PHYTOCHEMICAL COMPOSITION, PREPARATION AND CHARACTERIZATION OF BIOCHAR COMPOSITE FROM *Solanum incanum* FRUITS FOR CONTROL OF BACTERIAL WILT IN TOMATO PLANTS

LUCY NYAMBURA KARANJA

A Research Thesis Submitted In Partial Fulfilment of the Requirements of the Award of Masters of Science in Analytical Chemistry of Moi University

2021

DECLARATION

Student declaration

This thesis is my original work and has not been submitted for a degree in any other university. No part of this thesis may be produced without the prior written permission of the author and /or Moi University.

Lucy Nyambura Karanja Signature ……  ….. **DATE:** ……..9/10/2021
Reg No. MS/ACH/4311/20

Declaration by Supervisors

This thesis has been submitted for examination with our approval as University Supervisors.

Dr. Isaac K'Owino Signature  Date 13/10/2021

Masinde Muliro University, Kakamega, Kenya

Dr. Wangila Phanice Signature  Date 

University of Kabianga, Kericho, Kenya

Dr. Rose Ramkat Signature  Date 17/10/2021

Moi University, Eldoret, Kenya

DEDICATION

This work is dedicated to God for gift of good health, stable mind and guidance in my day-to-day activities, my beloved family for their moral and financial support, classmates and friends for their considerable contributions to attain this work.

ABSTRACT

Ralstonia solanacearum causes *b*acterial wilt in tomato plants thereby leading to severe losses of the crop. This has led to the overuse of chemical pesticides often resulting to environmental contamination and human health risks as well as development of resistant strains of bacteria to several chemical pesticides. Therefore, there is need for novel methods to control bacterial wilt. This study focuses on the use of biochar composites and *Solanum incanum* fruit extracts to control bacterial wilt in tomato plants. The specific objectives were to: determine the phytochemical composition of the extract; isolate and test the biochemical characteristics of *R. solanacearum*; characterize biochar prepared from *S. incanum* fruits biomass; and compare the effectiveness of the plant extract, biochar composite, and ampicillin in control of *R. solanacearum*. Maceration method using ethanol as the solvent was used in extraction. Phytochemical screening was done following standard methods. The extract was analyzed using Gas Chromatography-Mass Spectroscopy (GC-MS) and Fourier Transformed Infrared (FT-IR). *Ralstonia solanacearum* was isolated by plating the bacterial tuber exudate on Triphenyl Tetrazolium Chloride (TZC). The residual biomass was transformed into biochar at pyrolysis furnace temperatures of 300 ℃ and 500 ℃ and characterized using FT-IR and scanning electron microscope (SEM). *In vitro and in vivo* studies were done to determine the antibacterial activity of the plant extract, biochar composite, and ampicillin. Phytochemical screening showed the presence of alkaloids, flavonoids, phenols, saponins, tannins and terpenoids. FT-IR spectra showed peaks at 3415 cm^{-1}, 2931 cm^{-1}, 1596 cm^{-1}, and 1398 cm^{-1} for O-H, C-H, C=C, and C-O-H groups respectively. GC-MS results of the extract showed 15 compounds, with (9E)-1-Methoxy-9-octadecene (26.85%) as the major compound and others like 2-4-Di-tert-butylphenol, n-Tetracosanol-1, 1-Tridecene, E-15-Heptadecenal. The SEM images showed differences in morphological properties with the biochar prepared at 500 ℃ being more porous than that for 300 ℃. The *in vitro* antibacterial activity of the plant extract showed an inhibition zone of 30.75 ± 0.5 mm at a concentration of 0.15g/10 mL compared to ampicillin with a zone of inhibition of 35.00 ± 0.0 mm at the aforementioned concentration ($P< 0.05$). In the *in vivo* studies, distilled water treatment had a disease incidence of 100% 10 days post-inoculation (dpi). Plant extract and ampicillin treatment had a disease incidence of 100% and 60.0% respectively, 16 dpi. Activated biochars for 300 ℃ and 500 ℃ had a disease incidence of 80.0% and 66.7% respectively, 30 dpi. Biochar composite for 300 ℃ had a disease incidence of 73.3%, 64.5%, 60.0%, 22.2%, 13.3%, and 0% at a concentration of 1%, 2%, 3%, 4%, 5%, and 7% respectively, 30 dpi. Biochar composite for 500 ℃ showed disease incidences of 60.0%, 46.7%, and 22.2% at 1%, 2%, and 3% concentrations respectively, 30 dpi. No symptoms were observed at a concentration of 4%, 5%, and 7%. In conclusion, biochar composite is more effective in managing bacterial wilt than activated biochar, ampicillin, and plant extract. Biochar composite for 500 ℃ is recommended as a substitute for synthetic pesticides.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

DEFINITION OF TERMS AND ABBREVIATIONS

Actd B 300 ℃ - Activated biochar prepared at 300 ℃

Actd B 500 ℃ - Activated biochar prepared at 500 ℃

B 300 ℃ - Non- activated biochar prepared at 300 ℃

B 500 ℃ - Non- activated biochar prepared at 500 ℃

BC 300 ℃ - Biochar composite for biochar prepared at 300 ℃

BC 500 ℃ - Biochar composite for biochar prepared at 500 ℃

BCA - Biological Control Agent

CFU – Colony Forming Unit

DAP – Diammonium Phospahte

DNA – Deoxyribonucleic acid

DPI – Days Post Inoculation

FCR - Folin- Ciocalteu's reagent

FT-IR – Fourier Transform Infrared

GAE - Gallic Acid Equivalent

GC-MS – Gas Chromatography-Mass Spectrum

HPLC – High Performance Liquid Chromatography

MIC - Minimum Inhibitory Concentration

MSE – Minimum Square Error

NPR1 - Nonexpressor of Pathogenesis Related Genes1

PAA - Peroacetic Acid

QE - Quercetin Equivalent

RMSE – Root Mean Square Deviation

RPM – Revolutions per Minute

RT – Retention Time

SEM – Scanning Electron Microscope

TEM-Transmission Electron Microscope

TFC – Total Flavonoids Content

TOC - Total Organic Carbon

TPC - Total Phenolic Content

TZC - Triphenyl Tetrazolium

UV-Vis - Ultra-Violent-Visible

XRD - X-ray Diffraction

ACKNOWLEDGEMENT

I thank God for His protection and good health. I also want to express my gratitude to Africa Center of Excellence in Phytochemicals, Textile and Renewable Energy (ACE II PTRE) for the award of scholarship to pursue this Master's program in Analytical Chemistry. Most sincere gratitude goes to my supervisors Dr. Isaac K'Owino, Dr. Phanice Wangila and Dr. Rose Ramkat for their invaluable advice, support and corrections during the entire period of this research.

I also thank the entire staff of Moi University, Department of Chemistry and Biochemistry for their guidance and encouragement. Special thanks to my beloved family for their financial and moral support. Finally, I would like to thank my classmate specifically Decrah Moraa Nyangena for her company during the entire period.

CHAPTER ONE: INTRODUCTION

1.1 Background Information

Tomato (*Solanum lycopersicum*) is among the cultivated species of the genus Solanum in the family Solanaceae (Gutierrez, 2018). It is a rich source of vitamin C, pro-vitamin A, folate, β carotene, minerals such as iron, potassium, and phytochemicals such as lycopene, phytosterols, flavonoids, and polyphenols (Chaudhary et al., 2018). Processed tomato such as soup, paste, concentrate, juice, and ketchup contribute positively to human health by the content of the afromentioned compounds (Bergougnoux, 2014). Tomatoes possess remarkable antioxidant, antithrombotic and anti-inflammatory activities as they are rich sources of lycopene (Gull et al., 2020). Economically, tomatoes are a source of income to small holder and medium-scale commercial farmers (Moranga, 2016). Even then, their production is impaired by *Ralstonia solanacearum* that causes bacterial wilt.

Bacterial wilt is a severe soil borne disease of several economically important crops such as potato, tobacco, tomato, peanut, eggplant, banana and some ornamental plants such as rose plants (Chen et al., 2020). *Ralstonia solanacearum* is extensively distributed in tropical, subtropical, and some warm temperate regions of the world including Kenya (Kago et al., 2019; Khasabulli et al., 2017). The bacteria reaches the plant through wounds or emergence points of lateral roots and inhabits the vascular system which eventually leads to whole plant wilting and death (Kago et al., 2019). Bacterial wilt disease is the most limiting factor of tomato production in Sub-Saharan Africa (Aloyce et al., 2017). The disease is documented to contribute to 10-100% tomato yield loss (Din et al., 2016). In Kenya about 64% of

tomatoes grown in open field and 100% of tomatoes grown under greenhouse conditions are lost (Kambura, 2020). This severe loss of tomato production depend on type of cultivar, soil, climatic conditions, prevailing strain, and cropping system (Yuliar et al., 2015). The disease has remained a challenge despite advances in control methods. Among the control methods are cultural, physical, chemical, biological, integrated methods among others (Jiang et al., 2017; Yuliar et al., 2015; Lee et al., 2012; Hong et al., 2018).

Cultural methods such as crop rotation has proven inefficient since crop rotation with a non-host crop is of negligible value due to varied range of crop and weed hosts of the pathogen (Panth et al., 2020). Chemical methods such as the use of chloropicrin (Zhang et al., 2017), peroxyacetic acid (Hong et al., 2018), methyl bromide (Panth et al., 2020), and myriad copper based chemicals (Chen et al., 2019) have shown negative effects to the plant and also to the environment. The bacteria has also shown adaptation to the used bactericides. For instance, *Ralstonia solanacearum* has quick adaptation to copper stress which is among the used bactericides (Sing'ombe, 2019). The work by Ascarrunz et al., (2011) showed that cells of *R. solanacearum* exposed to 20 μM $CuSO_4$ exhibited notable recovery in culturability after incubation in natural and pasteurized soils for 36 h. Peroxyacetic acid has phytotoxic effect as it result in reduction of vegetative parts in the plant (Hong et al., 2018). Antibiotics such as tetracycline, streptomycin, penicillin, and ampicillin have been identified as having minute efficiency in curbing the growth of *R. solanacearum* (Namisy et al., 2019).

Therefore, there is need for a more effective and eco-friendly bactericides. Biopesticides are known to be effective in various cases and are equally eco-friendly. Hence, the current study

was undertaken to assess the antibacterial properties of the plant extract and biochar composite from *Solanum incanum* fruits against *R. solanacearum*.

1.2 Statement of the Problem

Globally, tomato is among the most utilized vegetables in kitchens with 13,000 million metric tonnes consumed in 2020/2021, due to its potential in producing juice, paste, ketchup amongst others (Osoyo, 2021). Moreover, it has high contents of vitamins A and C and is extensively used in numerous meals. Commercially, tomato in Kenya is of great importance from processing to fresh market and from beefsteak to grape tomatoes (Diaz et al., 2017). Even then, its production is impaired by *Ralstonia solanacearum* that causes bacterial wilt (Din et al., 2016). The disease contribute to 64% tomato yield loss in Kenya (Kambura, 2020) causing the country to import most of the crop from neighboring Uganda (Moranga, 2016). Low production of tomatoes due to bacterial wilt is of great concern to horticultural industries as well as to consumers. The lack of an effective solution for such challenges often contributes to reduced earnings for farmers thus leading to food insecurity and livelihood challenges (RSA, 2015).

Numerous attempts have been made to solve this challenge. Among the control methods are cultural, physical, chemical, biological, integrated methods among others (Jiang et al., 2017; Yuliar et al., 2015; Lee et al., 2012). These methods have been associated with several drawbacks. For instance, chemicals such as methyl bromide used in control of this bacterial parasite has been found to cause stratospheric ozone depletion linked with diseases such as skin cancer and eye cataracts (Sande et al., 2011). Methyl bromide is also volatile and thus

increased capability to move far distance from application area. This contributes to neurotoxicity to applicators and animals near and in field where they have been applied (Park et al., 2020). Use of resistance cultivar is another method which have gain consideration as the most effective, cost-effective and eco-friendly method of bacterial wilt disease control(Mamphogoro et al., 2020). Even then, the pathogen landrace and strain diversity has made breeding for resistant cultivars fruitless in the control of bacterial wilt (Diaz et al., 2017).

Cultural methods involve crop rotation offers the benefits of maintaining soil structure, organic matter, reduces the concentration of pests and diseases (Shah et al., 2021). Nonetheless, crop rotation with a non-host crop is of negligible value due to extensive range of crop and weed hosts of the pathogen (Baker, 2019). Biological control agents (BCAs) such as *Pichia guiller-mondii, Enterobacter cloacae, Candida ethanolica*, and *Bacillus megaterium* have also been used (Nguyen and Ranamukhaarachchi, 2019). Even though these spread on their own after primary establishment and are potentially self-sustaining in long term, they are unable to suppress soil borne diseases like bacterial wilt (Yuliar et al., 2015). In addition, BCAs have been reported to have adverse effect on non-target for example, parasitization on non-target insects (de Clercq et al., 2011).

Solarization and hot water treatments are examples of physical methods that control bacteria wilt by increasing soil temperature to about 10-15 ℃ thus affecting the survival of *R. solanacearum* in the soil (Kanaan et al., 2015). Nevertheless, the bacteria *R. solanacearum* is reported to survive at higher temperatures of up to 35 ℃ (Singh, & Kumar, 2017a). There is need therefore for an ecofriendly and effective method of bacterial wilt control.

1.3 Objectives

1.3.1 General Objective

To control bacterial wilt disease of tomato using extracts and biochar composite prepared from Solanum incanum fruits.

1.3.2 Specific Objectives

The specific objectives of the study are to

1. To isolate and profile the phytochemical composition of extracts from *Solanum incanum* fruits
2. To prepare and characterize biochar and biochar composites from *Solanum incanum* fruits
3. To assess the efficacy of biochar, biochar composite, and *Solanum incanum* extracts in the management of tomato bacterial disease.
4. To assess the application dosage and types of biochar, biochar composite, and *Solanum incanum* extracts in the management of tomato bacterial wilt.

1.4 Research Hypothesis

1. *Solanum incanum* fruits contain bioactive compounds that can be used to control bacterial wilt disease of tomato
2. Materials from *Solanum incanum* can be used to prepare biochar and biochar composite for the management of bacterial wilt disease of tomato

3. The efficacy of fruit extracts, biochar and biochar composite for the management of bacterial wilt disease of tomato is dose or concentration dependent

1.5 Justification of the Study

Infection of tomato plants by bacteria *Ralstonia solanacearum* has contributed to increased low production of tomatoes (542,000 tonnes) despite the increased demand (581, 000 tonnes) in Kenya (Ochilo et al., 2019). In attempt to meet the demand of tomato, farmers result to use of synthetic chemicals such as Methyl bromide. Use of synthetic chemicals has contributed to rigorous ecological consequences such as adverse effects on non-target organisms, obliteration of natural enemy fauna, increased bactericides residues in harvested yield as well as development of drug-resistant pathogens (Musyoka, 2014).

These unsustainable practices of disease control provides a good opportunity to identify safer, sustainable and eco-friendly methods of pest and disease management in tomato plants to meet the demand of tomatoes in the ever-growing population in Kenya and in the entire world. Use of plant extract biochar composites is one of eco-friendly and sustainable approach. Plant extract biochar composites have been previously used in inhibition of nitrification process in the soil (Reyes-Escobar et al., 2015). This activity is attributed to presence of phytochemicals such as tannins in the extract that act as source of labile Carbon that increases nitrogen immobilization and reduce nitrogen mineralization (Muñoz et al., 2014). This approach has not been used in control of bacterial wilt in tomato plants or any other plants. There is need therefore to evaluate its efficacy. This study aimed at evaluating the effectiveness of plant extract biochar composite prepared from *Solanum incanum* fruits

as a bacterial wilt diseases control option that could be useful in eliminating use of synthetic bactericides among small, medium and large scale tomato farmers.

1.6 Significance of the Study

The use of biochar composite significantly suppressed bacterial wilt diseases in tomato plants. This can contribute to increased raw material in horticulture sector, tomato processing industries and in fresh market. Horticulture sector being among the leading foreign exchange earner contributes enormously to food security and household income to smaller producers in the country. Increase of tomatoes in fresh market and tomato processing industry also contribute to economic growth.

Through use of natural remedies, African agriculture can be transformed to enable the continent to feed itself which is among the way of achieving the African Union Agenda 2063 Aspiration 1: A Prosperous Africa based on inclusive Growth and Sustainable Development. Kenya vision 2030, Sustainable Development Goal: achieve food and improved nutrition, end hunger and promote sustainable agriculture can be achieved. Kenya will also reduce importation of tomatoes from Uganda since farmers will be in a position to produce enough for the country.

CHAPTER TWO: LITERATURE SURVEY

2.1 Introduction

Vegetable refers to all comestible parts of certain herbaceous plants, including the leaves, stem, fruits, flowers, seeds and roots (Welbaum, 2017). These plants parts are either eaten raw or prepared in number of ways. Vegetable serves as vital source of vitamins mainly A and C, antioxidant, dietary fiber and minerals especially iron and calcium (Asaduzzaman & Asao, 2018). Vegetables are generally categorized according to plant part that is utilized as food (Dhaliwal, 2017). They comprise of root vegetable which includes carrots, radishes, beets, turnips, and sweet potatoes (Dhaliwal, 2017). The leaf and leaf stalk vegetables include spinach, celery, rhubarb, cabbage, lettuce, and Brussels sprouts (Cooper, 2018). Seed vegetables are usually legumes for example green peas and green beans (Blair et al., 2016). Among the bulb vegetable are onions, garlic, and leeks (Atif et al., 2020). Stem vegetable include kohlrabi, rhubarb, celery, and asparagus (Dhaliwal, 2017). Flower vegetable include broccoli, cauliflower, and artichokes. Fruits considered as vegetable by the virtue of their use include eggplant, sweet corn, cucumbers, okra, peppers, squash and tomatoes (FAO, 2020).

2.2 Tomato Farming and Production

Globally, tomato (*Solanum lycopersicum*) is among the most important vegetable plant (Gutierrez, 2018) as it is nutritionally well balanced since it contains large amount of vitamin A and C (Gull et al., 2020) and thus plays a vital role towards ensuring nutrition and food security (Tabe-Ojong et al., 2020). Worldwide, tomato ranks third in term of production

after potato and sweet potato and first in terms of processing (Oromia, 2019). In Kenya, tomato is ranked the second most vital vegetable after potato constituting between 18% to 20% of vegetable value and area under which it's produced (Ateka et al., 2021; Otipa & Gitonga, 2016). Tomato thrives well in tropical, subtropical, and temperate climatic conditions (Bergougnoux, 2014) and is cultivated in either open field or greenhouse conditions. In Kenya, tomatoes are grown in areas covering medium to high potential agro-ecological zones with Kirinyaga (14%), Kajiado (9.1%), Taita Taveta (7%), Meru (5.6%) Bungoma (5.5%), Kiambu (5.2%), Migori (4.6%), and Makueni (4.4%), counties being the top counties producing tomatoes (Geoffrey et al., 2014). Tomato farming is mostly done by small scale and medium scale farmers (Mwangi et al., 2015). It's grown in rain fed and in some areas under irrigation (Nabiswa, 2020). The most common grown varieties in Kenya include Kentom F1, Neeema 1400, Caltana, Zawadi F1 hybrid, Neema 1200, Fortune maker F1, Riograde, Onyx, Manset, Money maker, Nyota F1, Anna F1, Faulu Tomato, and Cal J but still there are new varieties that have been introduced to farmers (Shep plus, 2019). In terms of production, Kenya ranks among the leaders in Sub-Saharan Africa producing approximately 542, 000 tonnes of tomatoes per year which constitute 7% of its total horticultural production (Ochilo et al., 2019). However, the yield has remained low due to countless hindrances.

2.3 World Related Tomato Challenges

Over the years, tomato production has remained low due to many impediments, key among them being biotic factors comprising of fungal, arthropod pests, viral and bacterial disease

(Malherbe, 2016). Abiotic factors like unsteady rainfall, poor soils and higher temperature (Singh et al., 2017) also affect tomato production.

