and secondary metabolites can form water-soluble nitrogen containing heterocycles and phenolic once pyrolysis (Oni *et al*., 2019). Chicken manure used to charge biochar has high nitrogen in form of ammonia which particularly toxic to many of bacteria, this also could have contributed to high inhibition of *Ralstonia solanacearum* by charged biochar compared to their respective plain.

3.5 Conclusion

The study's findings indicated that all examined biochar extracts repressed the growth and multiplication of *Ralstonia solanacearum in vitro*, attributable to the alkalinity and chemical makeup.

CHAPTER FOUR

EFFICACY OF SELECTED CHICKEN MANURE CHARGED BIOCHAR ON MANAGEMENT OF BACTERIAL WILT (*Ralstonia solanacearum*), GROWTH AND YIELD OF POTATO (*Solanum tuberosum* L.)

Abstract

Potato being central staple food and source of in Kenya, is cultivated on a very large area though its production is still low compared to its consumption. Bacterial wilt caused by *Ralstonia solanacearum* is among the devastating pathogen that cause huge yield loss of potato. The aim of the study was to determine the efficacy of selected chicken-manure charged biochar on management of bacterial wilt, growth and yield of potatoes. Field experiment was conducted at Egerton University and Mau-Narok while pot experiment was conducted at Egerton, laid out in randomized complete block design (RCBD) with three replicates. Eucalyptus (EU), Rice husk (RH) and bean waste (BW) plain and charged were used as they performed well from the previous experiment. The data collected include: emergence, plant height, number of stems, number of tuber per plant and tuber weight. Disease incidence was obtained by counting the number of diseased plant in each plot and severity were assessed by scale of 0-4. Data collected was subjected to analysis of variance (ANOVA) using R software version 4.4.3 and general linear model mean separation was performed with Fisher's least significance (LSD) at $p \leq 0.05$ level of significance. In pot experiment the treatments reduced disease incidence and severity by 72.7% and 95.5% for plain rice husk biochar and 100% for the rest of treatments, respectively. In field experiments, Eucalyptus charged (EUCB) had 0% incidence while plain rice husk (RH) biochar had the highest disease incidences at 66.4%. Additionally, EUCB had 0% disease severity followed by bean waste charged (BWCB) at 9%. Treatments improved plant height by 34.5% for bean waste charged biochar and 13.8% for rice husk plain biochar in the field. Yield was increased by treatments at 54.6% and 15.8% for bean waste charged biochar followed by 51.8% and 8.9% for eucalyptus charged biochar compared to the negative and positive controls respectively. This investigation shows that biochar is good alternative means of controlling bacterial wilt and increasing yield of potato.

4.1 Introduction

Potato is the most consumed staple food for more than billion people in world. The average global produce of potato is projected at 376 million tonnes produced mostly in 159 countries under 18,132,694 hectares and China is the leading potato producer (Rodrigues *et al*., 2025). In Africa, this tuber crop is one of the most common and useful nourishment sources, as it can be consumed in different ways including boiled, fried, mashed or as ingredients in local dishes. East African countries like Kenya, Tanzania, Rwanda, and Uganda are major potato producers, with Rwanda having a high per capita consumption of 125 kg per year which makes potato a major staple food crop for Rwandan population (Kilonzi *et al*., 2024). Despite its production and consumption, the demand is still higher than supply. Low production is due to different factors such as disease, pests, poor agronomic practice and urbanization. Amongst the important diseases is bacterial wilt caused by the bacterium *Ralstonia solanacearum* and is the major constraints that can cause up to 100% yield loss under favorable conditions and susceptible potato variety. The pathogen is one of the most devastating and it is hard to control due its ability to withstand harsh environmental conditions (Arora & Khurana, 2014).

The synergetic effect of biochar with poultry manure has been reported by several studies to be positive in suppressing soil borne pathogen such as: *Pythium spp, Ralstonia solanacearum* and *Fusarium oxysporum* in crops like potato, tomato, eggplant, soybean, wheat due to biochar ability to increase populations of antagonistic microorganisms and enhance soil physical and chemical properties (El-Naggar *et al*., 2019). Enriched biochar improved vegetative growth significantly in crops such as tomato, potato and maize by enhancing nutrient availability and uptake efficiency this make biochar-manure combination to be effective not only in restoring soil fertility but also strengthening plant resistance to stress especially in degraded and pathogen infested soil (Akhtar *et al*., 2015). Several studies have demonstrated the synergistic benefits of combining biochar with poultry manure in enhancing plant growth, suppressing soil-borne diseases and improving crop yield.

Biochar, owing to its porous structure and high surface area, creates a favorable habitat for beneficial microbes, while poultry manure contributes essential nutrients and organic matter that stimulate microbial activity. This combination has been shown to suppress pathogens such as *Ralstonia solanacearum*, *Fusarium oxysporum*, and *Pythium spp.*, likely due to increased populations of antagonistic microorganisms and improved soil physicochemical properties (El-Naggar *et al*., 2019). The aim of this study was to determine how chicken manure charged biochar can influence the growth and yield of potato and its impacts in management of bacterial wilt.

4.2 Materials and Method

4.2.1 Site Description

Field experiment was done in Mau-Narok and Egerton University research and teaching field. Egerton University is located in Njoro, Nakuru County with the coordinate35° 35' E longitude, latitude of 0° 23'S (Bulitia, 2019). Mau narok is located on the latitude of -0.17344 and longitude of 35.86313 (Khan *et al*., 2020). All those sites experiences tow rain seasons: long rainy season range from March to June and Short rainy season occur in September to December (Kong'ani *et al*., 2018).The soil type in Mau Narok is dominated by clay loam, andosols from volcanic activity of rift valley, it is black, poorly drained that can make it flooded during high rain falls while soil type at Egerton is clay roam influenced by volcanic parent material. Soils tend to be Nitisols and they are deep, reddish in color, quite weathered (Watene *et al*., 2021) (Table 5).

Table 5: Characteristics of experimental sites

	Egerton University	Mau-Narok
Altitude (m.a.s.l)	2190	2,600
Temperature(^{o}C)	16.1	18.56
Rainfall (mm)	1000	1300
Soil type	Vertisols	Andosol

Source: (Mbabazize *et al*., 2023 ; Kamai, 2021)

4.2.2 Potato Variety

Shangi variety is the most grown and preferred potato variety in Kenya and has the history of being susceptible to bacterial wilt. It is a partial- vertical middle tall variety with height of almost 1 meter, abstemiously sturdy stalks green in color and light comprehensive leaves and pink flowers as shown on Plate 4 A). The variety produces well in high altitude of above 1500 meter above sea level in the places like Nakuru, Bomet, Narok and Kericho. It is known for its early maturity in about 3.5 months with the average yield of between (30-40 t ha^{-1}) in case of optimal conditions. The tubers shape is oval, skin is smooth and cream, while the eyes are medium to deep while flesh is white(Haro, 2022) (Plate 4 B) . *Shangi* is said to be prone to late blight. The variety was selected because is suitable for the study and are preferred by most of farmers in Nakuru, and overall Kenya in general.

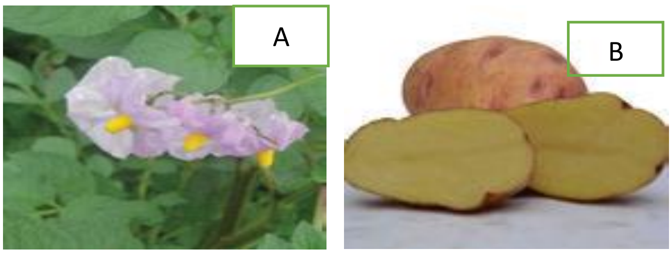

Plate 4: *Shangi* potato Variety: A - flowers and B – tubers. **Source:** (Irungu *et al*., 2022)**.**

4.2.3 Determination of *Ralstonia solanacearum* Population Density in Experimental Fields

Soil samples from experimental fields were collected at 10 cm depth and detected for the presence *Ralstonia solanacearum* inocula. Sterile distilled water was added to a test tube containing ten grams of soil sample, the mixture was 1:10 ratio shaken with rotary shaker at 150 rpm for 30 minutes to release microorganisms from the soil. The mixture was centrifuged at 1000 rpm for one minute to remove soil debris (Jaffee *et al*., 1992). The aliquot of 1ml was taken to a new test tube mixed with 9 ml of sterile distilled water and the process was repeated up to 10^{-4} serial dilution. An aliquot of 0.1 ml of the suspension was spread on plate with semi selective south Africa (SMSA) media composed by Peptone 10 g, Glucose 5 g, Agar-Agar 5 g, 2,3,5-Triphenyl Tetrazolium Chloride (TZC) 0.05 g in one litre of sterile distilled water and incubated at 28^{0}C for 72 hours to allow the bacteria to grow. The colonies with small size, fluidal, irregular shape and creamy whitish with reddish color in center were counted and population density per gram of soil calculated using the formula in equation 1descrobed by Ito *et al*. (2018).

$$\frac{CFU}{gram}\, of\ soil = (Number\ of\ colonies \times dilution) \div volume\ plated \ldots\ldots \text{ Equation 1}$$

4.2.4 Experimental Procedure and Layout

Land was prepared using a hoe and biochar was applied 15 tonnes per hectare rate two weeks prio to planting (Tian et al., 2021). Egerton research and teaching field 7 was planted on 19th October 2024 and Mau Narok on 21st October 2024. Tubers were planted at a spacing of 75 cm x 30 cm planting depth of 10 cm (Ninh et al., 2015). Plot size was 3 m x 2 m with four rows and six plants per row. The crops were grown on the same fields where potatoes were grown in the previous season in the two locations. Certified Potato seeds (shangi variety) used in the experiment were sourced from KALRO Molo. Experiment was laid out in randomized complete block design (RCBD) in both locations as presented in (Figure 2) and the treatments used as shown in (Table 6).