2.3.1 Abiotic Factors

2.3.1.1 Temperature

Tomato plants are sensitive to temperature and thrive well in optimum temperature condition ranging between 15-30 ℃ (Shamshiri et al., 2018). Higher temperature is associated with global change (Gonzalo et al., 2020) and largely affect quantity and quality of horticultural crops. Specifically, in tomato, higher temperature causes flower abortion and limit fruit set which contribute to reduced yield (Hernández et al., 2015). It has also been associated with decrease in pollen viability and protrusion of the style above the anther cone which negatively affect flower pollination (Xu et al., 2017). Extreme temperature modify tomato quality by changing the physical properties such as color and size of the fruit and also sensorial and nutritional quality (Kabir et al., 2020). It also increases incidences of tip burn and necrosis of the apical vegetative and reproductive tissues which has been correlated with insufficient water absorption and nutritional imbalance (Chung et al., 2010). Similarly, low temperature affects reproductive and vegetative growth of tomato. Tomato grown in low temperature exhibit reduction in growth rate, photosynthesis and delay in truss appearance, leaf formation and fruit growth (Sherzod et al., 2019).

2.3.1.2 Poor Soils

Tomato flourishes well in moist mineral soils, but they prefer deep, well-drained sandy loam (FAO 2020). In heavy clay type soils deep tillage can allow good root penetration (Correa et al., 2019). Tomatoes are moderately tolerant to acidic pH of 5.5 (Ahmed et al., 2017). Organic matter is necessary during tomato growth but excess organic matter is not recommended due to high moisture content. High moisture content especially during flowering causes flower loss without fruit set (Ozores-hampton et al., 2012). Over the years, use of synthetic fertilizers has increased and contributed to increased level of acidity in soil (Lin et al., 2019). With increased acidity, plants are not able to absorb nutrients for example phosphorous which is vital during plant growth and development (Cerozi & Fitzsimmons, 2016).

2.3.1.3 Erratic Rainfall

Erratic rainfall is among environmental factors contributing to reduced yield of tomato (Raza et al., 2019). Globally, protracted drought and flooding has been experienced following climatic changes attributed to global warming (Ngongeh et al., 2014). Prolonged drought and rainfall have adverse impact on agricultural crops as they provide favorable conditions for growth of pest and disease which attack plants leading to reduced amount of produce received (Garrett et al., 2013). Drought reduces soil moisture content and promote evapotranspiration which ultimately lead to stunted growth and thus reduced yield (Guodaar & Studies, 2015). On the other hand, heavy prolonged rainfall may saturate the soil and drown the roots if the soil cannot drain out easily (Mati, 2014).

2.3.2 Biotic Factors

2.3.2.1 Arthropod Pests

A wide range of insect pest hinders tomato production globally. Among them being mealy bugs (*Phanococcus* spp), African bollworm (*Helicoverpra armigera*), thrips (*Frankliniella* spp), aphids (*Aphis gossypii, Myzus persicae*), whiteflies (*Bemisia tabaci*), spider mites (*Tetranychus* spp), cutworm (*Agrottis* spp) among others (Ochilo et al., 2019). Abundance and diversity of arthropod pest is affected by factors such as cropping seasons, pest control practices, production system, agroecology and host plant species (Musyoka, 2014). Pest damage causes reduction in quality and quantity of yield thus contributing to food insecurity (Cerda et al., 2017).

2.3.2.2 Fungal Diseases

Fungal disease in tomato plant is caused by fungi. Example of fungal diseases affecting tomato production are; late bright (*Phytophythora infestans*), early blight (*Alternaria tomatophila* and *A.* solani), fusarium wilt (*Fusarium oxysporum*), septoria leaf spot (*Septoria lycopersici*), leaf mold (*Passalora fulva*), buckeye rot (*Phytopythora parasitica*), and anthracnose (*Colletotrichum coccoides*) (Sanoubar & Barbanti, 2017; Babadoost, 2011). Fungal disease contribute to vegetable yield decrease resulting to intense economic losses (Pavan Kumar et al., 2018).

2.3.2.3 Viral Diseases

Tomato is sensitive to viral diseases. Virus usually reach in the plantation through arthropod vectors such as aphids, thrips and whiteflies (Rotenberg et al., 2015; Dietzgen et al., 2016). Viruses cause damage that is usually much greater than that caused by insect vectors (Ong et al., 2020). Symptoms associated with viral disease are light coloured leaves, dwarfed growth, strange stem and leaf deformation and rosette formation (Damicone & Brandenberger, 2015). Examples of viruses reported on tomato plants are *Tomato mosaic virus, Tomato spotted wilt virus, Tomato yellow leaf curl virus, Tomato torrado virus, Pepsino mosaic virus* among others (Hanssen et al., 2010; Persley, 2012).

2.3.2.4 Bacterial Disease

Bacterial diseases are caused by bacteria. Bacteria infects the plant through scars, wounds, stomata and lenticels, weak spots or other mechanical injuries and in the soil they get into plant through root lesion caused by nematodes (Xue et al., 2020). They colonize the vascular system of roots, stems and leaves which eventually causes wilting of the plant (Caldwell et al., 2017). Some bacterial disease common in tomato plants are; bacterial spot caused by *Xanthomonas axonopodis pv. Vesicatoria,* bacterial canker caused by *Clavibacter michiganensis* and bacterial wilt (N. T. Vu & Oh, 2020; An et al., 2019). The current study sought to research on bacterial wilt caused by *Ralstonia solanacearum*.

2.4 Bacterial Wilt Disease

2.4.1 Causative Agents of the Bacterial Wilt Disease

Bacterial wilt disease is a weighty impediment in production of vegetables in tropic and sub-tropic regions (Kumar et al., 2018). *Ralstonia syzygii sub species indonesiensis* and *R. solanacearum* are the causative agents of the bacterial wilt disease (Álvarez & Biosca, 2017). Over the last years, *R. solanacearum* has been considered as the most complex species due to its persistence, broad geographical distribution, lethality, and wide host range (Meng, 2013). *Ralstonia solanacearum* is a gram negative bacteria, motile with single or more flagella or non-motile soil borne pathogen (Jibat et al., 2018). It is rod-shaped measuring 0.5-1.5µm in length. The bacteria has been categorized into four phylotypes (phylotype I, phylotype II, phylotype III, phylotype IV) (Sing'ombe, 2019; Namisy et al., 2019), six biovars (biovar 1, biovar 2 biovar 3, biovar 4, biovar 5, biovar 2T) (Meng, 2013; She et al., 2017; Garćia et al., 2019), and five races (race 1, race 2, race 3, race 4, race 5) (Sakthivel et al., 2016; Meng et al., 2015). These classification is based on host range, molecular characteristics and biochemical properties (Álvarez & Biosca, 2017).

Bacteria *R. solanacearum* have been reported to affect over 450 plant species belonging to more than 50 botanical families among them being Solanaceous crops such as tomato, potatoes , eggplants, tobacco, and pepper (Nguyen and Ranamukhaarachchi, 2019). The bacteria get into the plant through wounds and lateral root emergence points and increase speedily in the vascular system, which result to xylem vessels pervaded by bacterial cells which block xylematic flow, leading to yellowing of the leaves, general wilting and finally

death of the plant (Namisy et al., 2019). The bacteria survive in plant debris and can propagate from one field to the other through irrigation or flood water, workers, farm implements or weed which are along waterways (Chen et al., 2020). The impact of bacterial wilt is specifically injurious on tomato and potato since they are basic crops. It's documented that bacterial wilt contribute to 90% tomato yield loss and 30% potato yield loss (Din et al., 2016; Álvarez & Biosca, 2017). Several strategies have been adapted to curb this bacterial wilt disease.

2.5 Control Measures Against *Ralstonia solanacearum*

Numerous efforts have been made to resolve this challenge of bacterial wilt in tomato plants. Some of the measures that have found application include; cultural, physical, chemical, biological, use of plant extract, biochar, integrated methods among other.

2.5.1 Resistant Cultivars

Cultivars that are resistant to bacterial wilt have been in use. They are considered most effective, cost-effective and eco-friendly method of bacterial wilt disease control (Mamphogoro et al., 2020). For example, in a study by Lin et al., (2014) introduction of Arabidopsis NPR1 gene into tomato cultivar improved resistance to bacterial wilt and lowered the incidence of wilt by 70% 28 days after the inoculation. However, the yield quantity and quality decreased. The agronomic traits of the resistant cultivars are also not broadly acknowledged by consumers or farmers (Yuliar et al., 2015). Also landrace and strain diversity of the pathogen has made breeding for resistant cultivars ineffective in the management of bacterial wilt (Diaz et al., 2017).

2.5.2 Cultural Methods

2.5.2.1 Multi-cropping and Crop Rotation

Crop rotation is of benefit to the soil as it help in maintenance of the soil structure, its organic matter, reduces the concentration of pest and disease and also aid in reduction in soil erosion that is frequently related to unceasing row crops (Bowles et al., 2020). Crop rotation has been related with decrease in plant diseases caused by soil-borne pathogens (Jiang et al., 2017). For instance, in a trial in Ethiopia, season crop rotation involving common bean and maize after tomato yielded a decrease in the final wilt with an average of 6% and 16% respectively (Ayana & Fininsa, 2017). In second trial, growing tomato after bean- maize and maize-bean resulted in reduction of average wilt incidence by about 29%. Nonetheless, crop rotation with a non-host crop is of negligible value due to extensive range of crop and weed hosts of the pathogen (Of et al., 2012). Bacterium *R. solanacearum* can also survive in plant debris in absence of a host (Álvarez et al., 2010).

2.5.3 Physical Method and Bio-fumigation

Solarization and hot water treatments are examples of physical methods used to control *R. solanacearum* (Yuliar et al., 2015). Solarization involve use of plastic sheets on humid soil during periods of high ambient temperature. Plastic sheets permit the solar radiant energy to be trapped in the soil resulting to heating of the upper levels (Zeist et al., 2019). The soil temperature rise to about 10-15 ℃ this affects the survival of soil pathogen to a depth of about 20cm (Kanaan et al., 2015). Nonetheless, these physical methods have been found ineffective. For example solarization is ineffectual where there is low sun radiation or a

dense infestation of weeds (Dai et al., 2020). The bacterium *R. solanacearum* has also been reported to survive at higher temperatures of up to 35 ℃ (Singh et al., 2014).

Using biofumigants is also a method that is used in control of soil pathogen. Biofumigants increases soil fertility as well as prevents soil pathogen. In a study done by Coca et al., (2012), 5% chicken manure was used for biofumigation in a tropical climate region in Brazil and resulted to reduced incidence of *R. solanacearum* in potatoes and tomatoes. However, recent studies have indicated that the effectiveness of biofumigants varies and in some cases they enhance disease severity due to increased pathogen inoculum potential especially when the substrate serves to maintain saprophytic growth of plant pathogens (Gilardi et al., 2013).

2.5.4 Chemical Method

Pesticides such as fumigants (methyl bromide, metham sodium, chloropicrin and 1, 3-dichloropropene), algicide (3-[3-indolyl] butanoic acid), and plant activators producing systemic resistance on the plant (validoxylamine and validamycin A) (Lee et al., 2012; Abo-Elyousr et al., 2014; Diaz et al., 2017) have been utilized to deter bacterial wilt. Some of the compounds are used singly or combined with another chemical. In a study by Yuliar et al., (2015) a combination of 1, 3-dichloropropene, methyl bromide and chloropicrin meaningfully reduced bacterial wilt in the field from 72% to 100% and improved the produce of tomato and the tobacco. Nevertheless, these pesticides have adverse effect to the environment. For instance, Methyl bromide has been found to cause stratospheric ozone depletion and is correlated with solemn health effects like the skin cancer and eye cataract (Sande et al., 2011). Methyl bromide is also volatile and thus increased capability to spread

far area during and also after applications. Other pesticide example peroxyacetic acid has phytotoxic effect on a plant (Hong et al., 2018). Antibiotics such as streptomycin, tetracycline, penicillin and ampicillin have been listed as possessing minute effectiveness in curbing growth of *R. solanacearum* (Namisy et al., 2019).

2.5.5 Biological Methods

Use of biological control agent (BCA) and use of organic matter are examples of biological control that have been in use in control of bacterial wilt. The merits of biological control agent are: they spread on their own after primary establishment, potentially self-sustaining, lessen input of non- renewable resources and offers long-term disease suppression in an eco-friendly manner (Yuliar et al., 2015). The mechanism through which BCA reduce the effect of *R. solanacearum* are completion for nutrients and space, parasitism, antibiosis and induced synthetic resistance. Example of BCA are: *Pichia guiller-mondii, Enterobacter cloacae, Candida ethanolica Bacillus megaterium,* (Yuliar et al., 2015; Pathology, 2019). In a research by Nguyen and Ranamukhaarachchi , (2019) *Enterobacter cloacae, Bacillus megaterium, Candida ethanolica* and *Pichia guiller-mondii* presented notable potential for disease subdue and also increased plant height, fruit weight and biomass. In another study *Bacillus cereus, Bacillus thuringiensis* and a commercial formulation of some rhizobacteria curbed bacterial wilt in eucalyptus protecting the plants during the initial phases of development (Santiago et al., 2015). Use of biological control agents have been reported to be less capable of suppressing soil borne disease (Pal, 2006).

2.5.6 The Use of Plant Extract

Over the years plants have been synthesizing phytochemicals for protection against predators like insects and infection from pathogen (Wang et al., 2013). Natural pesticide effectively protect cultivated crops from obliteration by pests and disease, with less adverse effects compared to synthetic chemical pesticides (Aloo et al., 2019).

Phytochemicals properties of crude extracts from different plant parts have been assessed for their antibacterial properties against *Ralstonia solanacearum* that causes bacterial wilt diseases in tomato plants. In a study by Vu et al., (2017) isolated tannins i.e. gallic acid, corilagin, chebulagic acid, methyl gallate and chebulinic acid exhibited strong antibacterial activity against *R. solanacearum* with a minimal inhibitory concentration of MIC = 26–52 μg/mL. In greenhouse experiment, 2000 and 1000 μg/mL of the plant extract of S*apium baccatum* reduced the development of tomato bacterial wilt by 83 and 63%, respectively 14 days of infection

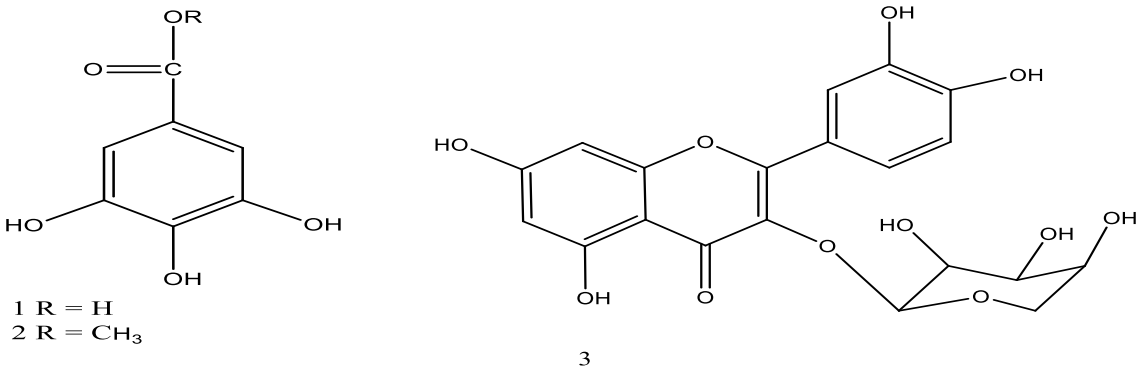


Figure 2.1: Chemical structure of isolated compounds from aerial parts of *Sapium baccatum* that had antibacterial activity against *Ralstonia solanacearum*. 1; gallic acid, 2; methyl gallate, 3; quercetin 3-O-α-L-arabinopyranoside (Vu et al., 2017).

Aloo et al., (2019) evaluated six plants for their antibacterial activity against *R. solanacearum*. In study, *Euphorbia tirucalli, Tagetes minuta*, *Solanum incanum*, and *Laurnea cornuta* extracts exhibited the greatest activity against *R. solanacearum.* The leaf extracts of *E. tirucalli* were the most potent while *Ageratum conyzoides* and *Opuntia monacantha* being the least potent with a means of inhibition of as low as 3.7 and 3.3 mm, respectively. Incorporation of the plant extracts into organic material for example biochar is one of the strategy for increasing their effectiveness of controlling soil borne pathogens such as *R. solanacearum* (Reyes-Escobar et al., 2015).

2.5.7 Use of Biochar

Recently, biochar has received notable agricultural interest as it improves soil fertility and productivity by increasing water retention, aggregation, cationic exchange capacity, and porosity, soils bioremediation, and climate change mitigation (Woolf, 2010). Biochar is produced by pyrolysis of biomass under oxygen limited condition (Reyes-Escobar et al., 2015). Biochar has been studied for its activity against *R. solanacearum.* For example, in a study by Lu et al., (2016), biochars made from peanut shell and wheat straw were added to *R. solanacearum* infected soil and they significantly reduced the disease index of bacterial wilt by 28.6% and 65.7%, respectively. In another study, 2% biochar made from wheat straw was added to the soil infected by *R. solanacearum*, and it significantly reduced the disease severity of bacterial wilt, increased soil total organic carbon, total nitrogen, Carbon: Nitrogen ratio, organic matter, available P, available K, pH, and electrical conductivity (Gao et al., 2019).

Hence, the aim of the current study was to evaluate the effectiveness of biochar composite from *Solanum incanum* fruits as a novel method of controlling bacterial wilt disease in tomato plants.

2.5.8 Use of *Solanum incanum* in Control of Plant Pathogen

Solanum incanum also known as bitter apple/ bitter garden egg/ thorn apple/ bitter tomato is a perennial soft wooded shrub that belongs to Solanaceae family (Mwaura et al., 2015). It is a species of nightshade which is native in Sub-Saharan Africa and the Middle East, eastward to India (Kaunda & Zhang, 2020). Its height is up to 1.8m with spines on the stem and calyces and with hairs on the foliage. The fruits are 2-3 cm in diameter and are dappled or barred green when unripe and turn to yellow or brown in color when ripe (Ofori et al., 2013). The flowers are either borne singly or as cluster at the axils of leaves and are characterized by pale to deep blue or purple color. The description of the plant is as shown on Figure 2.2. In most parts of Africa it is a common weed around houses, near roadsides and in over foraged grassland (Sbhatu & Abraha, 2020).

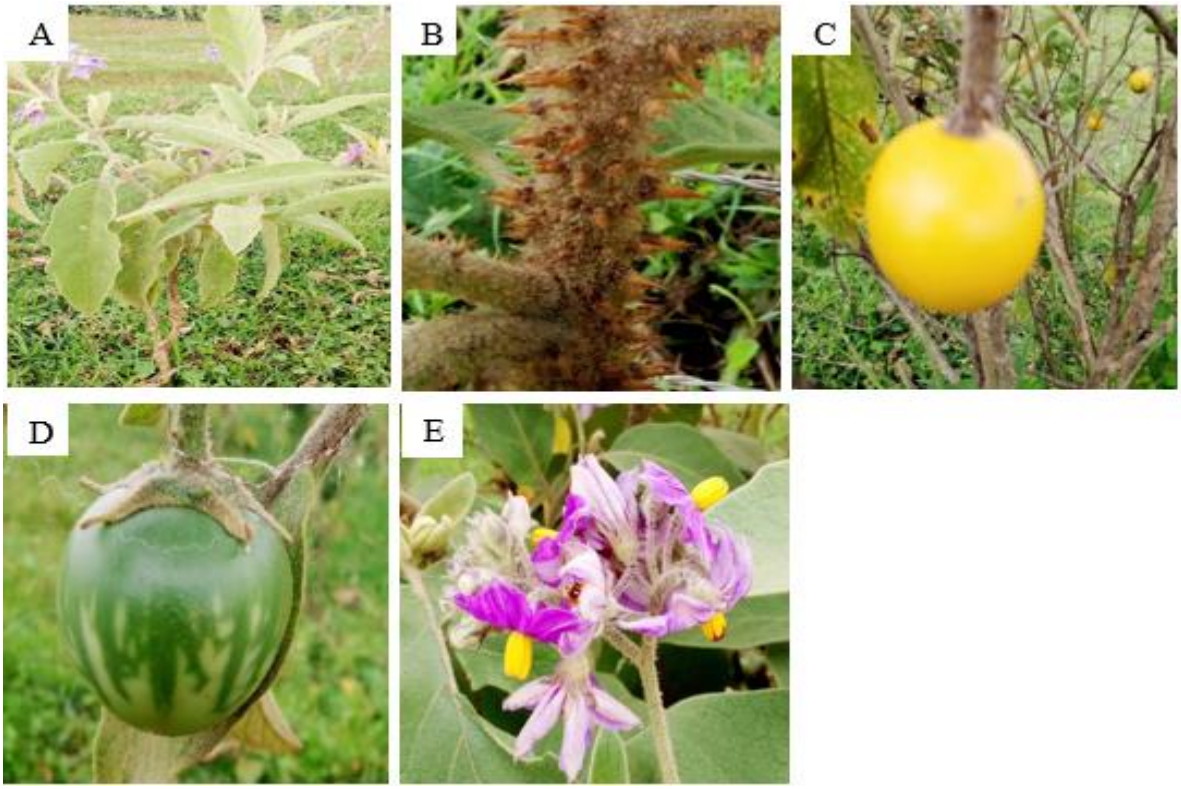


Figure 2.2: *Solanum incanum* plant (A), stem (B), ripe fruit (C), unripe fruit (D) and flowers (E). Images taken from Kesses, Eldoret, Kenya.