Table 6: Treatment Structure

Treatments	Description
T1	Bean waste plain (BWPB)
T2	Rice husk plain (RHPB)
T3	Eucalyptus plain (EUPB)
T4	Bean waste charged (BWCB)
T5	Rice husk charged (RHCB)
T6	Eucalyptus charged (EUCB)
T7	Positive control (DAP)
T8	Negative control (No treatment)

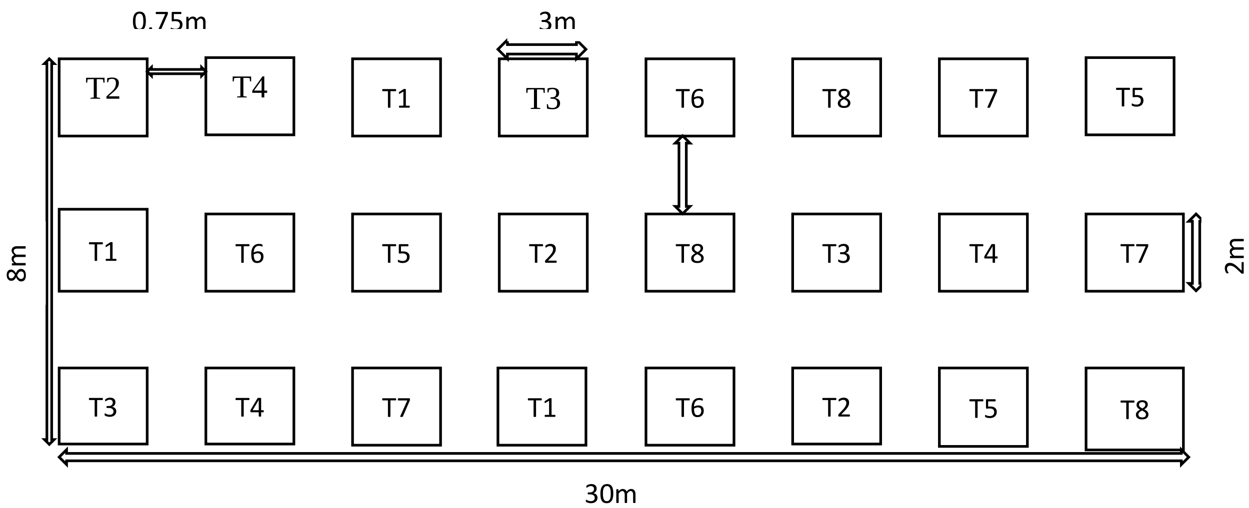

Figure 2: Field Experimental Layout

4.2.5 Agronomic Practices

Two weeks after fully emergence and at tuber bulking stage (70 days after planting) weeding was performed. Hilling up was performed at 5 weeks after planting to promote the roots to grow better underground and prevent the tuber from sunlight and turning green. Late blight was controlled by applying Ridomil Gold MZ WG which contain 40 grams per kilogram of Metalaxyl-M 640 grams per kilogram of Mancozeb at 14 days interval (Mburu Njoroge, 1985; Tedla, 1985)

4.2.6 Pot Experiment

The pot experiment was carried out at Egerton University teaching and research field 7 in a shade net structure. Pots of 35 cm height and 20 cm width was used, eight kilograms of sterile soil mixed with fine sand at the ratio of 1:2 ratio. An addition of 2% weight per weight (w/w) of each specific biochar was done and mixed with soil for two weeks before planting. The 2% w/w rate was chosen because it performed well in management of disease (Yadessa *et al.,* 2020). Potato tubers were washed with tap water, sterilized using 2% sodium hypochlorite and then washed with sterile distilled water, left for one hour at room temperature to allow them to dry. Potatoes were wounded by sterile scalpel, soaked in specific biochar extract overnight; the following day the tubers were left standing under shade to allow the sticking of active compound from biochar extracts to potato tubers. The tubers were also soaked in bacterial suspension of 1.6×10^8 colony forming unity (CFU) overnight and incubated for 24 hours at room temperature to enhance the sticking of the bacteria before planting (Abo-Elyousr & Asran, 2009). One certified potato seed was planted in pot containing specific biochar. The experiment was monitored daily from 14 days after emergence to see the appearance of wilting symptoms.

Experimental Model

$Y_{ijk}= \mu+T_i+B_j+L_k+B_jT_i+L_kT_j+Ti+T_iB_jL_k+\varepsilon ijk$

$Y_{ij=}$ Overall observation

μ: overall mean

T_i: Observation due to charged biochar

Bj: Observation due to block

Lk: Observation due to location

ε_{ijk}: Error term

B_jT_i: Interaction effect of biochar and block

L_kT_j: Interaction effect of location and biochar

$T_iB_jL_k$: Interaction effect of Biochar, block and location

4.3 Data Collection and Analyses

4.3.1 Disease Incidence

Disease incidence was measured by counting the number of symptomatic crop and divide by total number of crop assessed. Disease incidence was converted into percent disease incidence (PDI) by applying the formula below (Sharma & Singh, 2019):

$$PDI = \frac{Number\ of\ diseased\ plants}{Total\ number\ of\ plants\ assessed} \times 100 \text{..................equation 2}$$

4.3.2 Disease Severity

To know how severe disease is, a scale of 0 to 4 disease was used, where 0 = no symptoms of wilting, 1 = 1 to 25% of stems have wilting, 2 = 26 to 50% of stems have wilting, 3 = 51 to 75% of stems have wilting and 4 = 76 to 100% have wilting symptoms (Plate 5) (Ciampi-Panno *et al*., 2009).

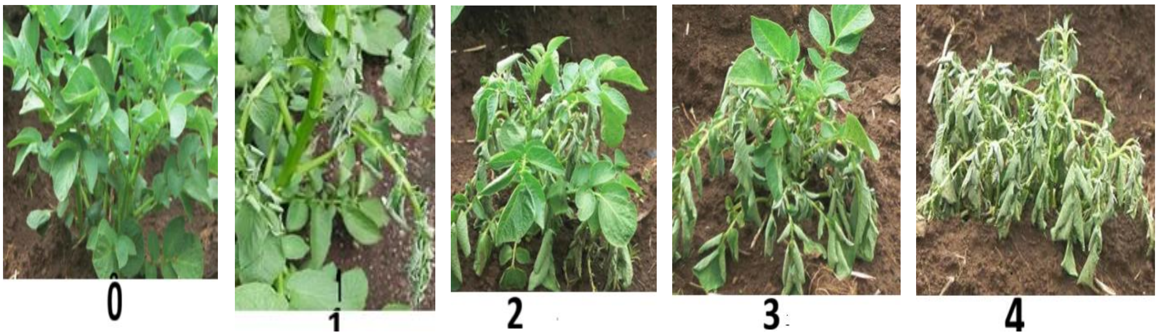

Plate 5: Pictorial representation for scoring disease severity .where: 0=no appearance of wilting symptoms, 1= 1-25% stems have wilting symptoms 2=26-50% of the stems have symptoms 3=50-75% have wilting symptoms, 4=76-100% have wilting symptoms. **Source:** (Wilson et al., 2002).

The formula by (Seem, 2004) was applied to get the disease severity index (DSI):

$$DSI = \frac{\sum individual\ numerical\ rating}{Total\ number\ of\ plant\ assessed \times maximum\ score\ in\ the\ scale} \text{.......... Equation 3}$$

From the disease severity index (DSI), area under disease progression curve (AUDPC) was calculated using the equation below:

$$AUDPC = \sum_{i=1}^{n=1} \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i) \text{..Equation 4}$$

Where; n number of observations, t: time of each reading in days, y_i the disease severity at time, y_{i+1}:disease severity on assessment date $(i + 1)$, t_i the time of observation (days after planting) and t_{i+1} is the second assessment date of two consecutive assessment (Hossain *et al*., 2020).

Pearson correlation analysis was used to assess how the disease is associated with growth and yield of potatoes. Data were analyzed using R software version 4.4.3 and general linear model (GLM) technique was used to conduct ANOVA. Means were separated by Fisher's least significance difference (LSD) at $p \leq 0.05$ level of significance.

4.3.3 Growth and Yield of Potato

Data collected on growth and yield included; Emergence which was done by manually counting the total number of plants emerged in each plot for each treatment 21 days after planting. Plant height was measured at the interval of 14 days starting from 35 until 65 days after planting using a ruler. Number of stems were counted at 65 days after planting. After harvesting, the average number of tubers per plant were counted for each treatment, assessed using electronic balancing gauge and was expressed into tonnes per hectare.

4.4 Data Analyses

Normality test of data was done with Shapiro Wilk test.

ANOVA was conducted by use of R. software version 4.4.3 and general linear model (GLM) technique. Pearson's correlation was used to analyze the correlation between disease, growth and yield and give the insights into how bacterial wilt affected potato productivity. The treatments mean separation was done by using Fisher's Least Significant Difference (LSD) at $P \leq 0.05$ level of significance.

4.5 Results

4.5.1 Determination of Population Density of Pathogen in Experimental Field

The inhabitants of *Ralstonia solanacearum* from Egerton site was found to be 1.8×10^6 CFU/gram of soil a while Mau-Narok site the density was 4.6×10^6 CFU/gram of soil which was found to be 175% higher than Egerton site.

4.5.2 Effects of Chicken Manure Charged Biochar on Percent Disease Incidence and severity in Pot Experiment

The first symptomatic crop was seen from untreated pot at 12 days after emergence The incidence of bacterial wilt in this experiment was 100% for the control as all the replicates of untreated pots showed the wilting symptoms during the time of experiment. For the applied treatments, rice husk biochar reduced disease incidence and severity by 33.3% and 57.1% respectively while the rest treatments reduced the incidences by 100%. Treatments also significantly reduced the severity of bacterial wilt in pot experiment (Plate 6). The treatments performed well by significantly reducing the disease severity of bacterial wilt in comparison to negative the control, the highest score was 5 in negative control where the plant wilted and dried up. This was followed by plain rice husk biochar which had a score of

3 with 68% of the crop showing the symptoms. The rest of the treatments scored zero as the crops treated showed no symptoms of wilting (Table 7).

Charged Eucalyptus biochar

Diammonium phosphate

Rice husk plain biochar

Negative control

Source: The author

Plate 6: Disease Severity of Bacterial Wilt in Pot Experiment

Table ity in pot experiment

Treatments	PDI (%)	DSI
Control	100.0^{a}	31.8^{b}
RHPB	33.3^{b}	1.4^{b}
BWCB	0.0^{b}	0.0^{b}
BWPB	0.0^{b}	0.0^{b}
DAP	0.0^{b}	0.0^{b}
EUCB	0.0^{b}	0.0^{b}

Treatments	PDI (%)	DSI
EUPB	0.0[b]	0.0[b]
RHCB	0.0[b]	0.0[b]

4.5.2 Effect of Selected Chicken Manure Charged Biochar on Growth and Yield in Pot Experiment

The treatments significantly affected the growth and yield of potato in pot experiment. Eucalyptus charged biochar increased plant height to 83 cm which was the highest as compared to untreated at 30.3 cm and positive control (DAP) at 71 cm. There was no significant difference between biochar treatments on stem number at $p \leq 0.05$ but bean waste charged biochar had the highest number of stems at 5 while untreated control had the lowest at 3. For number of tubers, positive control had the highest at 8.3 but was not significantly different from that of bean waste charged biochar at 7.6. Similarly, bean waste both charged and plain biochar had the highest tuber weight per hectare at 14.7 and 12.2 t ha^{-1} respectively followed by eucalyptus charged which produced 13.9 t ha^{-1} (Table 8).

Table 8: Effects of Selected Chicken Manure Charged Biochar on Growth and Yield in Pots Experiment

Treatment	Plant height (cm)	Branch number	Tuber number/plant	Tuber weight (t ha^{-1})
Control	30.3^{c}	3^{b}	0.0^{bcd}	0.0^{bcd}
RHCB	81.6^{a}	4^{a}	6.3^{b}	12.9^{b}
EUCB	83.0^{a}	4^{a}	6.7^{ab}	13.9^{a}
BWCB	80.0^{a}	5^{a}	7.6^{a}	14.7^{a}
DAP	71.5^{a}	5^{a}	8.3^{a}	12.5^{b}
BWPB	65.3^{a}	4^{a}	6.0^{b}	13.2^{a}
RHPB	52.0^{b}	4^{a}	6.0^{b}	9.6^{bc}
EUPB	62.0^{ab}	4^{a}	6.0^{b}	11.9^{b}
LSD	21.68	1.1	1.7	1.5
CV	17.3	13.7	15.5	7.1

Means followed by the same letter within a column are not significantly different at $p \leq 0.05$ according to Fischer's least significant difference (LSD test)

Key

RHCB: Rice husk charged biochar, BWCB: Bean waste charged biochar, DAP: Diammonium phosphate, EUCB: Eucalyptus charged biochar, EUPB: Eucalyptus plain biochar, RHCB: Rice husk charged biochar, CV: Coefficience of variation

4.5.3 Effects of Selected Chicken Manure Charged Biochar on Area Under Disease Progress Curve (AUDPC) in Pot Experiment

All the pots treated with biochar showed significant reduction in areas under disease progress curve at $p \leq 0.05$ compared to the untreated pot which exhibited the highest AUDPC of 128.6. Plain rice husk biochar reduced the areas under disease progress curve at 91.9% while the rest of treatments reduced the AUDPC by 100% as the pots treated with them did not show any wilting symptoms. The treatments show no significant effect on areas under disease progress curve among (Figure 3).

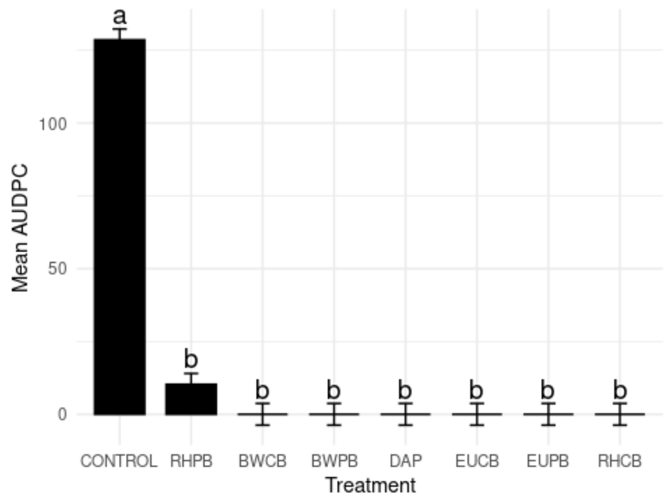

Figure 3: Effects of Selected Chicken Manure Charged Biochar on Area Under Disease Progress Curve (AUDPC) In Pots Experiment. Bars with same letter are not significantly different according to Fischer's least significant (LSD) test at $p \leq 0.05$.

Key

RHCB: Rice husk charged biochar, BWCB: Bean waste charged biochar, DAP: Diammonium phosphate, EUCB: Eucalyptus charged biochar, EUPB: Eucalyptus plain biochar, RHCB: Rice husk charged biochar.

4.5.4 Effect of Selected Chicken Manure Charged Biochar On Bacterial Wilt Disease Incidence Under Field Experiment

Comparing the two locations, the average disease incidences were higher in Mau-Narok at 5.13% than Egerton at 4.13%. Disease incidence for both locations were not high, the highest incidence of 12.5 was seen in the negative control while plots treated with charged eucalyptus biochar showed no symptoms in both locations. This is in line with the experiment conducted *in vitro* where eucalyptus charged biochar extract inhibited the growth of *Ralstonia solanacearum* at 100% in comparison with to the other tested biochar extracts. All the treatments significantly reduced the incidence of bacterial wilt at $p \leq 0.05$ compared to the negative control. Rice husk charged biochar and eucalyptus plain biochar resisted disease attack up to the 56^{th} and 53^{rd} days, respectively at Egerton. There were no incidences for the positive control (DAP) at Egerton but Mau-Narok showed remarkable incidences (Table 9).

Table 9: Effects of Selected Chicken Manure Charged Biochar On Percent Disease Incidence at Egerton and Mau-Narok

Location	Egerton					Mau Narok				
Treatments	Days After Planting					Days After Planting				
	42	49	56	63	70	42	49	56	63	70
CONTROL	12.5^{a}	12.5^{a}	12.5^{a}	12.5^{a}	12.5^{a}	4.2^{a}	8.3^{a}	8.3^{a}	12.5^{a}	12.5^{a}
BWCB	0.0^{b}	4.2^{b}	4.2^{b}	4.2^{b}	4.2^{b}	0.0^{b}	0.8^{b}	4.2^{b}	4.2^{b}	4.2^{b}
BWPB	0.0^{b}	4.2^{b}	4.2^{b}	4.2^{b}	4.2^{b}	0.0^{b}	0.0^{b}	4.2^{b}	4.2^{b}	4.2^{b}
DAP	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	1.4^{b}	5.5^{b}	5.5^{ab}
EUCB	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}
EUPB	0.0^{b}	0.0^{b}	0.0^{b}	4.2^{b}	4.2^{b}	0.0^{b}	0.0^{b}	4.2^{b}	4.2^{b}	4.2b
RHCB	0.0^{b}	0.0^{b}	4.2^{b}	4.2^{b}	2.7^{b}	0.0^{b}	0.0^{b}	0.0^{b}	5.0^{b}	5.0^{ab}
RHPB	0.0^{b}	0.0^{b}	4.2^{b}	4.2^{b}	4.2^{b}	0.0^{b}	0.0^{b}	0.0^{b}	4.2^{b}	8.3^{ab}
CV	9e-14	138.5	137.7	139.7	91.4	489.8	223.9	130.7	117.8	110.2
LSD	2e-15	6.2	7.7	9.7	11.7	4.5	4.5	6.8	9.9	8.0

Means followed by the same letter within a column are not significantly different at $p \leq 0.05$ (LSD test).