Solanum incanum has found a wide application in control of diseases in humans and in plants. In tropical Africa it has been used as a remedy for sore throat, angina, stomachache, colic, headache and in wounds dressing (Sbhatu & Abraha, 2020; Sahle & Okbatinsae, 2017). The plant has also been extensively used to lessen skin problems, such as warts, burns, whitlow, infections, sores, carbuncles, ulcers, wounds, ringworm, rashes, inflammations and benign tumors (Malik et al., 2018; Zinaye, 2020; Qureshi et al., 2019). Also, *Solanum incanum* has been used to control pest affecting plants for example; aphids, tingid bug, termites, nematodes, cutworms among others (Mvumi, 2019; Plazas et al., 2016; Mwangi, 2015). Additionally, it has also been used for control of plant diseases like bacterial wilt,

root knot disease, fusarium wilt, dry root rot disease, anthracnose, chocolate spot disease among other plant diseases (Villela, 2013; Rakha et al., 2020; Kipngeno et al., 2014; F. Boyaci et al., 2012). The plant has also been reported to be utilized in control of ticks in cattles (Madzimure et al., 2013). Its botanical effects against diverse pathogens is attributed to its phytochemical constituents. The herb has been reported to contain bioactive compounds such as solamargine, solasonine, phenylalkanoic acids, flavonoids, spirostanol saponin, chlorogenic acid, adenosine, kaempferol which have been experimentally proven to have effects against numerous pathogens (S. Anwar, 2018; Manase et al., 2012; Al Sinani et al., 2016). Therefore, the current study intended to explore other forms of plant parts preparation other than the extract in attempt to control bacterial wilt in tomato plants.

CHAPTER THREE: METHODOLOGY

3.1 Study Design

The study was both qualitative and quantitative using randomized experimental design.

3.2 Sampling Area

The study was carried out in Moi university farm. The farm is located approximately 40 km south of Eldoret town in Uasin Gishu County. The sampling site coordinates are 0˚17′05.3" N 35˚ 17′16.8"E. The farm lies at an altitude of 2160 m above sea level and rises to 2180 m at the top of the lower Uasin Gishu plateau. Rainfall is normally received at the month of April to September with the peak month being in August. Temperature ranges from 10℃ (mean annual minimum) to 23℃ (mean annual maximum) with an average annual temperature being 16℃. The farm soil is broadly grouped into two categories i.e. floodplain and footslopes basing on physiographic position. Generally, footslopes soil is well drained, reddish brown in color while that of floodplain is imperfectly drained, dark greyish brown soil. Footslopes soils are mainly utilized for cultivation of maize and wheat and that of floodplain is used for establishing grazing points.

3.3 Chemicals, Reagents and Equipment

3.3.1 Chemicals and Reagents

The chemicals and reagents used in this study were; Analytical grade Methanol, ethanol, Folin-Clocalteu's reagent (FCR), quercetin, gallic acid, HPLC grade ethanol, Mayer's reagent, distilled water, sulphuric acid, chloroform, sodium hydroxide, ferric chloride, sodium carbonate, sodium nitrite, aluminium chloride, hydrochloric acid, ammonium

sulphate, analytical standard KBr pellets, Kelman's medium. All chemicals and reagents were analytical grade and manufactured by Sigma Aldrich and supplied to Moi University Chemistry laboratory by Centrihex Limited.

3.3.2 Equipment

The equipment used during this study were; Hahnvapor HS-2005S Rotary evaporator, Beckman single beam Coulter Model DU^{R}720 UV-Vis spectrophotometer, Wise Therm furnace, Shimadzu FTS-800 FT-IR, HANNA H193127 pH meter, MRC DFO-36 oven, Shimadzu QP 2010 GC-MS, Mettler Toledo analytical weighing balance, Pressure Steam Sterilizer Electric Model No. 25X, Vega 3 Tescan Scanning Electron Microscopy, NutriBullet-900W electric blender and Norlake M2ph-0026-IAA-121 Refrigerator.

3.4 Sampling Procedure

The specimen sampling was done in January 2021 in Moi University farm Eldoret. Fresh samples of mature *Solanum incanum* fruits were collected by simple random sampling. The fruits were harvested by hand picking, stored in a clean sealable cotton bag and transported to the laboratory at Moi University Biological Sciences Department for confirmation of the species, later, they were taken to chemistry laboratory for preparation, extraction and analysis.

3.5 Sample Preparation

This was done following procedure as per (Desta et al., 2020). The fruits of *S. incanum* were washed using tap water and rinsed with distilled water. The fruit samples were sliced into four equal pieces and placed in an oven at 40 ℃ to dry for 5 days until a constant weight was

obtained. Finally, the dried samples were pulverized into fine powder using NutriBullet-900W electric blender. The fine powders were kept in dark airtight container in the laboratory freezer at 4 ℃ until used.

3.6 Extraction of Crude Extract from Fruits Using Maceration Method

This was done following procedure as per (Sbhatu & Abraha, 2020) with some modification. Crude plant extract was obtained through maceration using ethanol as the solvent. About 50 g of powdered sample was macerated using 200 mL ethanol for three days with occasional shaking and later filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator at 45 ± 2 ℃ and further concentrated to dryness on a water bath. The dried extract was stored at 4 ℃ in the Norlake M2PH-0026-IAA-121 refrigerator prior to use. The dried sample was weighed and total yield calculated using equation 3.1

$$\%\ \text{yield} = \frac{\text{mass of dried crude extract}}{\text{mass of the dried sample macerated}} \times 100 \quad \text{.....Equation 3.1}$$

3.7 Phytochemical Screening of the Extract

Chemical tests were carried out using the extracts from plant fruits following standard procedures to identify the phytochemicals present in the extract. Standard procedures were obtained from (Banso & Adeyemo, 2010).

3.7.1 Tests for Alkaloids

About 2 mL of the filtrate was put in a test tube and a drop of Mayer's reagent was added by the side of the test tube. Formation of creamy or white precipitate indicated the presence of alkaloids.

3.7.2 Test for Saponins

Exactly 5 mL of the sample was added into a graduated cylinder and diluted with distilled water to 20 mL. The suspension was shaken for 15 minutes. Formation of 1 cm layer of foam indicated the presence of saponins**.**

3.7.3 Tests for Flavonoids

A total of 5 mL of the filtrate was put in a beaker and treated with 2 drops of sodium hydroxide solution. Intense yellow colour was formed. On addition of 3 drops of HCl, the mixture turned colourless. This indicated presence of flavonoids.

3.7.4 Tests for Terpenoids

About 5 mL of the filtrate was put into a test tube and mixed with 2 ml of chloroform and 2 ml concentrated sulphuric acid to form a layer. Formation of reddish brown coloration of the interface indicated presence of terpenoids.

3.7.5 Test for Phenolic Compounds

About 2 ml of the filtrate was put into a test tube and diluted to 5 mL with distilled water. To this 3 drops of 5 % ferric chloride solution was added. Formation of dark green color indicated presence of phenolic compounds.

3.7.6 Test for Tannins

About 0.5 mg of dried powdered samples was boiled in 20 mL of water in test tubes then filtered. Three drops of 0.1 % ferric chloride was added and observed for brownish green or blue black coloration.

3.7.7 Test for Steroids

Crude plant extract (1 mg) was placed into a test tube and dissolved with 10 mL chloroform. Equal volume of concentrated sulphuric acid was added to the mixture by the side of the test tube. The upper layer in the test tube turned into red and sulphuric acid layer showed yellow with green fluorescence. This indicated presences of steroids.

3.7.8 Test for Glycosides

About 2 mL of the plant extract was placed in a test tube. Three ml of chloroform and 10% ammonia solution was added. Formation of pink color indicated presence of glycosides.

3.8 Quantitative Analysis of the Phytochemical Constituents

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) was determined following the procedures given below.

3.8.1 Determination of Total Phenolic Content

TPC was determined following two steps which involved preparation of standard gallic acid for calibration followed by preparation of the sample for determination of phenolic content.

3.8.1.1 Preparation of Standard Gallic Acid for Calibration

The total phenol content was determined with the Folin- Ciocalteu's assay using gallic acid as standard as described by (Phuyal et al., 2020). To prepare standard gallic acid stock solution, 0.25 g of analytical standard gallic acid was weighed using analytical balance (Mettler Toledo) and dissolved using 250 mL of methanol in a 250 mL volumetric flask. Various concentrations of gallic acid solutions in methanol (10, 20, 40 and 80 mg/mL) were

prepared from the standard solution. To each concentration, 5mL of 10% Folin–Ciocalteu reagent (FCR) and 4mL of 7% Na_2CO_3 were added making a final volume of 10 mL. The obtained blue colored mixture was shaken well and incubated for 30 min at 40 °C in a water bath. Absorbance was measured using BeckMann Spectrophotometer at 760 nm against blank. All the experiments were carried out in triplicates, and the average absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve.

3.8.1.2 Preparation of the Sample for Determination of Phenolic Content

About 0.01 g of dry sample was weighed using analytical balance (Mettler Toledo) and dissolved in 10 mL of methanol. The procedure as described for standard gallic acid (Refer Section 3.8.1.1) was followed, and absorbance of the plant extracts was recorded. The sample was prepared in triplicate and the average value of absorbance was used to calculate the concentration of extract using the regression equation of the calibration curve. Total phenolic content of the extracts was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). The total phenolic contents in the samples was calculated using Equation 3.2:

$$C = \text{Df.}\, c\frac{v}{m} \quad \text{.....Equation 3.2}$$

Where Df = dilution factor

C = total phenolic content mg GAE/g dry extract

c = concentration of gallic acid obtained from calibration curve in mg/mL

v = volume of extract in mL

m = mass of extract in gram.

Values were expressed in mean ± standard deviation in terms of phenol content per g of dry weight.

3.8.2 Determination of Total Flavonoid Content

TFC was determined following two steps which involved preparation of standard quercetin for calibration followed by preparation of the sample for determination of TFC.

3.8.2.1 Preparation of Standard Quercetin for Calibration

Total flavonoid contents in the extracts were determined by aluminum chloride assay using quercetin as the standard as described by (Baba & Malik, 2015). Various concentrations of quercetin solutions in methanol (10, 20, 40, 80 and 160 mg/mL) were prepared from the standard solution. One mL quercetin of each concentration was added to the test tube containing 4mL of distilled water. At the same time, 0.3 mL of 5% $NaNO_2$ was added to the test tube. After 5 min 0.3 mL of 10% $AlCl_3$ was added. Six minutes later, 2 mL of 1M NaOH was added to the mixture. The volume of the mixture was made 10 mL by immediately adding 4.4 mL of distilled water. Absorbance was measured at 420 nm against blank. The total flavonoids content was expressed as quercetin equivalents using the linear equation based on the calibration curve

3.8.2.2 Preparation of the Sample for Determination of Flavonoids Content

About 0.01 g of the dry sample was weighed and dissolved in 10 mL of methanol. The procedure as described for standard quercetin (Refer Section 3.8.2.1) was followed, and absorbance of the plant extracts was recorded. The sample was prepared in triplicate and the

average value of absorbance was used to calculate the concentration of flavonoids in the extract using the regression equation of the calibration curve. The flavonoid content was expressed as quercetin equivalent (mg QE/g). Values were expressed in mean ± standard deviation in terms flavonoid content per g of dry weight.

3.9 GC-MS Analysis of the Extract

The extracts of *S. incanum* was initially dissolved in HPLC grade ethanol and filtered through 0.45 μM syringe filters. They were then transferred into auto-sampler vials for GC-MS Shimadzu QP 2010 Model for further analysis. Carrier gas used was ultrapure Helium with the flow rate set at 1 mL / minute. A BPX5 non-polar column, 30m; 0.25 mm ID; 0.25 μM film thickness, was used for separation.

The GC-MS machine was set and programmed as follows: temperature of 60 ℃ (1 minute). This was subsequently increased at a rate of 10 ℃ /min up to 270 ℃ (18 minutes) with the total run-time being exactly 30 minutes. 1 μL of the sample was injected into the GC at 200 ℃ in split mode. These were in split ratios of 10:1 with the interface temperatures set at 250 ℃. The Electron Ionization (E.I) ion source was set at 200 ℃. Mass analysis, was done in full scan mode within the ranges 50-500 m/z and the detected peaks auto-matched against the National Institute of Standard and Technology (NIST) 14 library for possible identification. Both Fragmentation patterns and retention index, were used for matching.

3.10 Sample Collection of *Ralstonia solanacearum* Infected Potato Tubers

This was done by observing symptoms as described by (Campos & Ortiz, 2019). A total of 10 diseased potato tubers were collected from Matharu, Timboroa constituency, Kesses Sub

County, Uasin Gishu County with sampling site coordinates being (0.1015N, 35.4881E). Diagnosis of the infected potato tuber was done by critical observation of bacterial wilt symptoms which includes bacterial ooze which collects at tuber eyes or on the end of the stolon causing soil to adhere to the secretions. Cut tubers show brownish discoloration of the vascular ring, and slight squeezing forces a pus-like slime out of the ring, or it may exude naturally. The brown rot symptom on tubers can be confused with ring rot caused by *Clavibacter michiganensis subsp sepedonicus*. Thus a quick test was performed to differentiate between the two bacterial symptoms.

3.11 *Ralstonia solanacearum* Culture

This was done following procedure by (Lu et al., 2016) with slight modifications. *Ralstonia solanacearum* was isolated from a diseased tuber by plating the bacterial tuber exudate on modified Triphenyl Tetrazolium Chloride (TZC) agar. The plates were incubated at 28 ± 2 ℃ for 48 hours. The colonies in the medium was characterized by fluidal, irregularly round red colonies which were further streaked on TZC medium to get pure colonies of the bacterium. Pure colonies of the bacteria were further cultured on Nutrient Agar (NA). The bacterium was harvested from agar plates via sterile water flushing. Serial dilution was conducted to obtain a Colony Forming Unit ((CFU) of about 1.0×10^7 before inoculation.

3.12 Biochemical Characterization of *Ralstonia solanacearum*

3.12.1 KOH Test for Confirmation of *Ralstonia solanacearum*

The test was done following procedure by (Khasabulli et al., 2017). Bacteria was aseptically removed from petri plates with an inoculating wire loop, placed on the glass slide in a drop

of 3% KOH solution. This was mixed with a laboratory loop for 10 seconds. The formation of a milky thread upon lifting the loop indicated presence of *R. solanacearum.*

3.12.2 Gram Staining Test of *Ralstonia solanacearum*

Gram staining of *R. solanacearum* was done following procedure by (Khasabulli et al., 2017). A loop full of bacterium was spread on a glass slide and fixed by heating on a very low flame. Aqueous methylene blue solution (0.5%) was spread over the smear for 1 minute and washed with running tap water for one minute. Later was flooded with iodine for 1 minute and rinsed in tap water and finally decolorize with 95% ethanol until colorless. After washing, the specimen was counter-stained with safranin for about 10 seconds, washed with water, dried and observed microscopically at Mg X 10, Mg X 40 and Mg X 100 using oil.

3.12.3 Gas Production Test for *Ralstonia solanacearum*

This was done following procedure by (Khasabulli et al., 2017). The ability of *R. Solanacearum* to produce gas was tested by growing it in lactose broth media. The medium was distributed in test tube containing inverted Durham's tube. This was sterilized by autoclaving at 121 ℃ for 21 minutes. The tubes were later inoculated with 0.5 µL of bacteria suspension and incubated at temperature (28 ± 2 ℃) for seven days. Presence of air bubbles in the inverted Durham's tube was a positive indication of production of the gas by the bacterium.

3.12.4 Starch Hydrolysis Test for *Ralstonia solanacearum*

The capacity of *R. solanacearum* to hydrolyze starch was studied by growing it on nutrient agar containing 1% soluble starch following procedure by (Sharma & Singh, 2019). Nutrient

agar was sterilized in an autoclave (121℃ for 21 minutes) poured to sterilized petri plates and allowed to solidify. The bacterium culture was inoculated in the center of the plates and incubated for 7 days at room temperature (28 ± 2 ℃). The plates were flooded with Lugol's iodine. Clear zone around culture was an indication of positive test.

3.12.5 Catalase Oxidase Test of *Ralstonia solanacearum*

This was done following procedure by (Khasabulli et al., 2017). Young agar culture (18-24 hrs.) and 3% H_2O_2 was used to observe production of gas bubbles. Briefly, a loop full of bacteria culture was mixed with a drop of H_2O_2 on a glass slide and observed for the production of gas bubbles with unaided eye and under a dissecting magnification of 25X.

3.13 Biochar Production from *Solanum incanum* Fruits

Preparation of biochar was done as per (Jia et al., 2013). *Solanum incanum* fruits were first washed with tap water and rinsed with distilled water. They were sliced into equal size and air dried for 5 days until constant weight was obtained. After drying they were oven dried for 1 hour at 100 ℃. They were later transferred to the Wise Therm furnace and subjected to a step wise heating. Biochar was produced at two different pyrolysis temperature i.e. 300 °C and 500 °C. The starting temperature for the preparation of biochar at 300 °C was 200 °C, followed by elevation to 250 °C and finally to 300 °C with the temperature held constant for 15 minutes at each point. Next, the starting temperature for the preparation of biochar at 500 °C was 300 °C, followed by elevation to 350 °C, then to 400 °C, to 450 °C and finally 500 °C with the temperature held constant for 15 minutes at each point. The biochar was defined as the remaining mass after subsequent heating in a tightly aluminium foil to

constant weight at 300 °C and 500 °C respectively. Biochar was later grounded with an electric blender weighed using analytical balance and later sieved to obtain biochar with particle size 53-120 µM.

The percentage of biochar yield was calculated using Equation 3.3

$$\text{percentage yield} = \frac{\text{mass of biochar}}{\text{mass of raw biomass}} \times 100 \quad \text{.....Equation 3.3}$$

3.14 Characterization of Biochar

The ash content, volatile content, pH, moisture content, and fixed carbon content of the biochars was determined following procedures by (Sun et al., 2017)

3.14.1 Determination of pH

Two grams of the biochar was weighed out using an analytical balance. The weighed biochar was washed thoroughly for 5 minutes with 30 mL distilled water and filtered using a Whatmann filter paper number 1. The pH of filtrate was measured using a pH meter.

3.14.2 Determination of Ash Content

Two grams of the biochar was weighed and placed into a crucible. The crucible was reweighed with its content and heated in a furnace at 900 ℃ for 3 hours. The sample was cooled to room temperature in a desiccator and reweighed. Ash content was the mass of material remaining after incineration (Domingues et al., 2017). Ash content was calculated using equation:

$$\%\ \text{Ash content} = \frac{\text{mass remaining after incineration)}}{\text{initial weight (g)}} \times 100 \quad \text{.....Equation 3.4}$$

3.14.3 Determination of Moisture Content

Exactly 2.0 grams of the biochar weighed and placed into a crucible. The crucible and the content was reweighed and placed into an oven dried at a temperature of 105 ℃ for 5 hours until the sample weight was constant. The moisture content was determined using equation 3.5:

$$\% \text{ moisture content} = \frac{I-F}{I} \times 100 \quad \text{.....Equation 3.5}$$

Where I = initial weight before drying given in grams

F= Final weight after drying given in grams

3.14.4 Determination of Volatile Content

Volatile content of the biochar was determined by weighing 1 g and placing into a crucible, covered and placed in the furnace at 930 ℃ for 1 hour. The crucible and its content were cooled in a desiccator and the volatile component determined. The volatile content was then weighed and the difference in mass represented the mass of organic material present in the sample.