Key

CV = Coefficient of Variation; LSD = Least Significant Difference, BWCB = Bean waste charged biochar; BWPB = Bean waste plain biochar; DAP = Diammonium phosphate; EUCB = Eucalyptus charged biochar; EUPB = Eucalyptus plain biochar; RHCB = Rice husk charged biochar; RHPB = Rice husk plain biochar.

4.5.5 Effects of Selected Chicken Manure Charged Biochar on Disease Severity in Field Experiment

Comparing two locations, disease was more severe at Mau-Narok site with average percent disease severity of 6.4% while Egerton site recorded the average percent disease severity of 5.0%. Treatments showed significant effect in reducing disease severity compared to the negative control. Among treatments there was significant difference especially at Egerton site where charged eucalyptus biochar was significantly different from its corresponding plain biochar and other treatments. Charged bean waste performed better at Egerton with disease severity of 1.4 than Mau-Narok where it recorded 2.7 which is 92.8% higher than Egerton records. Diammonium phosphate did not show any symptoms at Egerton site while at Mau-Narok the severity recorded in plots treated with DAP was 8.3. Charged rice husk biochar performed well in reducing bacterial wilt severity compared to plain rice husk in both locations. Charged rice husk biochar recorded the severity of 1.4 and 4.2 at Egerton and Mau-Narok respectively while its corresponding plain recorded 2.7 and 5.5 that is 92.8% and 30.9% higher than rice husk charged biochar records on severity at Egerton and Mau-Narok respectively (Table 10).

Table 10: Percent Disease Severity for Egerton and Mau-Narok

Location			Egerton					Mau-Narok		
Treatments			Days After Planting					Days After Planting		
	42	49	56	63	70	42	49	56	63	70
CONTROL	6.9^{a}	12.5^{a}	15.2^{a}	20.8^{a}	27.7^{a}	1.4^{a}	4.2^{a}	6.9^{a}	11.1^{a}	22.2^{a}
BWCB	0.0^{b}	0.0^{b}	0.0^{b}	1.4^{b}	1.4^{b}	0.0^{b}	0.0^{b}	0.0^{b}	1.4^{b}	2.7^{b}
BWPB	0.0^{b}	1.4^{b}	1.4^{b}	1.4^{b}	4.2^{bc}	0.0^{a}	0.0^{b}	0.0^{b}	1.4^{b}	4.2^{b}
DAP	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	1.4^{b}	5.5^{b}	8.3^{b}
EUCB	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}
EUPB	0.0^{b}	0.0^{b}	1.4^{b}	2.7^{b}	2.7^{b}	0.0^{a}	1.4^{b}	1.4^{b}	2.7^{b}	4.2^{b}
RHCB	0.0^{b}	0.0^{b}	1.4_{b}	2.7^{b}	1.4^{b}	0.0^{a}	0.0^{b}	1.4^{b}	2.8^{b}	4.2^{b}
RHPB	0.0^{b}	0.0^{b}	1.4^{b}	2.7^{b}	2.7^{b}	0.0^{b}	0.0^{b}	1.4^{b}	2.7^{b}	5.5^{b}
CV	194.6	96.8	101.3	88.7	86.4	489.8	230.7	148.1	121.0	105.7
LSD	3.0	3.2	4.6	6.7	9.2	1.5	2.8	4.1	7.3	12.8

Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$.

Key

RHCB: Rice husk charged biochar, BWCB: Bean waste charged biochar, DAP: Diammonium phosphate, EUCB: Eucalyptus charged biochar, EUPB: Eucalyptus plain biochar, RHCB: Rice husk charged biochar.

4.5.6 Effects of Selected Biochar on Area Under Disease Progress Curve (AUDPC) In Field Experiment

High AUDPC was observed in the untreated plots compared to plots with treatments. Charged eucalyptus biochar had AUDPC 0% as the pots where it was applied plants did not develop any symptoms throughout the period of experiments (Table 11).

Table 11: Effects of charged biochar on areas under disease progress curve in field experiment

Treatments	AUDPC Mau-Narok	AUDPC Egerton
CONTROL	38.8[a]	88.0[a]
DAP	25.8[b]	0.0[f]
EUPB	17.7[c]	12.7[c]
RHPB	16.2[cd]	12.7[c]
RHCB	14.2[d]	27.4[b]
BWPB	8.2[e]	8.2[d]
BWCB	6.4[e]	4.9[e]
EUCB	0.0 [f]	0.0[f]

Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$.

Key

RHCB: Rice husk charged biochar, BWCB: Bean waste charged biochar, DAP: Diammonium phosphate, EUCB: Eucalyptus charged biochar, EUPB: Eucalyptus plain biochar, RHCB: Rice husk charged biochar.

4.5.7 Effect of Selected Chicken Manure Charged Biochar on Emergence and Number of Branches of Potato

There was no statistical difference in plant emergence across the treatments at $p \leq 0.05$. Rice husk and Bean waste plain biochar showed the highest emergence percentage (100%) at Egerton while Rice husk charged biochar showed poor emergence with 93.03%. At Mau-Narok, Bean waste and Rice husk plain biochar had emergence of 94.4% and 84.6%, respectively. The treatments had not significant effect on the number of branches when compare to the negative control. Bean waste both plain charged and DAP performed well in number of branches with average of 5 branches at Egerton site while in Mau-Narok site they recorded 4 and 6 branches for both plain and charged bean waste and DAP respectively (Table 12).

Table 12: Effects of Selected Chicken Manure Charged Biochar On Emergence and Number and of Branches of Potatoes

Location	Egerton		Mau-Narok	
Treatments	%Emergence	Braches	%Emergence	Branches
BWPB	100[a]	5[a]	84.6[b]	4[a]
RHPB	100[a]	4[a]	94.4[ab]	4[a]
CONTROL	98.5[ab]	4[a]	91.6[ab]	4[a]
BWCB	97.2[ab]	5[a]	93.03[ab]	4[a]
DAP	97.2[ab]	5[a]	93.03[ab]	6[a]
EUPB	97.2[ab]	4[a]	97.2[a]	4[a]
EUCB	94.4[ab]	3[a]	86.1[b]	4[a]
RHCB	93[b]	3[a]	90.2[ab]	4[a]
CV	4.6	22.8	13.7	6.4
LSD	6.9	1.3	1.6	10.7

Means followed by the same letters in the same column show no significance at $p \leq 0.05$

Key

RHCB: Rice husk charged biochar, BWCB: Bean waste charged biochar, DAP: Diammonium phosphate, EUCB: Eucalyptus charged biochar, EUPB: Eucalyptus plain biochar, RHCB: Rice husk charged biochar.

4.5.8 Effect of Selected Chicken Manure Charged Biochar on Plant Height at Different Growth Stages

Treatments did not significantly affect plant height at 35 days after planting but significant difference was seen at 50 and 65 days after planting. Comparing locations, treatments performed differently in two locations. DAP had the highest mean height of 40.57 and 60.5 cm at 50 and 65 days after planting respectively at Egerton site, while in Mau-Narok DAP recorded the mean height of 23.7 cm and 39.72 cm at 50 and 65 days after planting respectively which was the highest from the site. Bean waste charged biochar was the second in both locations though it performed better at Egerton with 40.5 cm and 57.5 cm at 50 and 65 days after planting respectively while in Mau-Narok it recorded 20 cm and 34.5 cm at 50 and 65 respectively. The shortest mean plant height was from untreated plots in both locations with 41.8 cm and 26.3 cm for Egerton and Mau-Narok respectively (Figure 4,5, and 6).

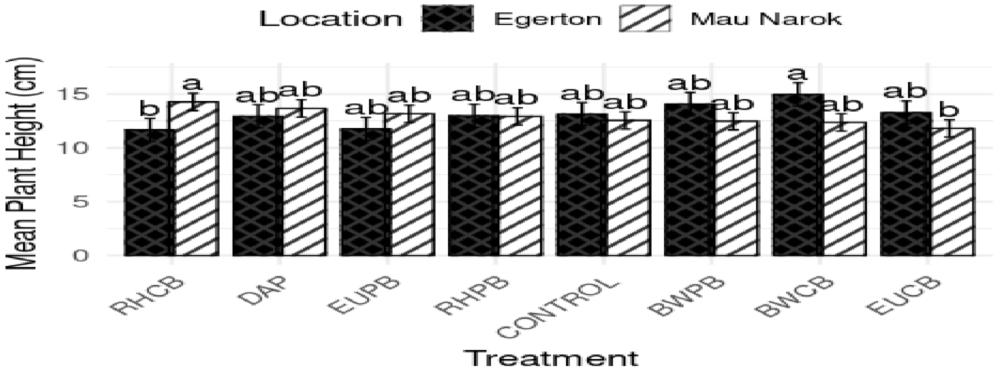

Figure 4: Effects of selected charged biochar on plant height at 35 days after planting (vegetative growth). Key: RHCB: Rice husk charged biochar, BWCB: Bean waste charged biochar, DAP: Diammonium phosphate, EUCB: Eucalyptus charged biochar, EUPB: Eucalyptus plain biochar, RHCB: Rice husk charged biochar.