3.14.5 Fixed Carbon Content

This was calculated following an equation given by (Domingues et al., 2017). The fixed carbon content of the biochar was calculated using equation 3.6:

$$\% \text{ Fixed carbon} = 100 - (\text{ash content} + \text{volatile matter}) \quad \text{.....Equation 3.6}$$

3.14.6 Identification of Functional Groups

For identifying functional groups, a Shimadzu Fourier Transform Infrared spectrophotometer, Model FTS- 8000, was used to study FT-IR spectra with signals provided in wave numbers (cm^{-1}) as usual in order to monitor the functional groups. The KBr pellets of samples were prepared by mixing 10 mg of samples, finely ground, with 250 mg KBr (FT-IR grade). The 13 mm KBr pellets were prepared in a standard device under a pressure of 75 kN cm^{-1} 2 for 3 min. The spectral resolution was set at 4 cm^{-1} and the scanning range from 400 to 4000 cm^{-1}.

3.14.7 Examination of Surface Morphology

The surface morphology of the biochars was examined using a Vega 3 Tescan Scanning Electron Microscopy (SEM) system operated at a 5 kV accelerating voltage potential.

3.15 Preparation of Biochar Composite

The initial step was the activation of biochar following procedure by (Yang et al., 2019). Biochar and 2M NaOH were thoroughly mixed with a solid–liquid ratio of 1:5. They were later resuspended for 12 h with a speed of 30 revolutions min^{-1} at 65 ℃. After, the mixture was filtered, and the precipitate was collected and rinsed with deionized water. Finally, the material was dried at 105 ℃. Dried biochar was mixed with plant extract at the rate of 1%, 2%, 3% 4%, 5% and 7 % w/w. Briefly, 1% biochar composite (BC) was prepared by weighing 1.98 grams of activated biochar and mixing with 0.02 grams (dissolved in 10 mL ethanol) of crude extract in a 50 mL beaker. The mixture was agitated at 500 revolutions per minute (rpm) at a temperature of 25 ℃. After 200 minutes the mixture was filtered and

residue air dried at room temperature (23 ± 2 ℃). Similar procedure was repeated for the preparation of 2%, 3% 4%, 5%, and 7 % BC.

3.16 Effect of Contact Time in the Adsorption of the Plant Extract

The adsorption experiment was conducted following procedure by (Lawal et al., 2021) with slight modification. The effect of contact time was carried out by weighing 1.86 grams of the activated biochar and mixing with 0.14 grams (dissolved in 10 ml ethanol) of crude plant extract and placed in a 50 mL beaker. The mixture was agitated at 500 rpm at a temperature of 25 ℃ for varying time ranging from 20-200 minutes. After each pre-determined time the mixture was filtered and the filtrate used to calculate the total phenolic compounds.

Concentration of phenols was determined using Folin-Ciocalteu method. The procedure involved adding 5 mL of 10% FCR reagent and 4 mL of 7% Na_2CO_3 into 1 mL of the filtrate making a final volume of 10 mL. The obtained blue colored mixture was shaken well and incubated for 30 min at 40°C in a water bath. Absorbance was measured at 760 nm against blank using UV-VIS spectrophotometer. The sample was prepared in triplicate and the average value of absorbance was used to calculate the concentration of phenolic compounds using the regression equation of the gallic acid calibration curve. The percentage reduction and adsorption capacity at given time was calculated.

The biochar sample adsorption capacity was calculated using the Equation 3.7

$$\boldsymbol{q_t} = \frac{\boldsymbol{C_0 - C_t}}{\boldsymbol{w}} \quad \text{.....Equation 3.7}$$

Where C_0 and C_t indicate the concentration of phenolic compounds during the adsorption experiment at the initial time and at time t, respectively. V represents the volume of plant extract solution and W represents the mass of the biochar sample utilized in the experiment.

The pseudo-first-order, pseudo-second-order, and Elovich kinetic models were used to investigate the kinetics of phenolic adsorption by biochar. The equations defining these models are as follow

Pseudo-first-order:

$\frac{dq_t}{d_t} = \mathrm{k}_1\ (\mathrm{q}_e \mathrm{q}_t)$……..Equation 3.8

Pseudo-second-order

$\frac{dq_t}{d_t} = k_2(\mathrm{q}_e \mathrm{q}_t)2$…….Equation 3.9

Elovich isotherm model

$\frac{q_e}{q_m} = \ K_E\, C_e\, \frac{\boldsymbol{q_e}}{\boldsymbol{q_m}}$……Equation 3.10

3.17 *In vitro* Antibacterial Activity of the Plant Extract

This was done following procedure by (Razmavar et al., 2014). Disc diffusion method was used for antibacterial activity on a nutrient agar. Plant extract was prepared by dissolving 0.01, 0.05 and 0.15 g in 10 mL of ethanol. Two µL of each dissolved plant extract was impregnated into sterile, blank discs 6 mm in diameter. The discs were allowed to dry before the next 2 µL was spotted to ensure precise impregnation. The positive control used was ampicillin antibiotic discs treatment with same concentration as that of plant extract.

Ethanol-loaded discs were used as negative controls. All discs were fully dried before the application on bacterial lawn. The plates were incubated in an upright position, at 28 °C for 48 hours. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the discs using a 30 cm ruler. The assay was repeated four times. Antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the fruits ethanol extract.

3.18 *In vitro* Antibacterial Test of Biochar Composite Against *R. solanacearum.*

This was done following procedures as per (Hu et al., 2019). Antibacterial activity of the biochar composites was determined for different duration i.e. 10, 20, 30, 40, 50, and 60 minutes. The preliminary results showed that at 60 minutes the biochar composites had good antibacterial activity. Thus the antibacterial activity of each concentration was determined as follows. Breifly, 5ml of cell suspension (1.07×10^7 CFU/ ml) was added to tubes containing 0.15g of 1%, 2%, 3%, 4%, 5%, and 7% biochar composite of both biochars produced at 300 ℃ and 500 ℃. A cell suspension without biochar was also included as a control. The tubes were incubated in a rotary shaker for 60 minutes (at 30 ℃ with 90 rpm shaking) and then allowed to stand for additional 60 minutes. An aliquots (100 µL) of the supernatant was spread on TZC (sterilized at 121 ℃ for 20 minutes) plate and incubated at 30 ± 2 ℃ for 48 hours. After the number of colonies were counted and recorded. All experiment was conducted in triplicate and the average number of observed colonies calculated.

3.19 Effect of pH on the Growth of *R. solanacearum*

This was done following procedures as per (Tomlinson et al., 2009). To measure the effect of pH on the growth of *R. solanacearum in vitro*, 100 ml TZC medium in 250 ml conical flasks were adjusted to pH 4.0, 5.0, 6.0, 7.0, and 8.0 by adding 1M NaOH or 1M HCl. The pH was rechecked after autoclaving and readjusted using sterile 0.1 M HCL or NaOH as necessary. All treatments were inoculated with 1 mL of bacterial suspension (1.07×10^7) and incubated in the oven at 30 ± 2 ℃ for 48 hours. Later the number of colonies on the plates was counted.

3.20 Germination of Tomato seeds

This was done following procedure by (Gao et al., 2019). Tomato seeds of the cultivar Rio Grande were sterilized using sodium hypochlorite and later rinsed with distilled water. They were later placed on moist filter paper in a petri dish and incubated at 28 ± 2 ℃ for seven days. After seven days, the germinated seeds were transferred in nursery soil (disinfected at 121˚C for 20 minutes in an autoclave) in experimental pots in the greenhouse.

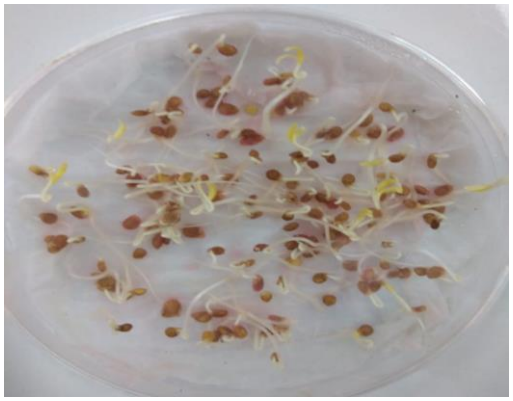


Figure 3.1: Germinated seeds in the incubator at 28 ± 2 ℃ for 7 days.

3.21 Greenhouse Set up

The experiment was arranged in a completely randomized design with three replications; each replicate having fifteen pots with 1 tomato plant each.

3.21.1 Greenhouse Experiment

This was done following procedure by (Khalifa & Thabet, 2015). The soil was collected from field at Moi University farm and first cleared of soil debris, and sterilized using Pressure Steam Sterilizer (Electric Model No. 25X) at 121 Pounds per Square Inch (psi) for 20 minutes. The soil was later filled into 3 liter plastic pots (15 cm in diameter). The pots were then separated into 10 groups (each of 15 pots and 3 replicates), depending on whether the potting soil is biochar amended, biochar plant extract composite (at different concentration i.e. 1%, 2%, 3% and 4 %, 5%, 7% BC) amended, plant extract amended (0.15g), distilled water amended (negative control) or ampicillin (0.15 g/10 mL) amended. Soil and biochar was mixed at a concentration of 2% w/w. Tomato seedling (49 days old) were transplanted individually into the pots and maintained under greenhouse conditions at temperature ranging between 28 and 32 ℃ and watered two times per day. *Ralstonia solanacearum* was inoculated by pouring 40 ml of the bacterial suspension at a cell density of 1.07×10^7 CFU/mL. The disease incidence was calculated at an interval of two days following the equation 3.11.

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of experimental subject}} \times 100 \quad \text{.....Equation 3.11}$$

3.22 Data Analysis

GC-MS data files were analyzed with reference to NIST 14 library, web-based resources (e.g. Chemspider) and published research papers. FT-IR data analysis was performed using Origin pro software. *In vivo* and *in vitro* data obtained were expressed as means ± standard deviation. The data were further subjected to statistical analysis using Microsoft excel. One-way analysis of variance (ANOVA) was used to statistically compare means between different treatments used against the bacteria.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Percentage Yield of the Extract from *Solanum incanum*

Amount of yield extracted in this study was 5.73 ± 0.2% and was calculated as shown in Equation 3.1. In a study by Kemei and Ndukui (2017), the amount of yield of *S. incanum* fruits extracted using distilled water was 5.3% while that of methanolic extract was 4.6%. Amount of yield extracted depends on methodology, solvent to solid ratio, extracting solvent, duration and particle size (Zhang et al., 2018). Higher solvent to solid ratio, longer duration, finer particles and polar solvents results to higher yield (Elisha et al., 2017).

4.2 Phytochemical Analysis of the Extract

Phytochemical analysis of *S. incanum* fruits ethanol extract tested positive for alkaloids, phenols, flavonoid, steroids, tannins, glycosides, terpenoids and saponins as shown in Table 4.1. Several other researchers had previously reported about the presence of alkaloids, flavonoids, tannins, glycosides, saponins, and steroids in the plant (Sbhatu & Abraha, 2020; Sahle & Okbatinsae, 2017; K. Kumar et al., 2019; Kaunda & Zhang, 2020). In addition, the identified phytochemicals have been reported previously in the fruits extract of *Solanum nigrum* (Mazher et al., 2016) and ethanolic extract of *Solanum melongena* (Imo et al., 2020) which are examples of plants belonging to family Solanaceae. Presence of the aforementioned phytochemicals in *S. incanum* ethanolic fruits extract implies that the plant can be utilized as novel source of biopesticides. The simple structure of phenols for example, can penetrate the microorganisms resulting to considerable damage to the cell metabolism (Kumar et al., 2014). On the other hand, flavonoids inhibit synthesis of nucleic acid, alters

the permeability of membrane, inhibits the function of cytoplasmic membrane among other effects (Xie et al., 2014). Similarly, alkaloids inhibit synthesis of nucleic acid and tannins destroy bacterial membrane and deters biofilm formation by its bacteriostatic properties (Roy et al., 2018). Thus *S. incanum* can be utilized in control of plants pests and pathogen that destroy crops leading to poor and low yield.

Table 4.1: Phytochemical compounds identified in *Solanum incanum* ethanol fruits extract.

S/N	Phytochemicals	Type of test performed	Inferences
1	Alkaloids	Wagner's test	+++
2	Saponins	Froth test	++
3	Steroids	Salkowskis test	+
4	Phenolics	Ferric chloride test	+++
5	Tannins	Ferric chloride test	++
6	Glycosides	Keller Kelliani test	+
7	Flavonoids	Alkaline reagent test	+++
8	Terpenoids	Salkowski's test	+

Key +++ = Highly present, ++ = Moderately present + = Low amount present

4.3 Quantitative Analysis of Ethanolic Extract of *Solanum incanum* Fruits

4.3.1 Total Phenolic Content

Figure 4.1 was used for the calculation of Total Phenolic Content (TPC). The correlation coefficient of the graph ($R^2 = 0.9967$) was obtained for the standard gallic acid curve.

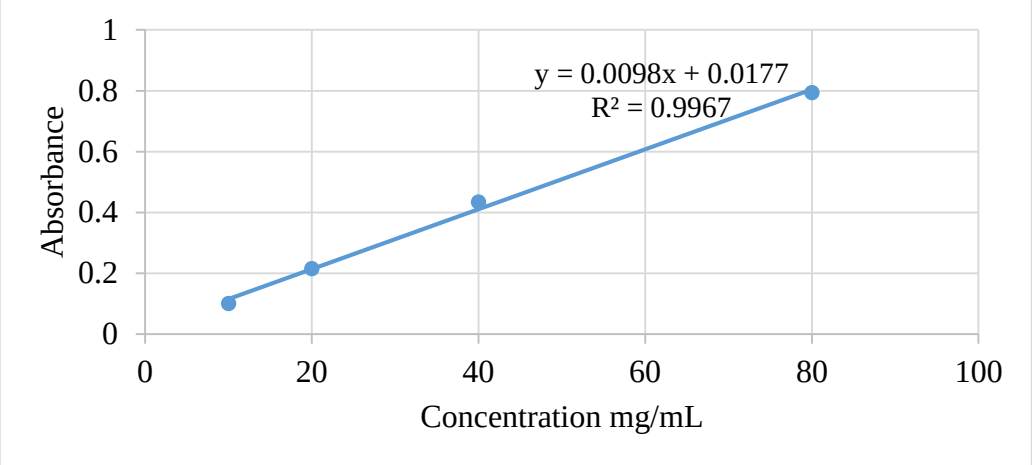


Figure 4.1: Standard gallic acid calibration curve.

The TPC in ethanol fruits extract was found to be 84.997 ± 0.2 mg/g of the dried sample. Comparing literature, Mongalo et al., (2018) reported a TPC of 490.7 ± 0.02 mg/g gallic acid equivalent (GAE) in *Solanum panduriforme*. This was six times higher compared to that reported in this study. On the other hand, Ghosal & Mandal, (2012) reported a total phenolic content of 2.306 ± 0.37 mg/g in fruits extract of *S. incanum.* This was 41 times lower compared to the TPC reported in this study. Further, Aryal et al., (2019) found the TPC to be 97.96 ± 0.62 mg GAE/ g dry extract weight of *S. nigrum* fruits extract which was slightly higher compared to the one reported in the current study.

Phytochemicals are polar or non-polar in nature, thus the type of solvent used for extraction is accountable for dissolving the compounds of the plant. Phenolic compounds contain hydroxyl group making them more soluble in polar organic solvent (Sobiesiak, 2017). Similarly, duration, abiotic environmental factors, geographical variation, methodology used for extraction can also cause difference in total phenolic content (Zlatić et al., 2019). Environmental factors, such as ultraviolent radiation, rainfall, temperature and soil

composition affects the concentration of phenolic compounds in the plant (Borges et al., 2013). According to Kouki & Manetas, (2002) availability of nutrients, water and light lead to preferential allocation of photo-synthetically absorbed carbon to the growth and development of the plant other than in concentration of secondary metabolites. Geographical regions with severe climatic conditions and low humidity have been reported to contribute to increase in total phenolic content in the plants (Monteiro et al., 2006).

4.3.2 Total Flavonoid Content

Figure 4.2 was used for the calculation of Total Flavonoid Content (TFC). As shown from the figure a good correlation coefficient (R^2 = 0.9998) was obtained from the standard quercetin curve. The equation of the line was Y = 0.0021X - 0.0055.

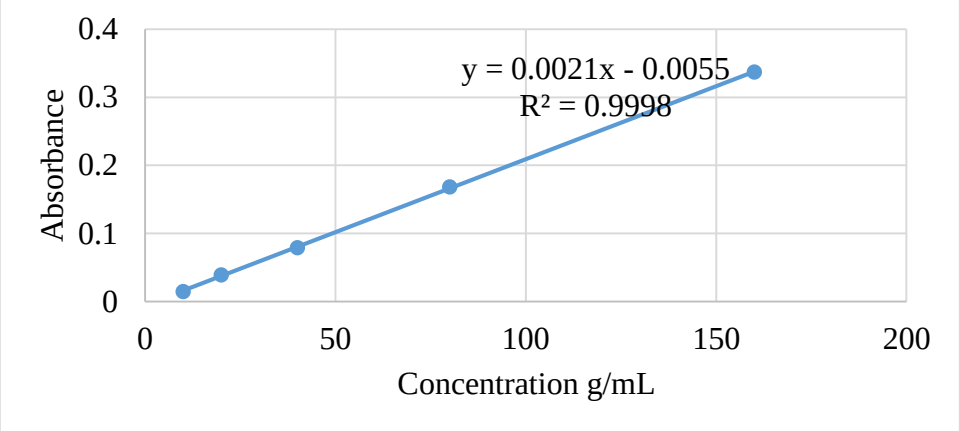


Figure 4.2: Standard quercetin calibration curve.

The TFC content of the plant extract was found to be 20.535 ± 0.2 mg/g quercetin equivalent (QE) of dried sample. In a survey of previous literature work, Mongalo et al., (2018) reported a TFC of 23.92 ± 0.92 mg/g QE dry weight of aqueous extract of *S. panduriforme*. These was slightly higher compared to the results of this study. Mitali Ghosal & Palsh Mandal,

(2019) reported a TFC of 0.207 ± 0.09 mg/g QE dry weight of methanolic fruits extract of *S. incanum.* This was lower compared to the TFC reported in this study. Similarly, Nwanna et al., (2014) reported low TFC content of 1.50 ± 0.17 mg/g QE of *S. incanum* aqueous fruits extract.

Environmental stressors contribute to a variation in amount of TFC in plant. For example, increased in UV-radiation, pathogen attack, nutrient deficiency and high light results to increased phenylpropanoids which is a general pathway in plant for flavonoid synthesis (Canyon et al., 2013). According to Wang et al., (2014) seasonal changes such as, shifting of temperature from low to high using proper frequency as well as moderate drought are very beneficial for accumulation of flavonoids in plants. Low level of nitrogen and phosphorous contribute to increased content of flavonoid in plants which is correlated to reduced content of soluble protein (Ibrahim et al., 2011). Soil condition example nutrition stress and low amount water also contribute to difference in amount of flavonoids present in the plant (Borges et al., 2013).

4.4 GC-MS Results of *Solanum incanum*

A Shimadzu QP 2010-SE GC-MS was used. The total run rime was 44 minutes. Several compounds were detected and their fragmentation patterns and retention indexes compared to NIST 2014, MS library. The GC chromatogram was as follows in Figure 4.3 and their molecular weight, molecular formula, area, area%, retention time, and name of the compounds summarized in Table 4.2.