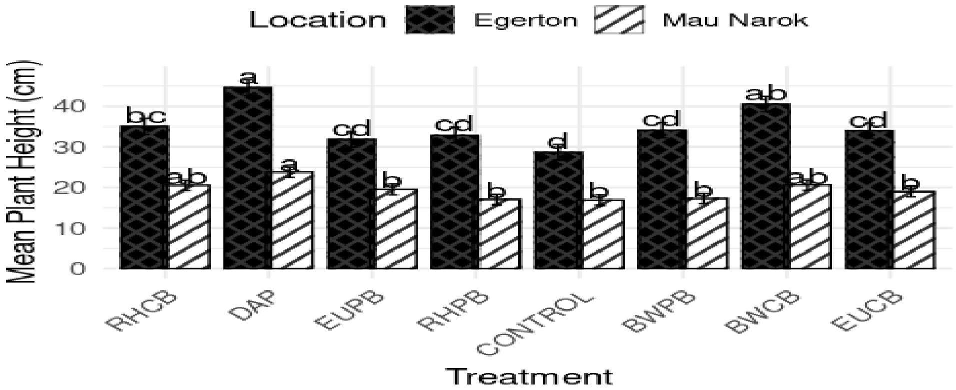

Figure 5: Effects of selected charged biochar on plant at 50 days after planting (flowering stage)

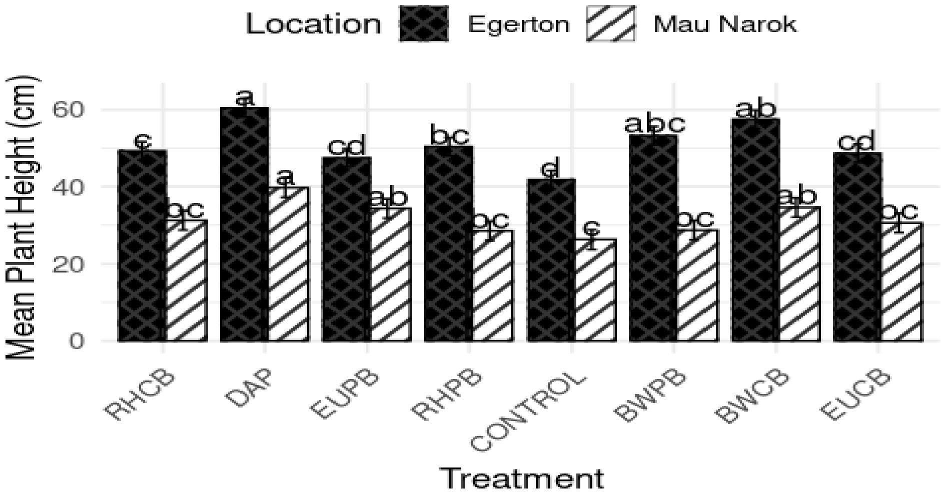

Figure 6: Effects of Selected Charged Biochar on Plant Height at 65 Days After Planting (Tuber bulking stage).

Bars with the same letter show no statistically difference at $p \leq 0.05$ level of significance. Key: RHCB: Rice husk charged biochar, BWCB: Bean waste charged biochar, DAP: Diammonium phosphate, EUCB: Eucalyptus charged biochar, EUPB: Eucalyptus plain biochar, RHCB: Rice husk charged biochar.

4.5.9 Effects of Chicken Manure Charged Biochar on Yield of Potato

Analysis of variance showed that the treatments yield was significantly improved comparing to the negative control in both locations. Comparing both locations, the treatments significantly improved the yield better in Egerton than Mau-Narok. Treatments effect on average number of tubers per plant were not significantly different from the negative control, but the highest number of tubers was produced by Diammonium phosphate with 8 tubers per plant followed by bean waste charged biochar and eucalyptus charged biochar with 7 tubers per plant at Egerton site. Mau-Narok, the highest number of tubers per plant recorded was 8 produced by charged bean waste followed by DAP and charged eucalyptus biochar which produced 7 while the lowest number was 4 recorded from the negative control. Tuber weight which is expressed in tonnes per hectare was significantly affected by the treatments across locations. BWCB produced the highest yield followed by DAP with 17.7 and 17.3 tonnes ha^{-1} respectively while the least was negative control with 8.03 tonnes ha^{-1} at Egerton University. Mau-Narok the highest yield recorded was 14.6 followed by 13.9 from charged bean waste and eucalyptus respectively while the control recorded the lowest 6.5 tonnes ha^{-1}. In Mau-Narok, DAP performed poorly in terms of yield up to 63.2% lower than that it produced at Egerton (Table 13).

Table 13: Effects of Selected Chicken Manure Charged Biochar on Yield of Potato

	Egerton		Mau-Narok	
Treatments	Tuber no	Tuber weight(t/ha)	Tuber no	Tuber weight(t/ha)
BWPB	7^{a}	14.6^{b}	6^{a}	11.7^{b}
RHPB	6^{a}	14.7^{b}	6^{a}	12.0^{b}
CONTROL	5^{ab}	8.0^{c}	4^{b}	6.5^{c}
BWCB	8^{a}	17.7^{a}	8^{a}	14.5^{a}
DAP	8^{a}	17.3^{a}	7^{a}	10.6^{b}
EUPB	7^{a}	14.5^{b}	6^{a}	11.9^{b}
EUCB	7^{a}	16.3^{ab}	7^{a}	14.0^{a}
RHCB	7^{a}	15.7^{ab}	6^{a}	13.2^{a}
CV	27.2	12.2	15.5	7.1
LSD	3.4	1.7	3.2	1.5

Means with the same letter in the same column are not significantly different at $p \leq 0.05$ LSD: Least significant difference, CV: Coefficient of variation. Key: RHCB: Rice husk charged biochar, BWCB: Bean waste charged biochar, DAP: Diammonium phosphate, EUCB: Eucalyptus charged biochar, EUPB: Eucalyptus plain biochar, RHCB: Rice husk charged biochar.

4.5.10 Correlation of Growth, Yield, Percent Disease Incidence and Areas Under Disease Progress Curve in Pots Experiment

Pearson correlation (r) analysis was done to analyze how plant growth (height, number of stem), yield were associated with disease incidence and areas under progress curve. The results showed that plant growth was negatively correlated with percent disease incidence and area under disease progress curve. Plant height, number of stem and branches were slightly negatively correlated with disease incidence with coefficient of correlation (r) of -0.24, -0.21 and -0.53 respectively which indicate weak negative correlation. Tuber number per plant and tuber weight had negative correlation with disease incidence with r -0.56 and -0.61 respectively. Tuber weight, disease incidence and AUDPC were strongly negatively correlated where r was -0.77 for disease incidence and -0.87 for area under disease progress curve. Disease incidence and area under disease progress curve displayed a strong positive correlation with r 0.91 (Table 14).

Table 14: Correlation of Growth, Yield, Disease Incidence and Area Under Disease Progress Curve in Pots Experiment

	Plant height	PDI	AUDPC	Branch no	Tuber no	Tuber weight
Plant height	1.0	-0.2	-0.6	0.5	0.3	0.6
PDI		1.0	0.9	-0.2	-0.6	-0.8
AUDPC			1.0	-0.4	-0.6	-0.9
Stem no				1.0	0.4	0.5
Tuber no					1.0	0.6
Tuber weight						1.0

PDI: percent disease index, AUDPC: area under disease progress curve

4.6 Discussions

The study revealed that selected biochar applied are effective in controlling bacterial wilt and improvement of potato yield. All the treatments used in the study reduced the incidence, severity and area under disease progress curve in both pot and field experiments. The physical and chemical properties of biochar such as: high pH, high nutrient content (organic carbon, total nitrogen, available phosphorus, CEC) contributed to its performance in suppressing *Ralstonia solanacearum* that cause bacterial wilt. Alkaline property of biochar is known to inconvenience pathogen by creating environment which is not favorable for pathogen's growth, this results in reducing plant pathogen attack and disease progress. Morgan *et al.* (2002) conducted a study determining the factors that influence the soil borne disease and concluded that most soil borne pathogens growth is favored by soil acidity which can be the reason why biochar inhibited the growth of pathogen and reduce the disease infection due to its buffering capacity. High porosity of biochar contributes to disease suppression due to its pores that provide shelter for beneficial microorganisms that help in pathogen antagonism. Biochar is able to limit the pathogen's mobility in the soil and reduce the infection as it can trap and immobilize pathogens due to its porous structure. Biochar can also reduce disease pressure by absorbing exudates and toxins released by pathogens (Are *et al.*, 2017).

High organic carbon, available phosphorus and total nitrogen found in biochar are the main nutrients that increase the plant vigor and help the plant to resist the pathogen attack. Organic carbon in biochar improved soil and microbial activity by increasing soil organic matter which stimulates beneficial microbes that suppress pathogens. It also improves nutrients cycling that reduce nutrients loss and make use of nutrients by plant. Organic carbon contributes to potato root and tuber development by enhancing soil aggregation, aeration and water-holding capacity due to improved soil structure caused by biochar. High organic carbon in biochar make it nutrient reservoir and serves as a slow-release source of nutrients both macro and micro-nutrients (Chan & Xu, 2019). Nitrogen, being a key component of proteins, chlorophyll and enzymes that supports vigorous vegetative growth make it one of the crop growth drivers as it promotes leaf development and chlorophyll contents that increase photosynthetic capacity and this results in better growth of potato. Biochar increased yield due to the supply of adequate nitrogen that supports stolon development and enhances tuber bulking (Hellmann *et al*., 2021). Available phosphorus in charged biochar, helps it to contribute to root development through stimulation of early root growth and improve the uptake of water and nutrients. Available phosphorus plays a major role in energy transfer as ATP and other phosphorus compounds drive metabolic and physiological processes that are essential for tuber formation. Phosphorus helps crop to tolerate stress by enhancing resistance to diseases, pests and other environmental stresses. It also encourages rapid establishment and uniform tuber set that results in early maturity and improved yield (Irungu *et al*., 2022).

High cation exchange capacity of biochar also influences the holding and exchange of cations like potassium (K), magnesium (Mg) and calcium (Ca) that are needed for plant defense mechanism. The adequate levels of certain cations, like copper, manganese, and zinc, can improve plant defense mechanisms by increasing production of lignin and phenolic compounds, which are involved in resistance to pathogens. Continuous application of DAP lead to soil acidity because of the breakdown of ammonia into nitrites and nitrates by microorganisms in process of nitrification that release hydrogen ions into soil and this results in soil acidity over time of use which favor the growth of the pathogen (Wang *et al*., 2022). This could be the reason of high incidence of bacterial wilt recorded from DAP treated plot in Mau-Narok.

Chicken manure used to activate biochar, being a nutrient rich substance contribute to health of plant and makes the plant withstand harsh conditions including pathogen attack. Increase of beneficial microorganisms' population density by chicken manure contribute to suppression of pathogen through mechanisms of competition and predation (Akhtar *et al*., 2015). Ninh *et al*. (2015) stated that chicken manure can act as soil fumigant and also when used with soil solarization reduce the diseases incidence and severity caused by different pathogens, this might have impacted the results from the study. Studies conducted by Feng *et al*. (2021); Messiha *et al*. (2007); Tian *et al*. (2021) revealed that minerals content in the ash fraction found in biochar can increase soil fertility and availability of nutrient, that result in reinforcement of plant defenses against pathogens. Besides, biochar contain volatile organic compounds that have antimicrobial and antifungal properties, contributing to pathogen suppression once they are released in the soil (Adekiya *et al*., 2022). All findings from the above stated research have contributed to the findings of this research. Biochar being highly porous can absorb poisons produced by pathogens and reduce their effect on plants (Agbede & Oyewumi, 2022).

Charged biochar has capacity to improve soil structure, aeration, water retention and creation of environment which is more favorable for plant growth by making nutrients available to plant and increase uptake due to its high porosity and CEC (Shao *et al*., 2023). High nitrogen content in biochar contribute to the building blocks of protein as nitrogen is a key component of amino acids, Proteins are important for several plant functions, like enzymatic activities, structural support and defense mechanisms. Nitrogen is also an essential component in chlorophyll production, which is indispensable for plant to make its own food. Organic carbon is a major component of soil organic matter that is accountable for plant growth by providing several benefits like: soil structure improvement, increase of soil porosity that facilitate water infiltration and aeration that supports root development and uptake of nutrient as it acts as reservoir of nutrients.

Phosphorus plays a crucial role in energy transfer within plants in the form of ATP. It is involved in several metabolic processes, such as: cell division, cell enlargement and sugar production (Mickiewicz *et al.*, 2022). The relationship of these three elements highlights the importance of managing these nutrients in balance for optimal plant growth and soil health. Nitrogen and phosphorus can affect the soil's organic carbon quantity with nitrogen input increasing the input of carbon from litter and phosphorus hindering its decomposition. Biochar being rich in all these three elements allow to it to contribute to better growth of potato (Adekiya *et al*., 2022).

Biochar can hold and mobilize plant nutrients by acting as a soil conditioner (Azeem *et al*., 2022). Yang *et al*. (2022) from his research found out that application of biochar as soil amendment, increased the population density of Arbuscular mycorrhizae fungi (AMF) which form a symbiotic relation with most terrestrial plant including potatoes. The AMF allow plant to access a wider range of nutrient by extending the adsorptive surface of the roots and allow plant to absorb and uptake enough nutrients for its growth and development. DAP being a fast releasing nutrients, make nutrient readily available to plant and this results the improvement of the early growth (Aramburu *et al.*, 2023).

The high produce of potato obtained from this study is due to biochar quality and nature Biochar application increase yield by improving soil fertility through its physical and chemical properties. Chicken manure used to activate biochar has contributed to biochar effectiveness in controlling disease and improving growth and yield of potato. Chicken manure being a natural and nutrients rich fertilizer, contributed to improved growth and yield of potato by enhancing soil and plant health (Poveda *et al*., 2021). Chicken manure can also act as non-chemical soil fumigant that involved in reducing the incidence and severity of the pathogen.

4.7 Conclusion

Both the pot and field experiments results showed that charged biochar effectively reduced the incidence and severity of bacterial wilt as well as improved growth and yield of potatoes. Those results are attributed to the biochar's' capacity to enhance soil and plant health by modifying soil structure, physical, chemical properties and enhancement of beneficial micro-organisms that help plant to withstand biotic stresses including soil borne diseases by increasing competition in colonizing the sites which results in improved plant defense system.

CHAPTER FIVE

GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1 General Discussions

Charged biochar used in this study retained significant physical characteristics compared to plain biochar, especially its high porosity**,** which is contributes in enhancing water retention, nutrient adsorption, and microbial colonization in soil (Morgan *et al*., 2022). Charging biochar with chicken manure made it darker, more cohesive structure, due to the adherence of organic and mineral residues on the biochar surface. This coating improves both microbial habitat suitability and nutrient exchange sites (Meisinger *et al.*, 2018). Additionally, charging biochar with chicken manure significantly improved its chemical properties, particularly its nutrient profile because chicken manure is a rich source of nitrogen (N), phosphorus (P), potassium (K), and micronutrients such as calcium (Ca) and magnesium (Mg) (Zhang, *et al*., 2021b).

Charged biochar supplies nitrogen directly and reduce the initial immobilization and mineralization that can happen if biochar was applied plain in the soil where it may absorb ammonium and nitrogen in organic forms. Charged biochar retain ammonium reducing leaching and volatilization due to its surface functional groups, microporosiy and its interaction with microbes that alter microbes which are responsible for nitrogen cycling. Application of charged biochar improve nitrogen use efficiency by making available nitrogen more stable over cropping season (Riegel & Noe, 2007). Biochar when charged with phosphorous rich materials, provide a slow-release phosphorus reservoir and reduce its loss through runoff and fixation due to its porous structure. Phosphorus in most conditions (highly weathered soil, acidic conditions) can be fixed to iron (Fe) and aluminium (Al) oxides by adsorbing it on its surfaces and make it unavailable to plant. Charged biochar facilitates solubilization of the fixed phosphates and allow early crop establishment and vigor (Song *et al*., 2020).

Charged biochar has a high cation exchange capacity compared to plain biochar due to oxidation in the process of charging that helps it to gain surface functional groups like carboxyl and phenolic that increase CEC. By retaining cations like K^+, Ca^{2+}, Mg^{2+}, $NH4^+$, charged biochar contributes to organic matter and make CEC more effective (Mikajlo *et al*., 2024). Biochar prepared from woody and crop residues feed stocks, contain ash with carbonates, oxides and hydroxides of minerals like Ca, Mg, K, Na that make them alkaline by nature. Applying biochar make nutrients more available to plants and reduce aluminium and manganese toxicity due to its liming effect that enhance phosphorus availability by reducing its fixation by Al^{3+} and Fe^{3+}, promote beneficial microbial activities that involve in nutrients cycling and disease suppression. More negative charges that are activated on biochar surfaces and organic matter increase biochar's contribution to improved cation exchange capacity due to raise of soil pH (Anbuganesan *et al*., 2024). Charged biochar controls soil borne diseases by creating an alkaline environment which is less conducive to them as most of them especially bacterial wilt thrives well in acidic soil. Suppression of disease and reduction of toxicity, combined with efficiency nutrient supply and uptake lead to healthier plants, strong root systems and yield increments (Chen *et al.*, 2013; Khan *et al*., 2020). When nutrients are adsorbed onto the biochar matrix, they are less prone to leaching and can be released gradually to support plant growth. The slow-release behavior supports the long-term nutrient needs of potato crops while minimizing losses (Kwak *et al*., 2018).

The pH of the charged biochar was alkaline and slightly moderated compared to uncharged biochar. This was due to incorporation of organic acids and ammonium forms during manure charging likely contributed to this pH adjustment (Vanitha *et al*., 2019). Such a buffering effect is especially beneficial in soils with high acidity, by helping in neutralizing the acidity of the soil, improving nutrient availability and microbial function. A notable enhancement was observed in the cation exchange capacity (CEC), reflecting an increased ability to retain and exchange essential cations such as K^{+}, Ca^{2+}, and Mg^{2+}. This aligns with findings by Abd Alamer *et al*. (2020) who reported that manure-charged biochar significantly improved CEC due to both surface modification and added organic matter.

Intense use of chemicals for crop production and crop protection has raised issue of environment and human health concern. Application of biochar from different feed stocks is an alternative way of managing bacterial wilt and other soil borne diseases instead of using synthetic fertilizer for production improvement and some chemical compounds in disease management. Biochar extracts mixed with the media inhibited the growth of *Ralstonia solanacearum in-vitro* at 100% for eucalyptus biochar both plain and charged due to its known concentration of essential oil which contain compounds that have strong antibacterial properties which interrupt bacterial cell walls and membranes resulting in leakage and growth inhibition (Hou *et al*., 2022).