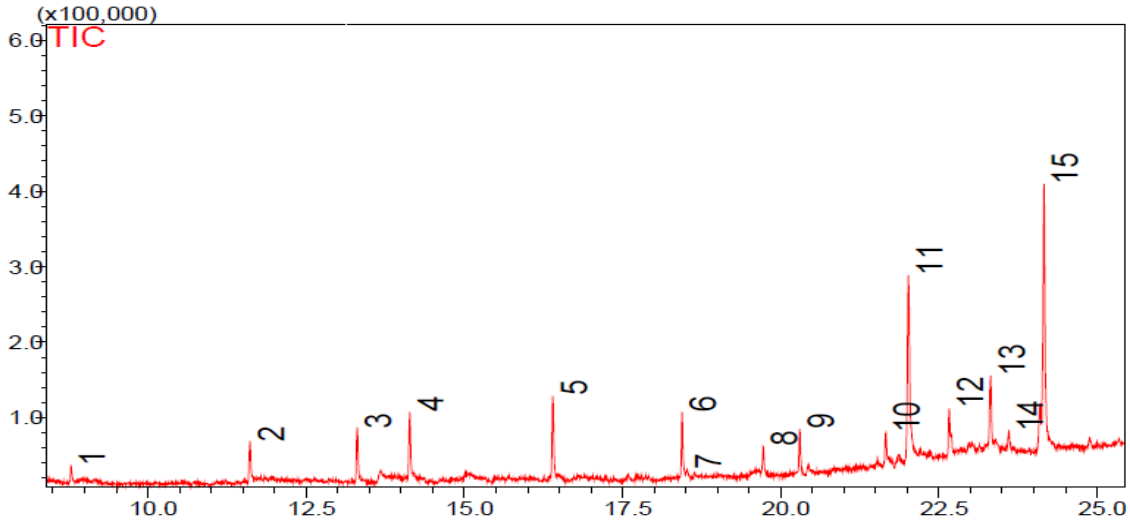


Figure 4.3: GC–MS peak report for *Solanum incanum* extract.

Table 4.2: A summary of information obtained from GC-MS showing compound names, chemical formulas, molecular weights and possible compounds based on compared NIST 2014 entries. GC retention time (RT) is also provided.

Peak #	R. time	Area	Area %	M.W	M.F	Name
1	8.782	36348	1.48	168	$C_{12}H_{24}$	1-Dodecene
2	11.611	115750	4.73	182	$C_{13}H_{26}$	1-Tridecene
3	13.307	121512	4.96	206	$C_{14}H_{22}O$	2,4-Di-tert-butylphenol
4	14.136	153861	6.28	238	$C_{16}H_{30}O$	E-14-Hexadecenal
5	16.397	178327	7.28	252	$C_{17}H_{32}O$	E-15-Heptadecenal
6	18.441	133601	5.45	354	$C_{24}H_{50}O$	n-Tetracosanol-1
7	18.519	6639	0.27	290	$C_{20}H_{34}O$	Thunbergol
8	19.722	57864	2.36	227	$C_{11}H_{17}NO_4$	1(2H)-Naphthalenone, octahydro-4a-hydroxy-7-methyl-4-nitro-,
9	20.300	91291	3.73	354	$C_{24}H_{50}O$	n-Tetracosanol-1
10	21.659	48905	2.00	192	$C_{11}H_{12}0_3$	Butanoic acid, 4-formylphenyl ester
11	22.018	524948	21.4	281	$C_{18}H_{35}NO$	9-Octadecenamide,

			3			(Z)-
12	22.663	133784	5.46	206	$C_{13}H_{18}O_2$	2,3-Dehydro-4-oxo-.beta.-ionol
13	23.315	152683	6.23	168	$C_{10}H_{16}O_2$	2H-Pyran-2-one, tetrahydro-6-(2-pentenyl)-, (Z)
14	23.606	36236	1.48	354	$C_{24}H_{50}O$	n-Tetracosanol-1
15	24.160	657531	26.85	282	$C_{19}H_{38}O$	(9E)-1-Methoxy-9-Octadecene
		2449280	100.00			

Key: M.W = Molecular Weight, M.F = Molecular Formula, R.Time = Retention Time,

GC-MS results showed that the extract was a complex mixture of 15 bioactive compounds; with some being present in trace amount. On the other hand, 1-methoxy-, 9-Octadecene (E) - (26.85%), 9-Octadecenamide, (Z) (21.43%), E-15-Heptadecenal (7.28%), E-14-Hexadecenal (6.28), n-Tetracosanol-1 (5.45%), 2,4-Di-tert-butylphenol (4.96%) were present in considerable amount. Each of identified structures are attached in the appendix 8.

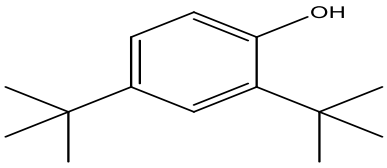
The compound 1-Dodecene is plant volatile compound which has been previously detected using GC-MS in *S. incanum*, *Oryza sativa*¸ *Ipomoea separia*, *Senecio coincyi*, *S. congestus* among other plants (Sakulnarmrat et al., 2018; Arrabal et al., 2011; Senthil et al., 2016). This compound has been reported to have antimicrobial and antioxidant activity (Abdelwahab et al., 2010). According to Senthil et al., (2016) 1-Dodecene has also found application in manufacture of detergents. 1-Tridecene is a plant metabolized fatty acid which has been reported to have antibacterial activity as well as use in treatment of respiratory irritation in human beings (Kumar et al., 2011; Shettar et al., 2017). It has been previously identified using GC-MS in Solanaceae species such as *Solanum spirale* and *S. incanum*

(Payum, 2020). Phenol, 2,4-di-tert-butyl- (2,4-DTBP) is a natural occurring lipophilic phenol which has been reported in about 169 species of organisms (Zhao et al., 2020). 2,4-DTBP has been reported to enhance disease resistance in *S. lycopersicum* (Passari et al., 2019). It has also been identified in *S. incanum* and *S. nigrum* (Arabia, 2017). The phenol has been reported to have antifungal, antibacterial, antioxidant, anti-Inflammatory, and antimalarial activity (Kusch et al., 2011; Sang & Kim, 2012).

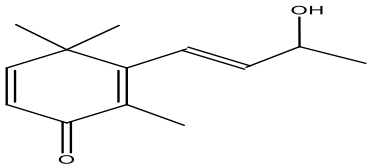
E-14-Hexadecenal and E-15-Heptadecenal are aldehydes which have been previously reported in solanum species such as *S. xanthocarpum* (Satyal et al., 2015). E-15-Heptadecenal have been reported to have antibacterial and antioxidant activity. The compound n-Tetracosanol is an alcoholic in nature and has been previously reported to have good antibacterial activity (Arockiasamy, 2014). N-Tetracosanol has been previously identified using GC-MS in *S. incanum* and *S. melongena* (Kalimuthu et al., 2016; Vambe, 2018; Boadu, 2018). Thunbergol belong to group of terpens with remarkable antioxidant activity and has previously reported in *Solanum dasyphyllum* (Oyinloye et al., 2020). 2,3-Dehydro-4-oxo-beta-ionol was previously reported in *Abutilion hirtum* (Gomaa et al., 2021). 2H-Pyran-2-one, tetrahydro-6-(2-pentenyl)-, (Z)- has been previously identified using GC-MS in *S. tuberosum* (Risticevic, 2012). 2H-Pyran-2-one, tetrahydro-6-(2-pentenyl)-, (Z)- has been previously used as precursor for organoleptic compounds especially for fragrance, flavour, masking agent as well as antimicrobial compound (Ch, 2002).

9-Octadecenamide is an amide derived from fatty acid oleic acid. It has a role as plant metabolite as well as human metabolite. The most recognized function of 9-Octadecenamide, (Z) is its sleep inducing effect as well as remedy for mood disorder and

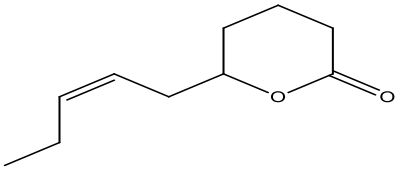
cannabinoid–regulated disorder (Naumoska et al., 2020). The compound has also been reported to have antibacterial and anti-inflammatory activity (Salah, Ali, & Imad, 2015). 9-Octadecenamide (Z) has previously been reported to be present in plants such as *S. torvum*, *S. xanthocarpum*, *S. nigrium* and *S. incanum* using GC-MS (Mohamed et al., 2010; Jasim et al., 2015). 1-methoxy-, 9-Octadecene (E)- is a plant hydrocarbon which has been previously identified using GCMS in plant such as *Terminalia catappa* and palm oil (Iheagwam et al., 2019; Nuylert et al., 2018).



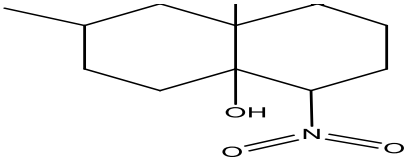
2,4-Di-tert-butylphenol



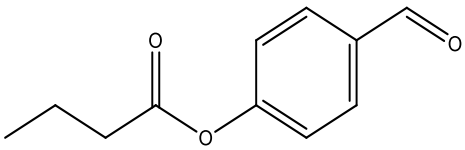
2,3-Dehydro-4-oxo-beta-ionol



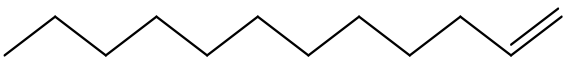
2H-Pyran-2-one, tetrahydro-6-(2-pentenyl)-, (Z)-



1(2H)-Naphthalenone, octahydro-4a-hydroxy-7-methyl-4-nitro-,



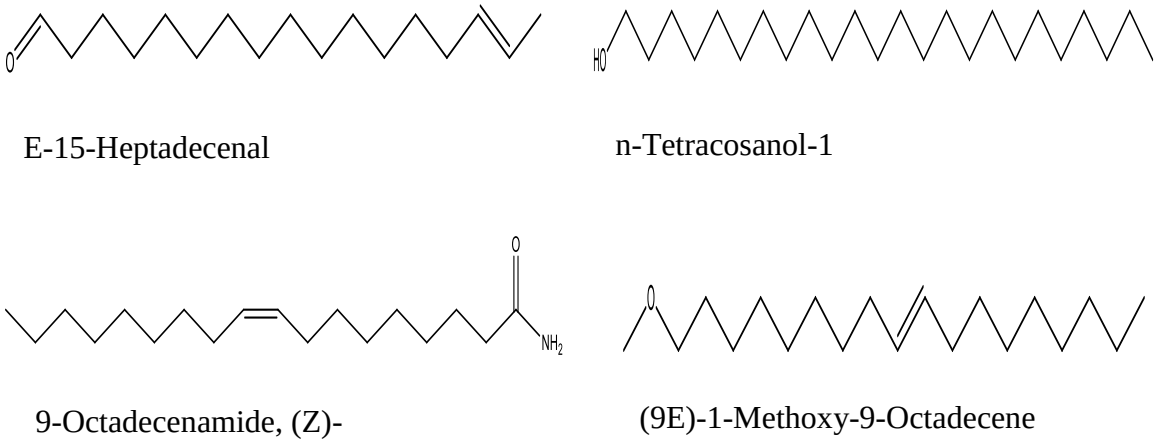
Butanoic acid, 4-formylphenyl ester



1-Dodecene

1-Tridecene

E-14-Hexadecenal



E-15-Heptadecenal

n-Tetracosanol-1

9-Octadecenamide, (Z)-

(9E)-1-Methoxy-9-Octadecene

Figure 4.4: Chemical structures of compounds identified using GC-MS in S. incanum fruits.

4.5 FT-IR Analysis of Crude Ethanol Extract of *Solanum incanum* fruits

The FT-IR spectrum (Figure 4.5) of the crude extract was used to identify the functional groups of the bioactive compounds present in *S. incanum* fruits. FT-IR analysis of the extract confirmed the presence of phenols, alkanes, aromatic, alkyl halide, alkene, primary and secondary amines and ethers. The description for the peaks assignment is given in Table 4.3. The peak at 3348 cm^{-1} corresponds to OH stretching in alcohol and phenol, 2931 cm^{-1} is attributed to C-H vibration of alkane groups. The peak at 1589 cm^{-1} is due to amides N-H characteristics of enzyme and protein, the peak at 1396 cm^{-1} represent C-O-H bending mode, peak at 1218 cm^{-1} is attributed to C-O stretching mode. The peak at 1110 cm^{-1} is assigned to alkyl substituted ether C-O stretch. The peak at 995 cm^{-1} is assigned to C-H out-of-plane bending mode, peaks at 702 cm^{-1}, 601 cm^{-1}, 524 cm^{-1}, and 493 cm^{-1} are attributed to alkyl halide (C-Cl). These functional groups are integral parts of most bioactive compounds such as tannins, flavonoids, polyphenol, alkaloids and terpenoids (Poojary et al., 2015).

Functional groups in *S. incanum* fruits makes them a potential source of therapeutic products such as for anti-cancer, anti-ulcers, headache, stomachache, and as source of antimicrobial compounds (Ralte et al., 2021).

Table 4.3: Functional groups observed on the FT-IR spectrum of crude extract.

S/N	Wave number (cm^{-1})	Functional group
1	3348	OH stretching in alcohol and phenol
2	2931	C–H vibration in alkane group
3	1589	Amides N-H
4	1396	C-O-H bending mode
5	1218	CO stretch
6	1110	Alkyl substituted ether C-O stretch (C-O-C)
7	1072	Carboxylic acid
8	995	N-H stretch vibration, primary and secondary amines
9	702	Chloro compounds (C-Cl stretching)
10	601	Iodo compounds (C-I stretching)
11	524 – 493	C–Cl stretching mode

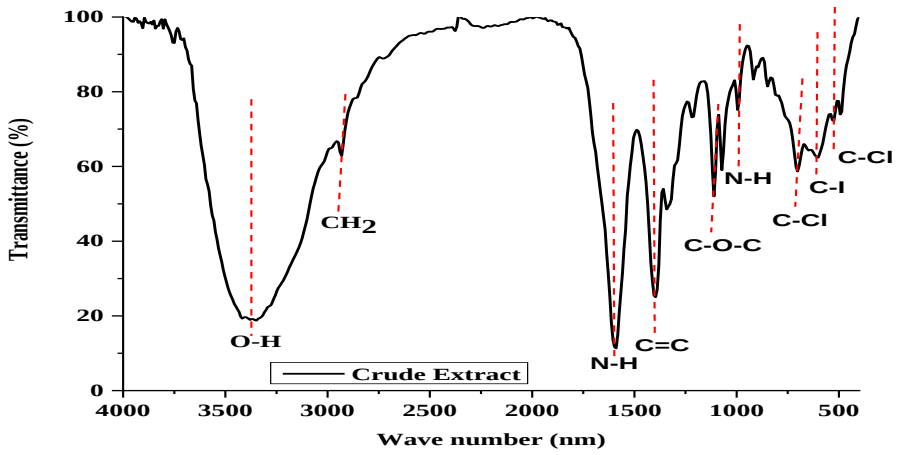


Figure 4.5: FT-IR spectrum of *S. incanum* fruits crude extract.

4.6 Correlation between FT-IR and GC-MS results

The functional groups of the identified compounds by GC-MS were given by the FT-IR spectrum. It is highly possible the presence of OH functional groups corresponded to 2,4-Di-tert-butylphenol, 2,3-Dehydro-4-oxo-beta-ionol, n-Tetracosanol-1, 1(2H)-Naphthalenone, octahydro-4a-hydroxy-7-methyl-4-nitro-, and Thunbergol identified by GC-MS as each of these compounds has hydroxyl group on their structure. 1-Tridecene and 1-Dodecene may have been the source of C=C stretching mode in the FT-IR spectra. Similarly, 2H-Pyran-2-one, tetrahydro-6-(2-pentenyl)-, (Z) and 1-methoxy-, 9-Octadecene (E)- may have been sources of CO stretch and Alkyl substituted ether C-O-C stretch. E-14-Hexadecenal and E-15-Heptadecenal corresponded to the presence of C=O functional groups while butanoic acid, 4-formylphenyl ester corresponded to carboxylic acid stretch.

N-H stretch vibration on the other hand may have come from 9-Octadecenamide, (Z)- due to presence of nitro group on its structure.

4.7 Isolation of Ralstonia solanacearum from Diseased Tuber

Ralstonia solanacearum was isolated from diseased potato tuber showing typical signs of bacterial wilt as seen in Figure 4.6. Such signs were: collection of bacterial ooze at tubers eyes which causes soil to attach to the secretions. Upon cutting the tuber, it showed a brownish discoloration of the vascular ring. Slight squeezing forced out a pus-like slime out of the ring.

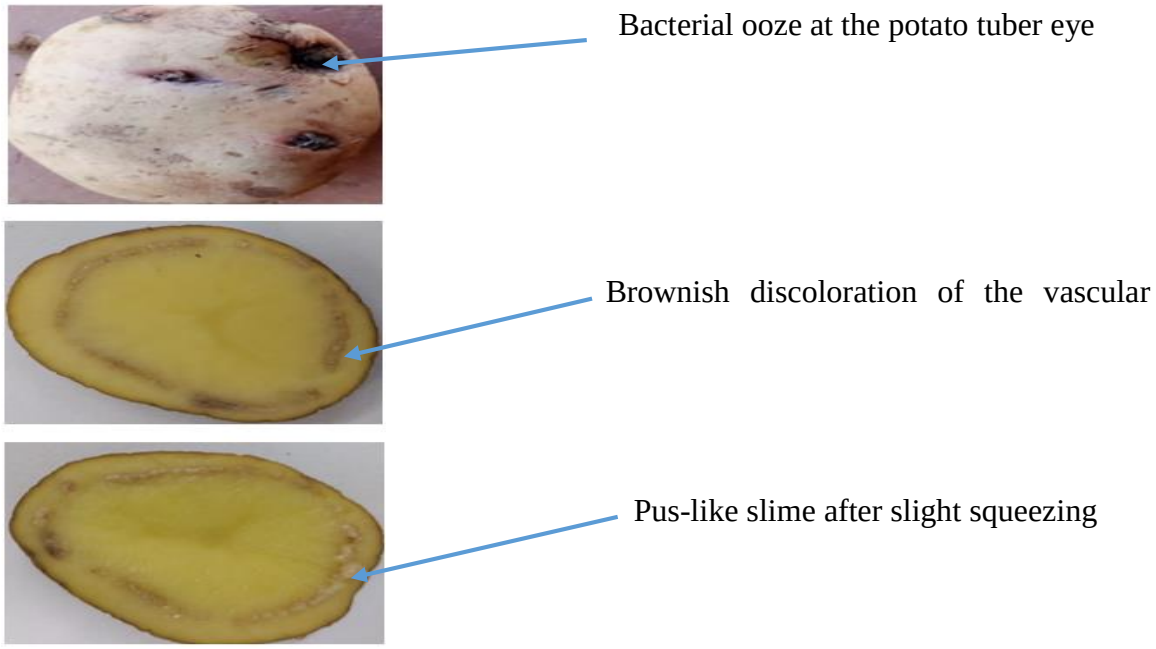


Figure 4.6: Symptoms of bacterial wilt in diseased potato tuber.

Isolation of the bacteria was done by plating bacterial exudate on Triphenyl Tetrazolium Chloride (TZC) medium. The colonies were characterized by deep red color on the TZC medium and dirty white on nutrient agar medium as seen in Figure 4.7. This results agreed

with that reported by (Pawaskar et al., 2014). According to Chaudhry & Rashid, (2011) TZC medium is used to differentiate *R. solanacearum* among other bacteria during isolation. Moreover, when it's used, virulent and avirulent colonies of *R. solanacearum* can be differentiated, where the avirulent are dark red while virulent are white with pink center (Figure 4.8) (Khasabulli et al., 2017). The bacterial culture on a nutrient agar were smooth raised and dirty white in color.

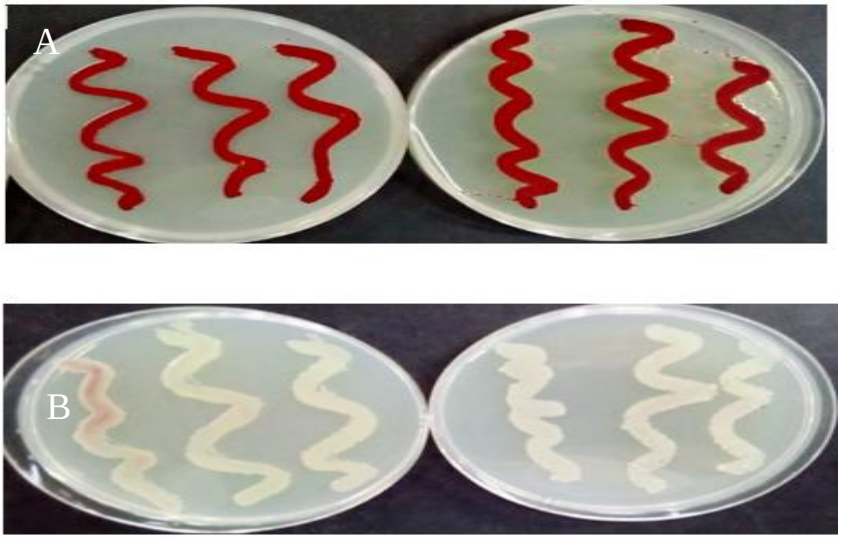


Figure 4.7: Appearance of *Ralstonia solanacearum* on TZC agar (A) and Nutrient agar (B).

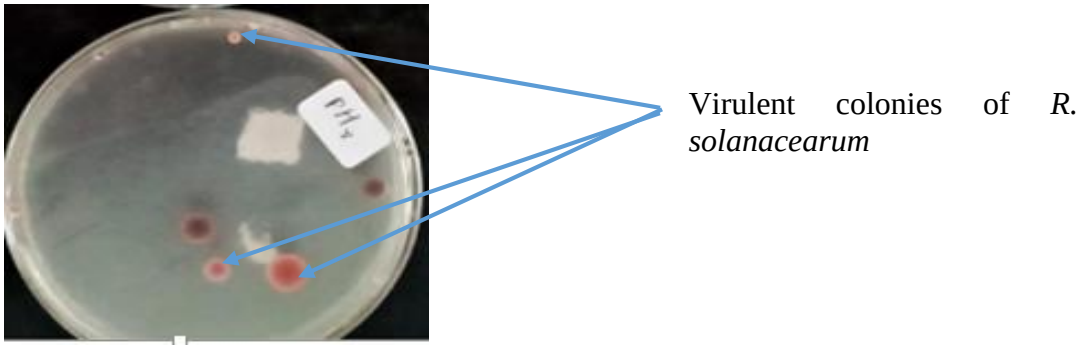


Figure 4.8: Appearance of virulent colonies of bacteria *R. solanacearum*.

4.8 Biochemical Profiling of *Ralstonia solanacearum*

Table 4.4 shows summarized observed biochemical characteristics of *R. solanacearum* isolated from potato tuber with typical signs of bacterial wilt. Gram staining, KOH test, starch hydrolysis, gas test, catalase oxidase test, and ooze test confirmed the biochemical characteristic of the isolated bacteria.

Table 4.4: Profiling of *Ralstonia solanacearum* isolated from diseased potato tuber.

Type of test performed	Observation	Inferences
Gram staining	➢ Retention of the red color of safranin ➢ Short curved rod	-
KOH test	➢ Slime thread a few centimeter from the glass slide	+
Gas production test	➢ Gas space and gas bubbles observed in the Durham's tubes	+
Catalase oxidase test	➢ Gas bubbles were observed with unaided eyes	+
Starch hydrolysis	➢ No clear zone at the margin	-
Ooze test	➢ Pus-like slime exude from tuber eye	+
Growth on TZC medium	➢ Deep red colonies	+

Key + = Positive reaction - = Negative reaction

4.8.1 Gram Staining of *Ralstonia solanacearum*

Figure 4.9 shows the morphology of *R. solanacearum* after gram staining observed under light microscope (Mgx 100). The bacteria *R. solanacearum* retained the reddish color of the counter stain i.e. safranin. This was an indication that the bacteria cells are gram negative in

reaction. They were also characterized by short curved rods, measuring 1.5–3.12µ X 0.25–2.5µ. These results are supported by findings of other researchers like Khasabulli et al., (2017), Pawaskar et al., (2014), Chaudhry & Rashid, (2011) who reported similar morphology and staining reaction of *R. solanacearum*. Most of the plant pathogenic bacteria are gram negative in reaction with an exception of Clavibacter and Streptomyces (Tancos et al., 2013; Polkade et al., 2016). Gram staining is a vital preliminary step for the identification and classification of bacteria isolated from any source (Thairu et al., 2014). Gram negative bacteria have thinner fragile cell wall which stain red to pink while gram positive bacteria have thick mesh-like cell wall composed of peptidoglycan that stain purple (Biosciences, 2012).

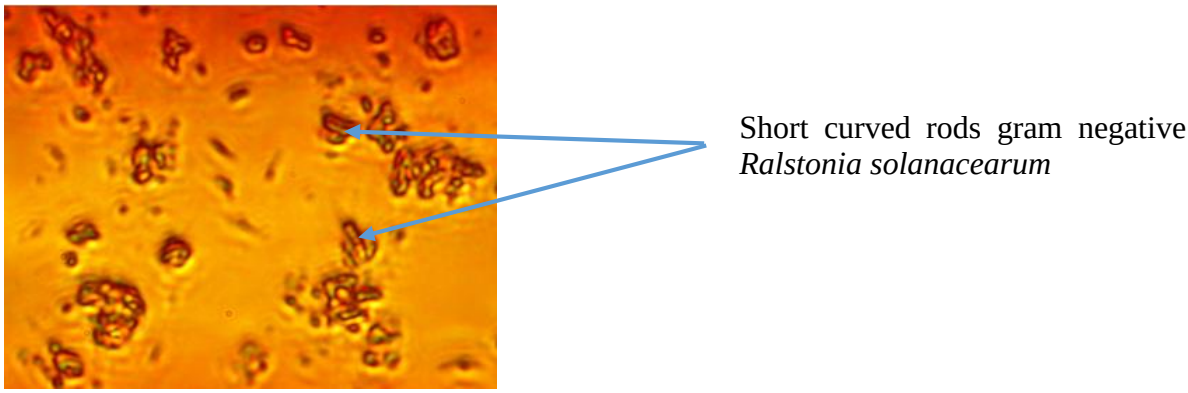


Figure 4.9: Appearance of *Ralstonia solanacearum* cells after gram staining.

4.8.2 KOH Test

Figure 4.10 shows a slime thread few centimeters from glass slide as observed when the sterile loop was raised from the bacterial solution. This has been proven to happen when *R. solanacearum* is treated with 3% KOH in studies by Pawaskar et al., (2014) and Chaudhry & Rashid, (2011). These results further compliment the gram-negative test and affirms that

gram-negative bacteria have an outer membrane that bounds fragile cell walls. Exposure of this outer membrane to 3% KOH results to its disruption thus releasing viscous Deoxyribonucleic Acid (DNA) (Ahmed et al., 2013). Therefore, slime threads are actually DNA and thus the test is lytic release of DNA.

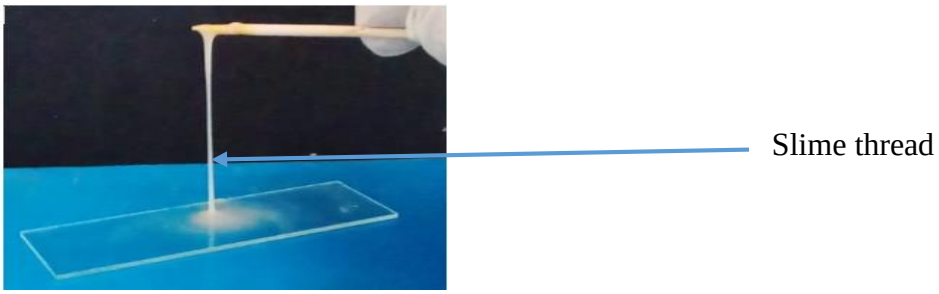


Figure 4.10: Slime thread formed after treating with 3% KOH for 10 seconds.

4.8.3 Catalase Oxidase Test

Figure 4.11 shows gas bubbles formed when the bacteria *R. solanacearum* was treated with a drop of 3% H_2O_2 that served as an indication that the bacteria was aerobic (Oljira & Berta 2020). This is because *Ralstonia solanacearum* have KatE a catalase hemi enzyme capable of decomposing hydrogen peroxide into water and oxygen (Tondo et al., 2020). Catalase enzyme is produced by microorganisms living in oxygenated environment to neutralizing the bactericidal effects of oxygen metabolites for example; hydrogen peroxide (Nandi et al., 2019). These results therefore confirm *Ralstonia solanacearum* as aerobic bacteria (Ślesak et al., 2016).

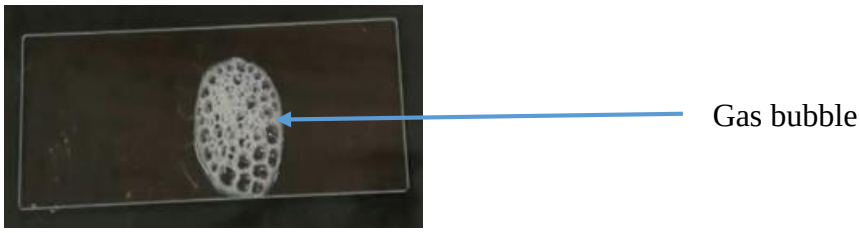


Figure 4.11: Gas bubbles formed after mixing the bacteria with H_2O_2.

4.8.4 Gas Production

Figure 4.12 shows gas space formed in the Durham's tubes after incubation of the bacteria in lactose broth medium for seven days. Pawaskar et al., (2014) and Khasabulli et al., (2017) reported similar results in gas production with nutrient broth containing 2% glucose. *Ralstonia solanacearum* have enzyme system which oxidize and utilize simple sugars and glucose resulting to production of gas (Chaudhry & Rashid, 2011). Different bacteria's have different enzyme system that results to different utilization of energy source in the medium (Ferdeş et al., 2020). The characteristics features of the enzyme production in the bacteria enables them to use diverse carbohydrates and this in turn aid in the identification of unknown bacteria (Khasabulli et al., 2017).

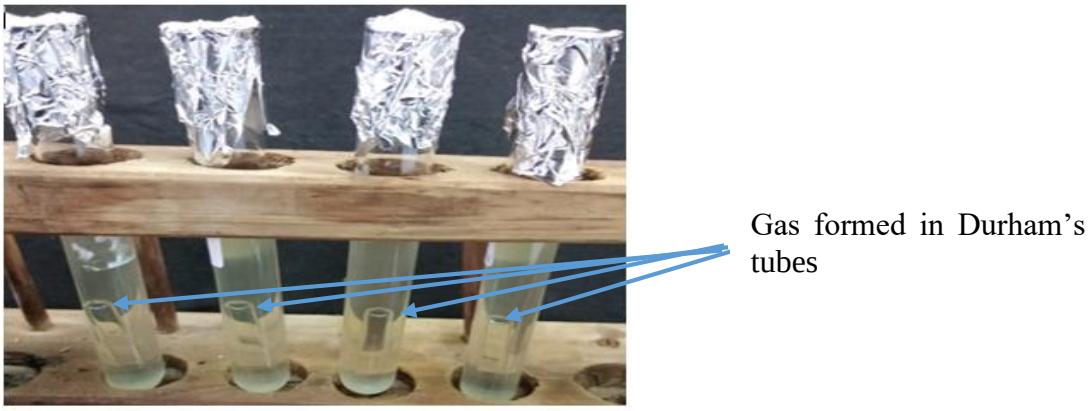


Figure 4.12: Image showing gas space in Durham's tubes.

4.8.5 Starch Hydrolysis

Figure 4.13 shows no clear zone at the margin of the bacteria after flooding the plate with Lugol's iodine solution. This suggests that *R. solanacearum* was incapable of hydrolyzing starch. This results were similar to that reported by (Khasabulli et al., 2017; Pawaskar et al., 2014; Sharma & Singh, 2019). This depicts that the bacteria did not produce exoenzymes i.e. amylase and oligo-1, 6-glucosidase which have the ability to degrade starch (Gopinath et al., 2017). Exoenzymes are secreted by some bacteria to degrade starch molecules into smaller subunits that can be utilized by the microorganism (Flint et al., 2012).

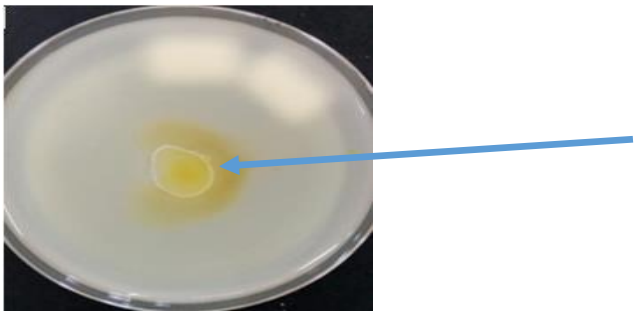


Figure 4.13: Image showing absence of clear zone at the margin.

4.9 Characterization of the Biochar

4.9.1 Effect of Temperature on Basic Biochar Characteristics

Basic characteristics of a biochar include ash content, pH, volatile content, moisture content, and fixed carbon content. Figure 4.14 presents the results of basic characteristics of the biochar prepared at two different pyrolysis temperature i.e. 300 ℃ and 500 ℃.

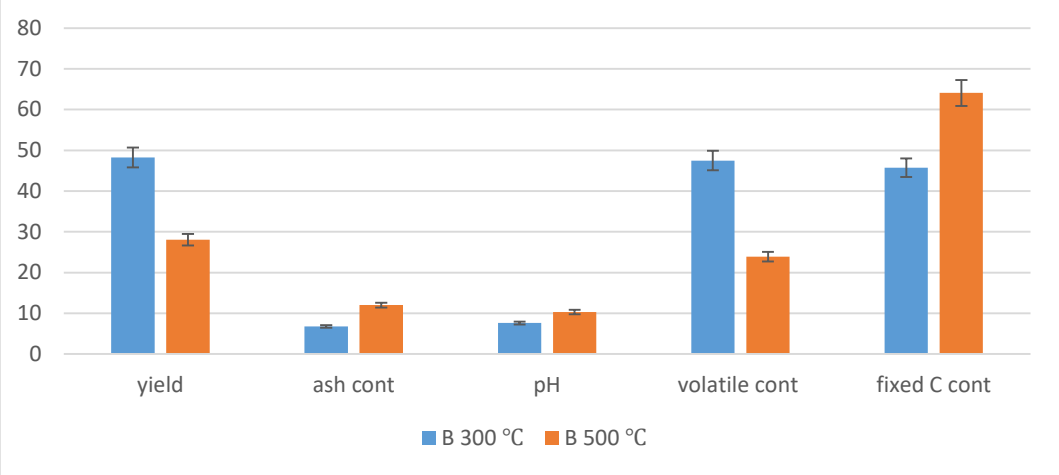


Figure 4.14: Basic characteristics of the biochars at different pyrolysis temperature.

Effect on Percentage Yield: At a pyrolysis temperature of 300 ℃ the percentage biochar yield was 48.26 ± 0.9 grams. At the pyrolysis temperature of 500 ℃, the biochar yield was 28.04 ± 0.3 grams. Biochar yield decreased by 20.22% as the pyrolysis temperature increased. This results were comparable to that in literature where the biochar yield decreased from 42% to 28% for biochar produced from natural plants matter at a temperature of 300 ℃ and 500 ℃ (Sun et al., 2017). This can be attributed to decomposition of lignocellulosisc materials in the biomass at high temperature (Zhao et al., 2017). At higher temperature rapid carbonization, condensation and biomass vaporization took place thus lowering yields (Liu et al., 2020).

Effect on Biochar pH: The pH of biochar prepared at 300 ℃ was 7.6 ± 0.0. The pH of the biochar prepared at 500 ℃ was 10.3 ± 0.0. As the pyrolysis temperature increased, pH of the biochar increased by 2.7. According to Mohd Hasan et al., (2019) pyrolysis temperature of 500 ℃ gives a biochar with a pH value around 10.0. Mohd Hasan et al., (2019) recorded that, increase in pyrolysis temperature leads to increased ash content which is alkaline in

nature thus the basicity of the biochar. Relative concentration of non-pyrolyzed organic content present in the biomass is also a contributor to the increase (Jindo et al., 2014). The core reason of alkaline pH in the biochar is the formation of carbonates and total base cations which are formed with increasing temperature (Ding et al., 2014). *Solanum incanum* is non-woody and most non-woody derived biochar are basic due to presence of salts such as chlorides of potassium, carbonates and calcium in ash (Mukome et al., 2013).

Effect on Biochar Ash Content: The ash content of the biochar prepared at 300 ℃ was 6.75 ± 0.4% while that of 500 ℃ was 12.00 ± 0.7%. These results depicts that ash content increased with increase in pyrolysis temperature. The increase of the ash content from 300 ℃ to 500 ℃ was by 5.25%. In a study by Elnour et al., (2019) biochar produced from palm feedstock showed an increase of ash content from 12.74 ± 0.02% to 16.21 ± 0.20% at temperature range from 300 ℃ and 500 ℃. According to Zhao et al., (2017) ash content increases as the temperature increase due to continuous concentration of inorganic components and combustion of organic matric residues.

Effect on Volatile Content: The volatile content (VC) of biochar prepared at 300 ℃ was 47.5 ± 1.3% while that for 500 ℃ was 23.9 ± 1.0%. Volatile content decreased by 23.6% as the pyrolysis temperature increased. This results closely agreed to that of biochar prepared from apple tree branches which had a volatile content of 60.77 ± 0.86% and 23.19 ± 0.34% at 300 ℃ and 500 ℃ respectively (Zhao et al., 2017). It is believed the volatile fractions were broken further into lower molecular weight gases and liquids as temperature increased (Ronsse et al., 2013). Moreover, higher temperature may have resulted into thermal degradation of lignin and dehydration of hydroxyl groups (Zhang et al., 2015).

The Fixed Carbon Content: The fixed carbon content of the biochar prepared at 300 ℃ was 45.75 ± 1.3%. Biochar prepared at 500 ℃ had a fixed carbon content of 64.1 ± 0.3%. These are supported by works of Zhao et al., (2017) who reported an increase in fixed carbon content from 32.50 ± 0.86% to 66.75 ± 0.28% as the pyrolysis temperature increased from 300 ℃ to 500 ℃. It has been shown that higher pyrolysis temperature leads to higher degree of polymerization resulting to a more condensed carbon structure in biochar (Domingues et al., 2017). Moreover, the presence of higher lignin content in the biomass increases carbonization thus higher carbon content (Wang et al., 2015).

Effect on Moisture Content: The moisture content of the two biochars prepared at 300 ℃ and 500 ℃ was 0.00. This was attributed to evaporation of moisture content in biochar during pyrolysis.

4.9.2 Fourier-Transform Infrared Analysis (FT-IR) of Biochars

4.9.2.1 Effect of Pyrolysis Temperature on Spectral Features of Biochar

The FT-IR spectra of the biochars prepared at two different pyrolysis temperature is presented in Figure 4.15. The description for the peaks assignment is given in Table 4.5. From the FT-IR spectra, the OH, CH, C=O and C-O-H functional groups were identified.

Spectral Features of OH Group: As the pyrolysis temperature increased from 300 ℃ to 500 ℃ the intensity of a broad band at the range of 3,675-3,000 cm^{-1} decreased. The OH peak maxima for the biochar prepared at 300 ℃ (B 300 ℃) was 3404 cm^{-1} while that of the biochar at 500 ℃ (B 500 ℃) was 3415 cm^{-1}. This showed shifting of the peaks as the

pyrolysis temperature increased. This suggest that OH groups are very unstable at higher temperature (Bavariani et al., 2019). According to Tomczyk et al., (2020) decrease in OH functional group is also attributed to increased dehydration of the biomass as pyrolysis temperature increased.

Spectral Features of C-H Group: C-H peak was exhibited at 2984 cm^{-1} (B 500 ℃) and at 2729 cm^{-1} at (B 300 ℃).

Spectral Features of C≡N stretching mode: A peak at 2363 cm^{-1} for B 300 ℃ shifted to 2369 cm^{-1} for the B 500 ℃. These peaks are attributed to C≡N stretching mode.

Spectral Features of aromatic C=C stretching: The peak at 1596 cm^{-1} is attributed to aromatic C=C stretching. The intensity of the peak decreased as the pyrolysis temperature increased. According to Claoston et al., (2014) decrease of the intensity of peak at 1596 cm^{-1} is attributed to degradation of phenolic and carboxylic acid in lignin as the pyrolysis temperature increased.

Spectral Features of C-O-H bending mode: The peaks at 1392 cm^{-1} (B 300 ℃) and at 1398 cm^{-1} (B 500 ℃) is attributed to C-O-H bending mode (Acemioğlu, 2019). The intensity of the peak decreased as the pyrolysis temperature increased. Similar results were reported by (Yuan et al., 2011).