Phenolic compounds, alkaloids found in numerous biochar extracts have antibacterial and antifungal effect that contributed to the applied extracts to inhibit the growth of bacteria. The high nutrient contents especially organic carbon, nitrogen and phosphorus in biochar more so in those charged with chicken manure contributed to the improvement of potato performance in both pot and field experiments. Biochar contributed to reduction of nutrient leaching by improving soil structure. This was in line with the report study by Kilonzi *et al*. (2024) who reported that biochar has high nutrient content and water holding capacity that allows it to improve soil aeration and increase microbial activities thereby increasing plant growth. Biochar physical and chemical properties contributed to reduction of incidence of bacterial wilt in both pot and field experiments. Contribution of charged biochar to disease suppression is due to its ability to stimulate beneficial microbes that otherwise antagonize pathogens through completion for food and space, parasitism and antibiosis. Charged biochar disrupt disease establishment by adsorbing and inactivating toxins and signaling molecules produced by pathogens and improving plant vigor with better nutrients and water relations that increase plant resistance and tolerance to infections. Biochar can have direct suppressive effects on soil-borne pathogens when charged with well composted and integrated materials that release ammonia and volatile fatty acids compounds that are toxic to pathogens thus results in disease reduction (Bekchanova *et al*., 2024).

High pH in biochar make environment less conducive to pathogens resulting to the reduction of its growth and multiplication as it has been reported by Ahmad *et al*. (2023). High porosity of biochar enhances absorption of different substances including pathogens that make them less available in the soil. This limits pathogen growth as they will not access nutrients from the soil (Mansfield *et al*., 2012). High cation exchange capacity in biochar improves the movement of cations and anions between soil and crop that increase the availability of nutrients to plant and improve its growth and health that make it able to withstand the pathogen's attack. Biochar has the ability to change the rhizosphere exudates chemistry that repel the pathogen from colonizing the root zone of the crop preventing it from causing the disease.

Manasa *et al*. (2024) reported that the application of biochar improved the enzymatic activities such as: sucrose, urease, and dehydrogenase that indicate the metabolism of active microbes and reflect health and fertility of soil. This contributes to the improved growth, yield and health of plant that make it able to withstand pathogen attack. Under dry conditions, potato with its shallow roots benefit from improved moisture availability which is favored by biochar through improvement of soil aeration, root penetration, water and nutrients retention. Besides, it facilitates nutrient uptake and tuber development that is enhanced by biochar porous structure and high surface area. With its porous structure, biochar is able to hold water that enhance tuber filling at critical conditions as potato is resilient to short period of drought (Guo *et al*., 2025). Potato indirectly benefits from biochar for growth, biochar release growth promoting compounds and supports nutrients cycling through enhancement of soil microbes, increase their activities and improve the structure of soil by contributing stable carbon.

A study conducted by Hou *et al*, (2024) on the effect of combining biochar and organic fertilizer on rhizosphere microbial diversity concluded that combination of biochar with organic improved the multiplicity of microbes and microbial groups in potato rhizosphere soil thus elevate the potato yield. The rhizosphere bacterial community was dominatedby*Proteobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Chloroflexi*, and *Bacteroidetes* that are beneficial and help to improve growth and yield. Charged biochar provide a continuous supply of nutrients especially nitrogen, phosphorus and other nutrients essential for tuber initiation and bulking as it serves as a slow-releasing fertilizer and thus increase yield of potato (Fornes *et al.,* 2024). Chicken manure used to activate biochar is known positively to impact growth, yield and disease management in various crops including potatoes. Chicken manure has essential nutrients that boosts water and nutrient retention as well as improve soil structure and fertility that enhance growth and yield of potato. By improving soil microbial activity and soil conditions, chicken manure plays a critical part in defeating pathogen responsible for disease (Black *et al.*, 2021).

5.2 Conclusions

i. All tested biochar extracts from different feedstock inhibited the growth with plain and charged eucalyptus biochar exhibiting100% inhibition of *Ralstonia solanacearum in-vitro* when mixed with nutrient agar media before solidification.
ii. Selected charged biochar applied at 15 tons/ha reduced the incidence and severity of bacterial wilt by 28% -100% and 69.5-100% respectively under field conditions. In pot experiment, biochar reduced incidence by 66.7%-100% and severity by 95.6%-100% respectively when applied at 2% w/w.
iii. Chicken manure charged biochar improved growth and yield of potato by 44.6% and 54.9% respectively when applied at 15 tons /ha two weeks before planting.

5.3 Recommendations for Further Studies

From the findings of the study, the following are recommended for further studies:

i. To study the tested biochar extracts by using ethanol or methanol as solvents.
ii. To assess the presence and concentration of phenolic, organic acids, volatile organic compounds in the studied biochar extracts.
iii. To determine the minimum inhibitory concentration (MIC) of the studied biochar extracts.
iv. Assessment of biochar extracts as foliar spray in controlling bacterial wilt.
v. To study the effects of combining biochar as soil amendment and biochar extracts foliar spray in management of bacterial wilt.
vi. To study the effects of selected chicken-manure charged biochar on the soil microbiome

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APPENDICES

Appendix 1: Analysis of variance for in vitro

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	12	139341	11612	6038	<2e-16 ***
Residuals	26	50	2		

Appendix 2: Analysis of variance for percentage emergence Across locations

		Df	Sum Sq	Mean Sq	F value	Pr(>F)
	Replication	2	1.5833	0.79167	0.8926	0.4317
Egerton	Treatment	7	7.3333	1.04762	1.1812	0.3730
	Block	2	1.5833	0.79167	NaN	NaN
	Residuals	14	12.4167	0.88690		
	Replication	2	0.5833	0.2917	0.1907	0.82852
Mau-Narok	Treatment	7	27.3333	3.9048	2.5525	0.06429 .
	Block	2	0.5833	0.2917	NaN	NaN
	Residuals	12	21.4167	1.5298		

Appendix 3: Analysis of variance for plant height at different days after planting (Egerton)

DAP	Source	Df	Sum Square	Mean Square	F value	Pr(>F)
	Replication	2	6.349	3.1745	0.9235	0.4200
35	Treatment	7	25.215	3.6021	1.0479	0.442
	Block	2	1.5833	0.79167	NaN	NaN
	Residuals	12	48.122	3.4373		
	Replication	2	35.26	17.628	1.6161	0.233624
50	Treatment	7	537.52	76.789	7.0397	0.001027**
	Block	2	6.349	3.1745	NaN	NaN
	Residuals	12	152.71	10.908		
	Replication	2	158.17	79.084	4.6764	0.027830 *
65	Treatment	7	727.54 1	3.935	6.1459	0.001992 **
	block	2	158.17	79.084	NaN	NaN
	Residuals	12	4.122	3.4373		

Appendix 4: Analysis of variance for plant height at different days after planting (MAU-NAROK)

DAP	Source	Df	Sum Square	Mean Square	F value	Pr(>F)
	Replication	2	18.100	9.0501	4.6953	0.02752 *
35	Treatment	7	12.997	1.8568	0.9633	0.49277
	Block	2	18.100	9.0501	NaN	NaN
	Residuals	12	26.985	1.9275		
	Replication	2	10.458	5.2291	1.0011	0.39231
50	Treatments	7	114.094	16.2992	3.1205	0.03325 *
	Block	2	10.458	5.2291	NaN	NaN
	Residuals	12	73.126	5.2233		
	Replication	2	50.26	25.130	1.3246	0.29728
65	Treatment	7	389.97	55.710	2.9364	0.04096 *
	Block	2	50.26	25.130	NaN	NaN
	Residuals	12	265.61	18.972		

Appendix 5: Analysis of variance for number of stems Egerton

	Source	Df	Sum Square	Mean Square	F value	Pr(>F)
	Replication	2	0.7500	0.37500	0.6632	0.5307
Stems	Treatment	7	2.9583	0.42262	0.7474	0.6378
	Block	2	0.7500	0.37500	NaN	NaN
	Residuals	12	48.122	3.4373		

Appendix 6Analysis of variance for number of stems and branches (Mau-Narok)

	Source	Df	Sum Square	Mean Square	F value	Pr(>F)
Stems	Replication	2	6.2500	3.1250	8.6066	0.003652 **
	Treatment	7	4.2917	0.6131	1.6885	0.191328
	Block	2	6.2500	3.1250	NaN	NaN
	Residuals	12	5.0833	0.3631	0.1727	

Appendix 7: Analysis of variance for yield parameters (Number of tubers and tuber weight)

	Source	Df	Sum Square	Mean Square	F value	Pr(>F)
Tuber no	Replication	2	5.083	2.5417	2.6196	0.10804
	Treatment	7	32.667	4.6667	4.8098	0.00609 **
	Block	2	5.083	2.5417	NaN	NaN
	Residuals	12	13.583	0.9702		
	Replication	2	1.268	0.6338	0.8171	0.4617
Weight	Treatment	7	152.910	21.8442	28.1623	3.284e-07 ***
	Block	2	1.268	0.6338	NaN	NaN
	Residuals	12	10.859	0.7757		

Appendix 8: Analysis of variance for yield parameters (Number of tubers and tuber weight)