Biomass nature was reflected by the presence of a peak around 1118 cm^{-1} in both biochars which was assigned to Si-O-Si (Zhu et al., 2012). Silica is well known to be the most vital

constituent for plant phytoliths as it prevent degradation of plant carbons (Nawaz et al., 2019).

The presence of hydroxyl and carboxyl functional groups in both biochars suggested that both biochars can be used as soil amendment and as potential adsorbent (Ambaye et al., 2020). This is because this are active-oxygen included functional groups which can act as electron transfer platform for example, being an electron donor or acceptor (Lee & Park, 2020). These functional groups have been reported to play as nucleophile or electrophile in the process of the reactions involved such as adsorption (Xiao et al., 2021).

Table 4.5: Functional groups observed in the FT-IR spectra of biochar prepared at 500 ℃ and 300 ℃.

Wave Number (cm^{-1})		**Characteristics vibration and functional groups**
B 500℃	**B 300 ℃**	
3415	3404	O-H stretching (hydrogen bonded hydroxyl group), indicates presence of phenols and alcohols
2984	2729	Aliphatic CH_2/-CH_3 stretching
2369	2363	C=O ketone group
1596	1596	Aromatic C=C and C=O stretching of conjugated ketones and quinones
1398	1392	C-O-H stretching of carboxylic group
1118	1118	Aromatic Si-O-Si stretching
736	736	Out-of-plan bending of aromatic C–H

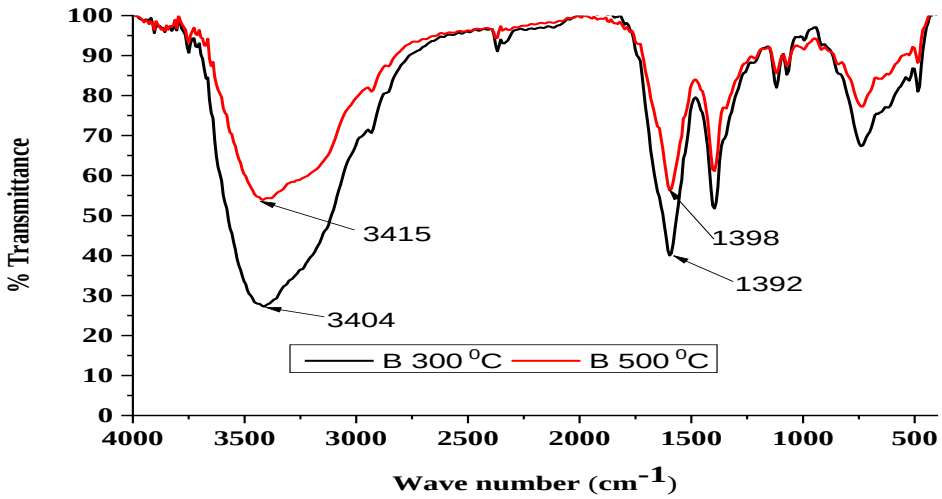


Figure 4.15: FT-IR spectra of biochar prepared at 300 ℃ (B 300 ℃) and 500 ℃ (B 500 ℃).

4.9.2.2 Effect of Activation on Spectral Features of Biochar

FT-IR Spectra of Activated Biochar Prepared at 300 ℃: An overlay of FT-IR spectral features of activated and non-activated biochar prepared at 300 ℃ is presented in Figure 4.16. The spectra of non-activated biochar prepared at 300 ℃ (B 300 ℃) was more intense at the band between 3,675-3,000 cm^{-1} compared to that of activated biochar (Actd B 300 ℃). This could be due to less prevalent O-H stretching as a result of carbonization and activation process (Saad et al., 2020). Weak peak associated with stretching vibrations of C-H bond at 2729 cm^{-1} on B 300 ℃ spectrum diminished on the activated biochar (Actd B 300 ℃). The intensity of the peak at 1596 cm^{-1} attributed to C=C stretching mode slightly increased. This was caused by the NaOH reactions results to the condensation of aromatic ring (Almeida et al., 2017).

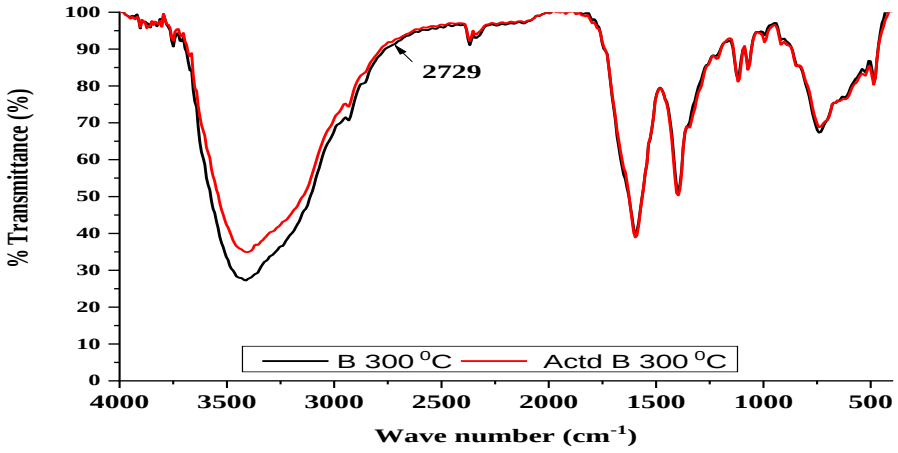


Figure 4.16: FT-IR spectra of non-activated (B 300 ℃) and activated biochar prepared at 300 ℃ (Actd B 300 ℃).

FT-IR spectra of activated biochar prepared at 500 ℃: Figure 4.17 present the FT-IR spectra of non-activated and activated biochar prepared at 500 ℃. Spectral features for the activated biochar prepared at 500 ℃ (Actd B 500 ℃) was less intense at the band 3675-3000 cm^{-1} than that of non-activated biochar (B 500 ℃). The results were similar to findings reported by Acemiogulu (2019) and are attributed to hydroxyl group provided by NaOH (Tayibi et al., 2021).

Weak peak associated with stretching vibrations of C-H bond of the methylene groups (-CH_2-) observed at 2984 cm^{-1} (B 500 ℃) diminished on the spectra of activated biochar. This was attributed to deformation of C-H bond to create aromatic C=C bond during activation process (Zhu et al., 2012). An increase in the intensity of the peak around 1596 cm^{-1} which is attributed to C=C stretching mode was observed on the spectrum of activated biochar (Actd B 500 ℃) and is also attributed to the condensation of aromatic ring likely due to the reaction with NaOH (Almeida et al., 2017). After activation, the intensity of a peak around

1400 cm^{-1} which denoted C-O-H bending mode showed a slight increase in intensity on spectrum of Actd B 500 ℃.

Arguably the activation of biochar is a method that increases physical characteristics and absorption properties of biochar as a result of increases in pore density and specific surface area of the biochar. The findings of this study suggested that both activated and non-activated biochar have similar functional groups which mostly remained intact during activation with NaOH (Bakhtiari et al., 2014).

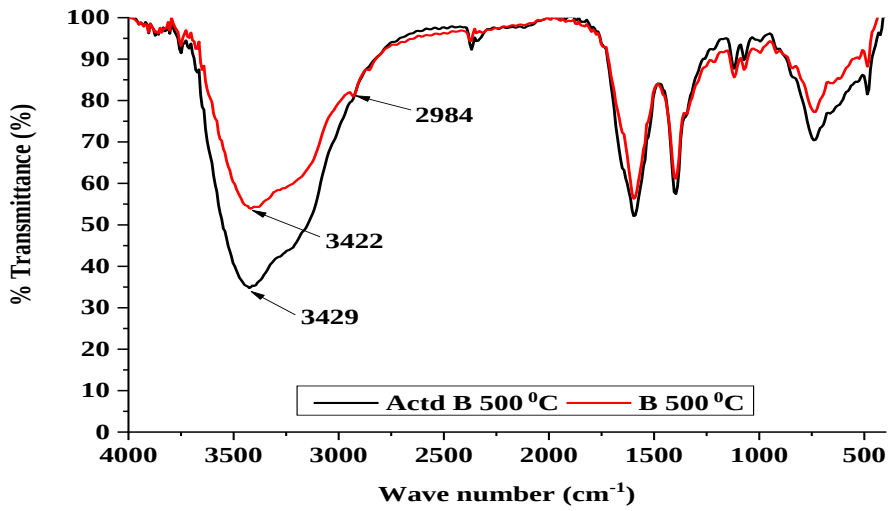


Figure 4.17: FT-IR spectra of non-activated (B 500 ℃) and activated biochar prepared at 500 ℃ (Actd B 500 ℃).

4.9.2.3 FT-IR spectra of biochar composite

Significant differences between the spectra of activated biochar and that of plant extract adsorbed biochar was observed.

FT-IR Spectra of Plant Extract Biochar Composite (BC 300 ℃): FT-IR spectrum of BC 300 ℃ is as shown in Figure 4.18. After the adsorption of the plant extract, the peak at 3404 cm^{-1} on Actd B 300 ℃ shifted to 3330 cm^{-1}. The peak is attributed to OH functional group. The intensity of the peak decreased which suggests that the plant extract was adsorbed onto the biochar. The shifting and decrease in the intensity of the OH peak is caused by the consumption of the hydroxyl group during the process of adsorption (Li et al., 2020). The intensity of the peaks at 1596 cm^{-1}, 1398 cm^{-1} and 1118 cm^{-1} increased. The peaks are attributed to C=C stretching mode, C-O-H bending mode, and C-O stretching mode respectively. The peak at 736 cm^{-1} in Actd B 300 ℃ shifted to 708 cm^{-1}. The changes of the functional group intensity and the shifting suggest that there was chemical interaction between the plant extract and the biochar.

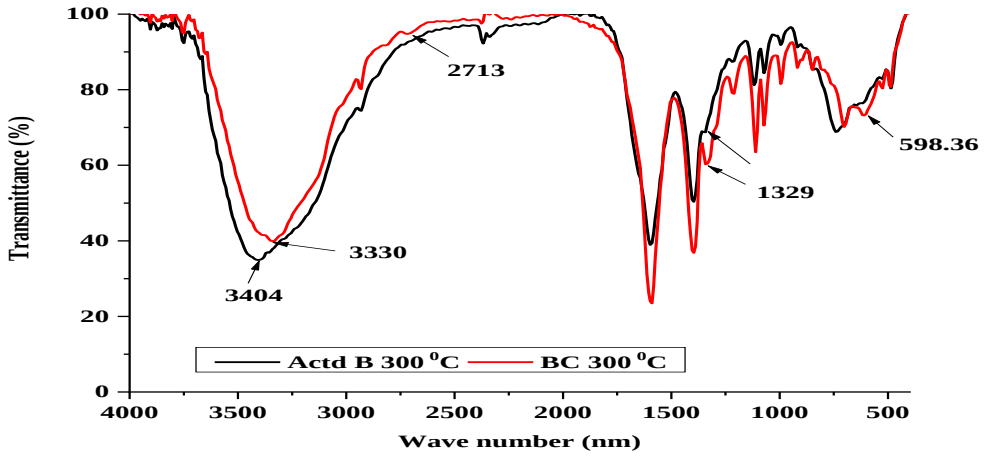


Figure 4.18: FT-IR spectra of activated biochar prepared at 300 ℃ (Actd B 300 ℃) and that of plant extract biochar composite BC 300 ℃.

FT-IR spectra of Plant Extract Biochar Composite (BC 500 ℃): The FT-IR spectrum of BC 500 ℃ is as shown in Figure 4.19. The peak at 3415 cm^{-1} on activated B 500 ℃ shifted to 3373 cm^{-1} on BC 500 ℃ spectrum. The peaks are attributed to OH group. The intensity of the peak increased after adsorption. New peaks were observed at 2936 cm^{-1} and 2713 cm^{-1} on the spectrum of BC 500 ℃. This may be as a result of C-H vibration in the plant extract. The intensity of the peaks at 1596 cm^{-1}, 1398 cm^{-1} and 1118 cm^{-1} increased. The peak at 741 cm^{-1} on the spectrum of Actd B 500 ℃ shifted to 702 cm^{-1} on the spectrum of BC 500 ℃. New peaks were also observed on the spectrum of BC 500 ℃ at 1336 cm^{-1}, 1215 cm^{-1} and 598 cm^{-1}. This could correspond to C-O-H bending mode, C-O stretch, and C-I stretch respectively present in the plant extract. The FT-IR results shows that chemical interaction may have occurred between the functional groups of the activated biochar and that of the plant extract.

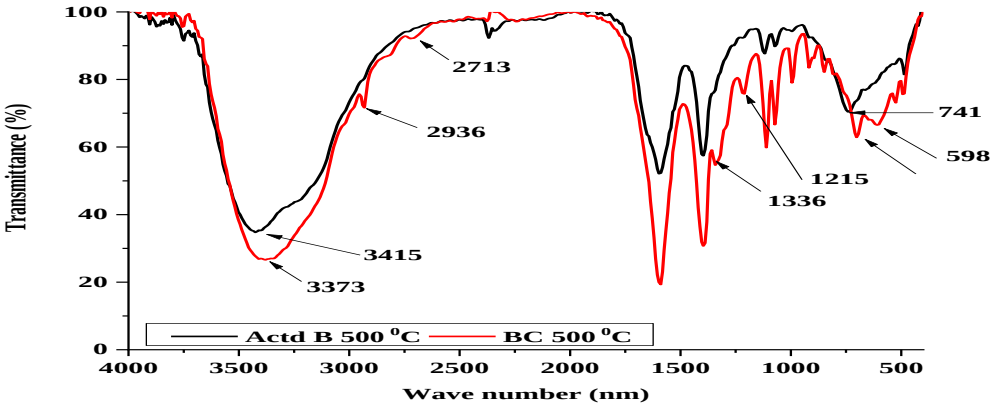


Figure 4.19: FT-IR spectra of activated biochar prepared at 500 ℃ (Actd B 500 ℃) and biochar composite BC 500 ℃.

4.9.2.4 FT-IR analysis of the soil Structure

The infrared spectroscopy of the sterile soil and soil treated with biochar composite (BC 500 ℃ and BC 300 ℃) is as shown in Figure 4.20 and Figure 4.21. Both sterile soil and soil treated with biochar composite spectrum shows peaks at 3381 cm^{-1}, 2937 $cm^{-1}$and 1591 cm^{-1} which are characteristics of soil humic compounds (Cox et al., 2000). The bands are assigned to OH, aliphatic C-H and aromatic C=C and/ or amide C=O respectively. The peaks at 1393 cm^{-1}, 1118 cm^{-1}, 1027 cm^{-1} and 703 cm^{-1} were assigned to aliphatic C-H, symmetric stretch vibrations of COO, Si-O stretch of silicate material and NH_2 stretch of primary amines respectively (Nuzzo et al., 2020; Dong et al., 2017).

Addition of biochar composite (BC 500 ℃) in the soil led to shift of peak at 3338 cm^{-1} to 3388 cm^{-1}. The shifting of the peak may be due to increase in concentration of hydroxyl group after the addition of biochar into the soil. The peak intensity slightly increased while the intensity of the peak at 1591 cm^{-1} slightly decreased. The peak at 1027 cm^{-1} shifted to 1070 cm^{-1}. A new peak occurred at 540 cm^{-1}. For the soil treated with biochar composite (BC 300 ℃) new peak was observed at 535 cm^{-1}.

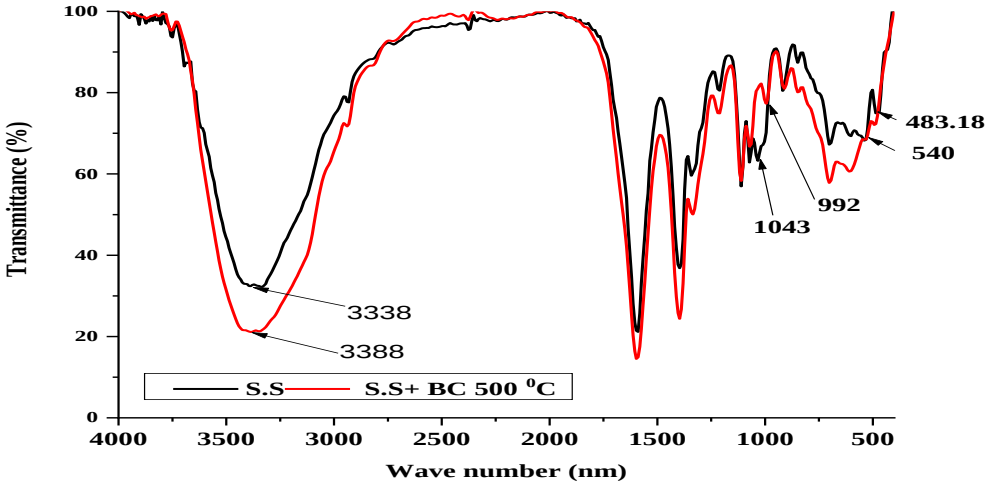


Figure 4.20: FT-IR spectra of sterile soil (S.S) and soil treated with biochar composite (S.S + BC 500 ℃).

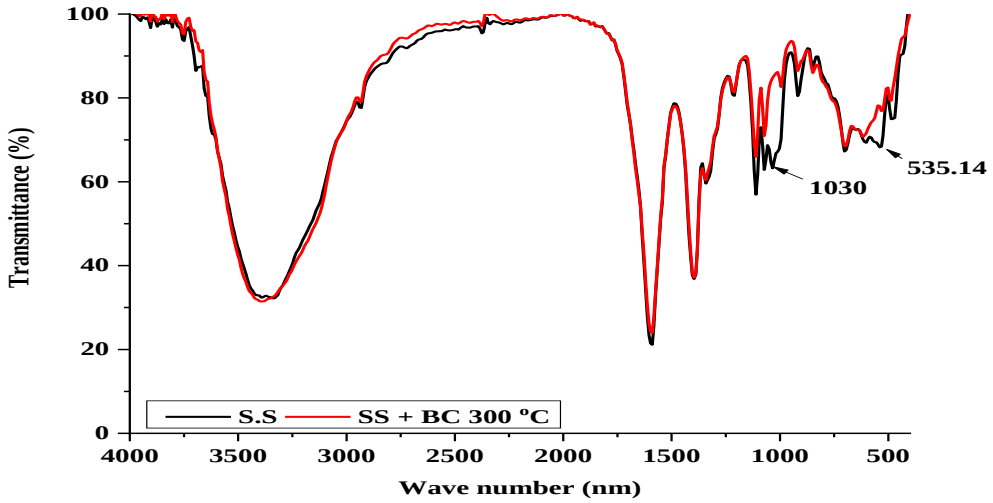


Figure 4.21: FT-IR spectra of sterile soil (S.S) and soil treated with biochar composite (S.S + BC 300 ℃).

4.9.3 Scanning Electron Microscopy (SEM) Analysis

4.9.3.1 SEM Analysis of Non-activated Biochar

Figure 4.22 shows the morphology of the biochars prepared at two different pyrolysis temperature i.e. 300 ℃ and 500 ℃. Non-activated biochar prepared at 300 ℃ (B 300 ℃) had fewer pores with large size while that prepared at 500 ℃ (B 500 ℃) had more pores with smaller sizes. This results are similar to work previously done by (Shaaban et al., 2013). Lianget al., (2016) also reported increased quantity of pores as pyrolysis temperature increased from 300 ℃ to 700 ℃ which changed the surface morphology of the particles. This is further backed by studies by Liu et al. (2020) that demonstrated that surface structure became looser and surface roughness of the biochar increases with increases in temperature.