	Source	Df	Sum Square	Mean Square	F value	Pr(>F)
	Replication	2	19.750	9.8750	2.6126	0.1086
Tuber no	Treatment	7	23.958	3.4226	0.9055	0.5292
	Block	2	19.750	9.8750	NaN	NaN
	Residuals	14	52.917	3.7798		
	Replication	2	36.801	18.4004	5.6709	0.0157048 *
Weight	Treatment	7	187.393	26.7704	8.2505	0.0004567 ***
	Block	2	36.801	18.4004	NaN	NaN
	Residuals	12	45.426	3.2447		

Appendix 9: Analysis of variance for Disease severity (Field 7)

DAP	Source	Df	Sum Square	Mean Square	F value	Pr(>F)
42	Replication	2	5.741	2.8704	1.0000	0.392696
	Treatment	7	127.403	18.2004	6.3407	0.001715 **
	Residuals	14	40.186	2.8704		
49	Replication	2	10.15	5.075	1.486	0.2599
	Treatment	7	390.61	55.802	16.338	9.667e-06 ***
	Residuals	14	47.82	3.415		
56	Replication	2	30.45	15.226	2.192	0.1485
	Treatment	7	554.40	79.200	11.402	7.866e-05 ***
	Residuals	14	97.25	6.946		
63	Replicate	2	92.69	46.343	3.1225	0.0756248.
	Treatment	7	1000.33	142.905	9.6287	0.0002005 ***
	Residuals	14	207.78	14.841		
70	Replicate	2	192.76	96.378	3.4951	0.0587187.
	Treatment	7	1814.60	259.229	9.4008	0.0002283 ***
	Residuals	14	386.05	27.575		

Appendix 10: Analysis of variance for Disease severity (Mau-Narok)

DAP	Source	Df	Sum Square	Mean Square	F value	Pr(>F)
42	Replication	2	1.470	0.735	1.00	0.3927
	Treatment	7	5.145	0.735	1.00	0.4706
	Residuals	14	10.290	0.735		
49	Replication	2	10.116	5.0579	1.9620	0.17736
	Treatment	7	46.343	6.6204	2.5681	0.06309.
	Residuals	14	36.091	2.5779		
56	Replication	2	17.556	8.7781	1.6154	0.2338
	Treatment	7	110.551	15.7931	2.9063	0.0424 *
	Residuals	14	7.077	5.4341		
63	Replicate	2	96.26	48.132	2.5922	0.11022.
	Treatment	7	487.76	69.679	3.7527	0.01687*
	Residuals	14	247.137	17.653		
70	Replicate	2	288.03	144.016	2.6773	0.10361
	Treatment	7	959.08	137.011	2.5471	0.06471 .
	Residuals	14	753.09	53.792		

Appendix 11: Analysis of variance for Area under disease progress curve A (Field 7)

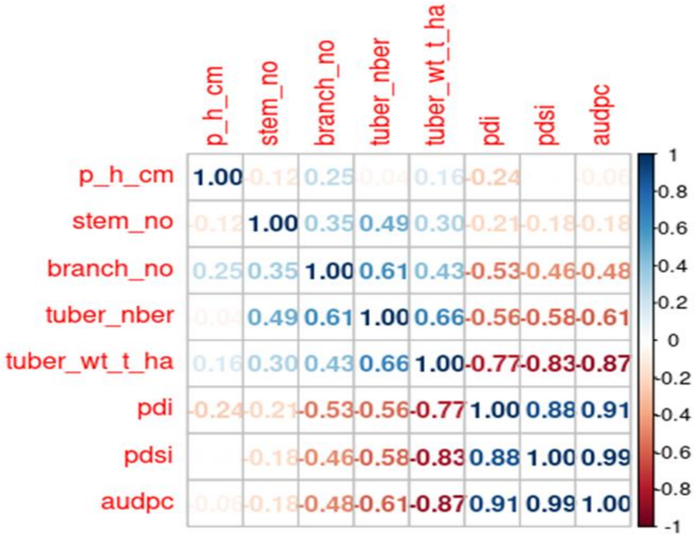
Source	Df	SumSq	MeanSq	Fvalue	Pr(>F)
Replication	2	7.9	8.96	3.2158	0.07092 .
Treatment	7	17841.9	2548.85	914.91	2e-16 ***
Block	2	17.9	8.96	NaN	NaN
Residuals	12	39.0	2.79		

Appendix 12: Analysis of variance for Area under disease progress curve B (MAU-NAROK)

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	6.54	3.27	1.9527	0.1787
Treatment	7	3104.04	443.43	264.66	8.885e-14 ***
Block	2	6.54	3.27	NaN	NaN
Residuals	12	23.46	1.68		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix 13: Correlation matrix for pot experiment



Appendix 14 Combined analysis of variance of disease incidence for Egerton and Mau-Narok

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	7	1395.12	199.303	20.3223	1.928e-08 ***
Block	2	186.80	93.398	9.5235	0.0009703 ***
Location	1	0.00	0.002	0.0002	0.9890872
Treatment: Block	14	337.29	24.092	2.4566	0.0270569 *
Residuals	23	225.56	9.807		

Appendix 15: Combined analysis variance of plant height for Egerton and Mau-Narok

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	7	539.5	77.1	5.723	0.000635 ***
Block	2	30.7	15.3	1.139	0.337408
Location	1	3022.0	3022.0	224.395	2.35e-13 ***
Treatment: Block	14	43.2	3.1	0.229	0.996815
Residuals	23	309.7	13.5		

Appendix 16: Combined analysis of variance on number of stem for Egerton and Mau-Narok

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	7	1.250	0.179	0.242	0.969936
Block	2	1.625	0.812	1.099	0.349990
Location	1	12.000	12.000	16.235	0.000523 ***
Treatment: Block	14	7.375	0.527	0.713	0.741101
Residuals	23	17.000	0.739		

Appendix 17: Combined analysis of variance for tuber number for Egerton and Mau-Narok

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	7	53.98	7.711	3.857	0.00645 **
Block	2	3.04	1.521	0.761	0.47871
Location	1	7.52	7.521	3.762	0.06479.
Treatment: Block	14	44.96	3.211	1.606	0.15179
Residuals	23	45.98	1.999		

Appendix 18: Combined analysis of variance for tuber weight (Egerton and Mau-Narok)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	7	329.2	47.03	16.496	1.37e-07 ***
Block	2	20.6	10.28	3.606	0.0434 *
Location	1	64.4	64.40	22.592	8.61e-05 ***
Treatment: Block	14	19.3	1.38	0.485	0.9185
Residuals	23	65.6	2.85		

Appendix 19: Combined analysis of variance for emergence (Egerton and Mau-Narok)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	7	21.00	3.000	82.091	0.086081.
Block	2	1.29	0.646	0.450	0.643045
Location	1	27.00	27.000	18.818	0.000242 ***
Treatment: Block	14	15.38	1.098	0.765	0.692941
Residuals	23	33.00	1.435		

Appendix 20: National commission for science-technology and innovation (NACOSTI) research license.

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Appendix 21: Abstract of published article from objective one

Effect of Selected Chicken Manure-Charged Biochar on Growth of *Ralstonia Solanacearum in Vitro*

Ernestine Niyonsaba*, Joseph Mafurah Juma, and Patrick Murerwa

ABSTRACT

A study was conducted *in vitro* to assess the antibacterial effect of specific chicken manure-charged biochar extracts against *Ralstonia solanacearum* at the microbiology laboratory of Egerton University. The study tested charged biochar extracts from various feed stocks: maize cobs (MC), maize straw (MS), rice husks (RH), bean wastes (BW), and eucalyptus branches (EU). The extracts consisted of plain biochar and charged biochar with poultry manure. The physico-chemical properties of biochar were assessed according to established standard procedures. The antibacterial efficacy of charged biochar extract was evaluated by mixing nutrient agar (NA) in petri dishes with 0.5 ml of the extracts prior to solidification, followed by the spread of 0.1 ml of bacterial suspension on each plate. Sterile distilled water served as a negative control, while copper oxychloride was utilized as a positive control. The plates were incubated at 28°C for 48 hours in growth chamber. The experiment was arranged in a completely randomized design (CRD) with three replicates. Charged biochar exhibited higher nitrogen content than that of plain, with bean waste charged biochar having the highest at 1.5% and plain eucalyptus biochar lowest at 0.4%. For available phosphorus, maize cob charged biochar exhibited the highest with 1846 mg/kg while the lowest was plain rice husk biochar at 671.5 mg/kg. Organic carbon at 74.2% for maize cob charged biochar was the highest and EUPB exhibited the lowest 23.5%. Porosity was high in plain biochar as compared to charged, while charged biochar exhibited higher density than plain with the lowest 0.6 for MCPB and highest 1.0 for rice husk plain biochar. The antibacterial assay indicated that all biochar extracts significantly suppressed the growth of *R. solanacearum* compared to the negative control. Charged eucalyptus biochar reduced pathogen growth by 100% which was the highest inhibition while plain maize straw was lowest at 1.19%. These results proved that charging biochar, especially that derived from bean wastes and eucalyptus, with chicken manure not only increases the nutrient content but as well enhances its potential of controlling bacterial wilt.

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1. Introduction

is placed second among plant pathogenic bacteria in

Appendix 22: Photos of different activities of research work