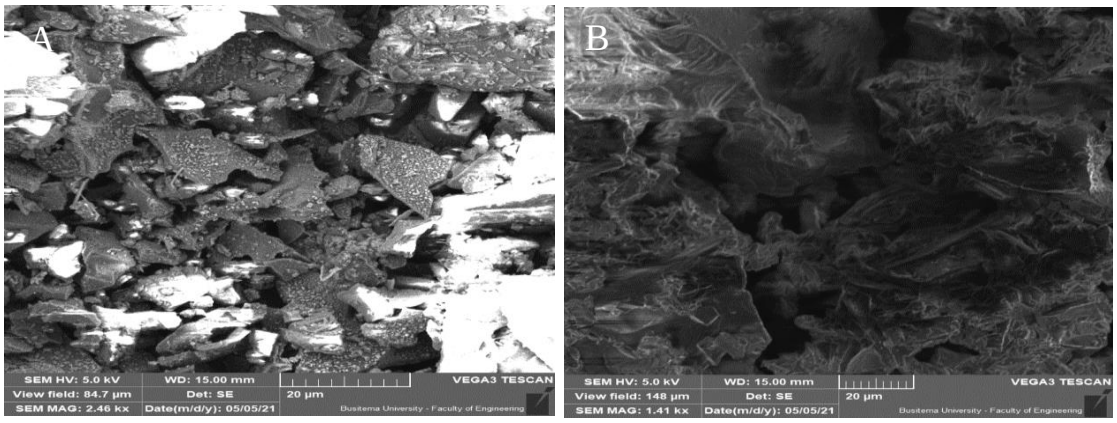


Figure 4.22: SEM images of biochar prepared at 500 ℃ (A) and 300 ℃ (B).

4.9.3.2 SEM Analysis of Activated Biochar

Figure 4.23 presents changes in morphological features after activation with NaOH. Generally, activated biochar gave different micro and mesopore concentration compared to

non-activated biochars. Activation of biochar with NaOH that led to removal of volatile carbon portion of carbonized precursors in the biochar matrix (Liu et al., 2015). Consequently, the specific surface area of activated biochar gets enhanced (Sakhiya et al., 2020).

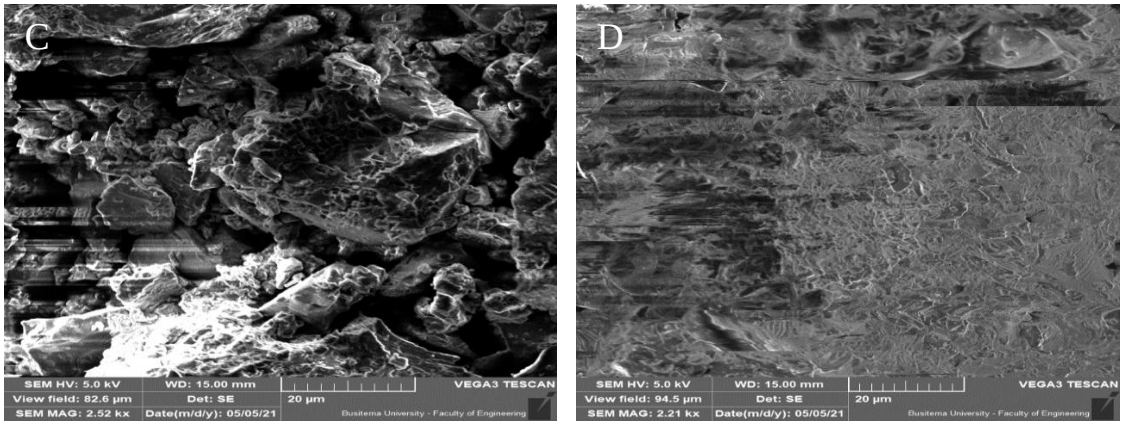


Figure 4.23: NaOH activated biochar Actd B 500 ℃ (C) and Actd B 300 ℃ (D).

4.9.3.3 SEM Analysis of Biochar Composite

SEM images after adsorption of the plant extract on the two biochars is as shown in Figure 4.24.

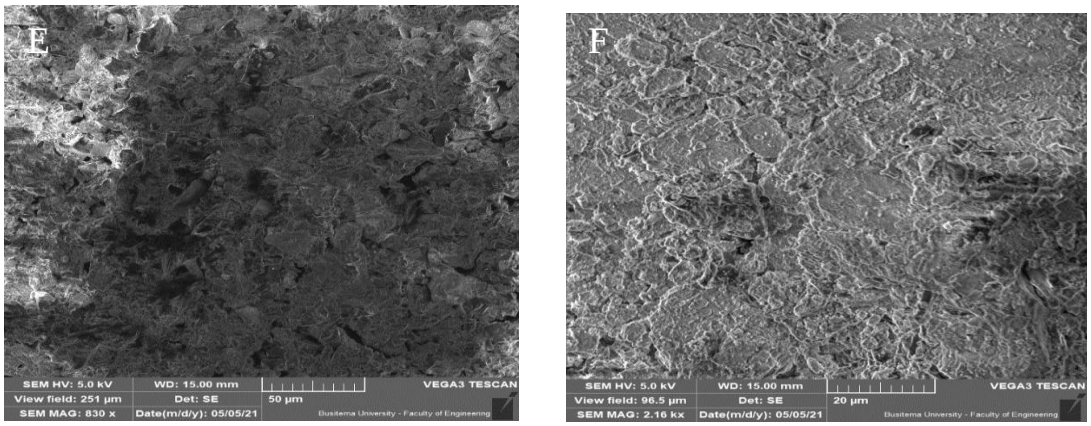


Figure 4.24: SEM images for biochar composite BC 500 ℃ (E) and BC 300 ℃ (F).

Image E and F shows that the plant extract was adsorbed on the surface of the biochar. Before adsorption SEM images of activated biochars (Figure 4.23 C and D) had a porous structure. The porous structure have pores which serves as cavity sites for the adsorption. In the images E and F (Figure 4.24) above the porous structures of the biochar have disappeared meaning that the plant extract was adsorbed on the surface of the biochars and embedded themselves into the pores.

4.9.3.4 SEM analysis of Soil Morphology After addition of Biochar Composite

Impact of biochar application into the soil was observed on the SEM micrograph as seen in Figure 4.25. Biochar being a porous material, when added to the soil it increases soil porosity (Adekiya et al., 2020). As seen in the SEM micrograph soil treated with Actd B 500 ℃ was more porous compared to that treated with Actd B 300 ℃. This could be attributed to increased porosity of the biochar as pyrolysis temperature increase (Tomczyk et al., 2020). The change in the porosity in biochar treated soil is as a result of rearrangement of soil particles as well as formation of macropores (Hseu et al., 2014). Similar results were reported by (Laird et al., 2010). According to Duarte et al., (2019) biochar increases soil micropores due to its high internal microporosity and thus reduces the average pore size of the soil. This enable the soil to hold water against gravity thus increased water retention (Mao et al., 2019).

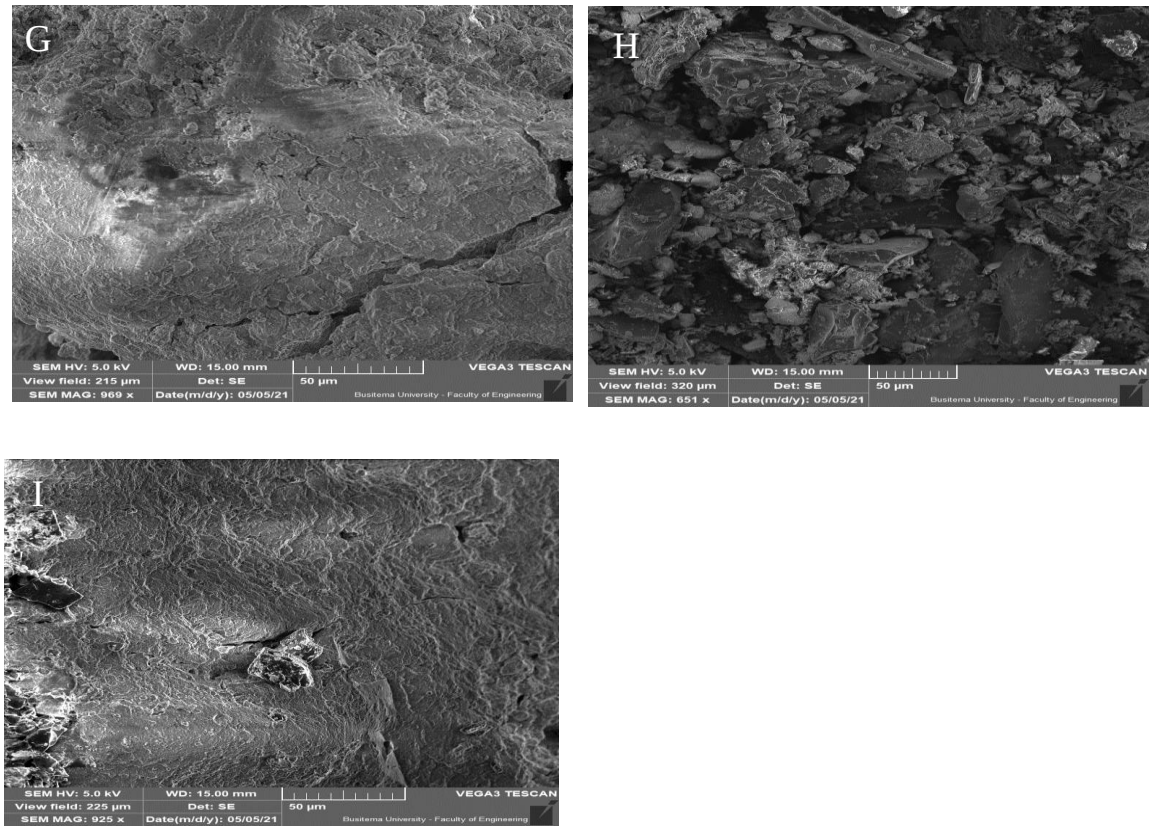


Figure 4.25: SEM image of sterile soil (G), soil treated with biochar composite (BC 500 ℃) (H) and BC 300 ℃ (I).

4.10 Effect of Biochar Contact Time on Adsorption of Plant Extract

Non-linear pseudo 1st order, pseudo second order and elovich kinetic models were used to analyze kinetics for the adsorption data of the experiment. Experimental data and calculated data were compared using statistical tests such as R Pearson, R^2_{adj}, R^2, chi-χ^2, MSE (Minimum Square Error) and RMSE (Root-Mean-Square deviation) as summarized in Table 4.6. This, was done using CAVS adsorption software.

For the biochar prepared at 300 ℃, the best model that fits the adsorption kinetics was Pseudo 1st order > Pseudo second order > elovich model. For the biochar prepared at 500

℃, the best model that fits the adsorption kinetics was Pseudo-second-order > Pseudo-first-order > elovich model. The order is based on statistical analysis where the kinetic model that has the highest value of R Pearson, R^2_{adj}, and R^2 and lowest values of chi-χ^2, MSE and RMSE is considered the best model that fits the experimental results best (Markandeya et al., 2015).

Contact time is a very critical operating parameter with an impact on adsorption of the adsorbate. Variation of adsorption capacity with variation of time is as shown in Figure 4.26 and Figure 4.27. From the dynamics of adsorption process, three stages were clearly observed. In the first stage, adsorption of phenolic compounds rapidly increased. This is probably due to availability of adsorption site as well as difference in concentration gradient between phenolic compounds and biochar surface (Huang et al., 2018). The second stage adsorption was comparatively slower compared to the first stage. The moderate rise was due to decrease of adsorption site with strong affinity. In the final stage there was equilibrium behavior of the adsorption process. This means that the adsorption sites were saturated (Lawal et al., 2021).

There was difference in the amount of phenolic compound adsorbed on the two biochars. More of phenolic compound (85.31 mg/g) was adsorbed on the biochar prepared at 500 ℃ than that prepared at 300 ℃ (30.52 mg/g). This is due to presence of more adsorption sites on the biochar prepared at 500 ℃ than that prepared at 300 ℃. As seen in Figure 4.10 C, biochar prepared at 500 ℃ is porous which is very beneficial in the adsorption process.

Table 4.6: Kinetic parameters and adsorption analysis for the adsorption of the phenolic compounds on BC 500 ℃ and 300 ℃.

	BC 500 ℃	BC 300℃
Pseudo-first-order		
K_f (h^{-1})	0.025541	0.019133
q_e	86.44641	32.17143
R Pearson	0.97033	0.987446
R^2	0.933914	0.966125
R^2_{adj}	0.915032	0.956447
X	3.629134	1.532436
RMSE	3.882672	1.368776
MSE	15.07514	1.873549
Pseudo-second-order		
K_s (g mg^{-1} h)	0.0003	0.000432
q_e	102.6998	41.53434
R Pearson	0.970303	0.971985
R^2	0.941204	0.937384
R^2_{adj}	0.924405	0.919494
X	2.350977	2.352756
RMSE	3.662252	1.860961
MSE	13.41209	3.463176
Elovich model		
α	6.656704	1.000943
β	0.045462	0.087531
R Pearson	0.961734	0.950818
R^2	0.924913	0.900998
R^2_{adj}	0.90346	0.872712
X	2.513091	3.29488
RMSE	4.138629	2.340002
MSE	17.12825	5.475609

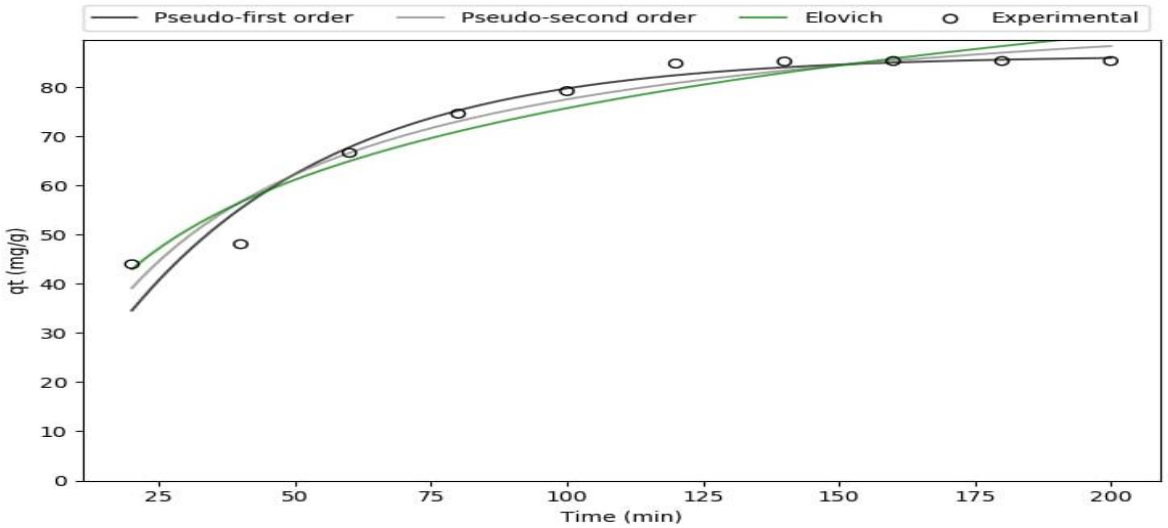


Figure 4.26: Kinetic modelling for the adsorption of plant extract onto the biochar (Actd B 500 ℃).

Figure 4.27: Kinetic modelling for the adsorption of plant extract onto the biochar (Actd B 300 ℃).

4.11 *In vitro* Antibacterial Activity of the Plant Extract

The ethanolic extract of *Solanum incanum* fruit produced auspicious results with the capability to deter the growth of *R. solanacearum.* A clear zone around the agar disk was observed. The diameter of the zones of inhibition (Appendix 1) depicts the relative vulnerability of the bacterium toward the antimicrobial agent. The results on antibacterial activity against *R. solanacearum* is presented in Table 4.7. The zones of inhibition yielded by varying concentration of ethanolic fruits extract of *S. incanum* against *R. solanacearum* was 20.75 ± 1.3 mm, 25.75 ± 0.5 mm, 27.25 ± 0.5 mm and 30.75 ± 0.5 mm at a concentration of 0.01, 0.05 0.10 and 0.15 g/10 ml respectively. This was significantly comparable (P< 0.05) to ampicillin which showed an inhibition zone of 26.75 ± 0.5 mm, 28.75 ± 0.5 mm, 31.75 ± 0.4 mm and 35.00 ± 0.0 mm at the aforementioned concentrations. Negative control (Ethanol) showed no inhibition. In a study by Aloo et al., (2019) aqueous flower extract of *S. incanum* showed an inhibition zone of 9.3 ± 0.0, 10.0 ± 0.0, and 10.0 ± 0.6 mm at a concentration of 0.01, 0.05, 0.15 g l^{-1} against *R. solanacearum* respectively. This was higher inhibition compared to that of positive control (amoxicillin) which had an inhibition zone of 9.9 ± 1.1 mm.

Most of the secondary metabolites are soluble in ethanol which may have contributed to the profounding activity of the extract (Indhumathi & Mohandass, 2014). Major antimicrobial compounds i.e alkaloids, tannins, saponins, phenols, terpenoids were reported to be present in the extract. GC-MS analysis identified presence of compounds such as E-15-Heptadecenal, 2,4-Di-tert-butylphenol, 9-Octadecenamide, (Z)-, Thunbergol, n-Tetracosanol, and 1-Dodecene (Figure 4.4) which have been previously reported to have

antibacterial activity. Compounds such as flavonoids are phenolic compounds which have been reported to have the capacity of coagulating bacteria cell proteins and affecting enzymes which are responsible for synthesis of amino acids in the bacteria (Panche et al., 2016). Tannins directly destroy the cell membrane resulting to death of the bacteria (Mainasara et al., 2012). Steroidal saponins hinders bacteria growth by reacting with membrane sterol and halting membrane function (Orczyk et al., 2020). Alkaloids acts upon DNA intercalating agent which sometime causes poisoning (Baikar & Malpathak, 2010). Terpenoids act upon phospholipids bilayer of cells affecting process like protein translocation, electron transport, phosphorylation steps and enzyme dependent reaction which eventually disrupt the cell membrane thus hindering growth of the bacteria (Din et al., 2016). It can be concluded that, the inhibition of the bacterial growth can be attributed to the presence of the stated phytochemicals in the ethanolic fruits extract of *S. incanum.*

Table 4.7: Mean zones of inhibition (mm) of ethanolic fruits extract, ampicillin and ethanol.

Treatment	Concentration	Mean zones of inhibition (mm)
Ethanolic fruits extract	0.01 g/10 mL	20.75 ± 1.3
	0.05 g/10 mL	25.75 ± 0.5
	0.10 g/10 mL	27.25 ± 0.5
	0.15 g/10 mL	30.75 ±0.5
Ampicillin (Positive control)	0.01 g/10 mL	26.75 ± 0.5
	0.05 g/10 ml	28.75 ± 0.5
	0.01 g/10 mL	31.75 ± 0.5
	0.15 g/10 mL	35.00 ± 0.0
Negative control	Ethanol	0.00

4.12 *In vitro* Antibacterial Activity of Biochar Composites

From the results obtained in this study, it was clear that as the concentration of biochar composite (BC) increased, the number of bacteria colonies decreased as shown in Figure 4.28. Decrease in the number of colonies was related with increase in duration with the highest decrease in the number of colonies observed at 60 minutes of exposure as shown in Figure 4.29. At a concentration of 1%, 2%, 3%, 4%, 5%, and 7% biochar composite (BC 300 ℃) the number of colonies observed on the agar plates were 91.33 ± 0.6, 83.0 ± 1.0, 72.33 ± 1.5, 58.0 ± 1.0, 49.0 ± 3.0 and 20.0 ± 2.0 respectively (Refer Appendix 3). This was significantly comparable ($P< 0.05$) to that of BC 500 ℃ with number of colonies being 76.0 ± 1.7, 64.67 ±2.1, 50.67 ± 2.1, 29.33 ± 1.5, 20.0 ± 2.0, and 4.0 ± 1.0 at the aforementioned concentrations respectively. This observation was similar to that reported by (Hu et al., 2019; Zeng et al., 2019).

The difference in antibacterial activity of two biochar composite may be as a result of increased specific surface area of the biochar with increasing pyrolysis temperature, pH, as well as number of surface charges (Jia et al., 2018). The pH of biochar prepared at 500 ℃ was 10.3 ± 0.0 while that of biochar prepared at 300 ℃ was 7.6 ± 0.0. *Ralstonia solanacearum* survive at an optimum pH ranging from 6.0-7.0 (He et al., 2014). The high pH for B 500 ℃ might have contributed to this observed activity. As shown in the SEM micrograph (Figure 4.23) porosity increased with increase in pyrolysis temperature thus opening the adsorption sites (Ambaye et al., 2020). Thus more plant extract was adsorbed on the biochar prepared at 500 ℃ which may also contribute to its high antibacterial activity.

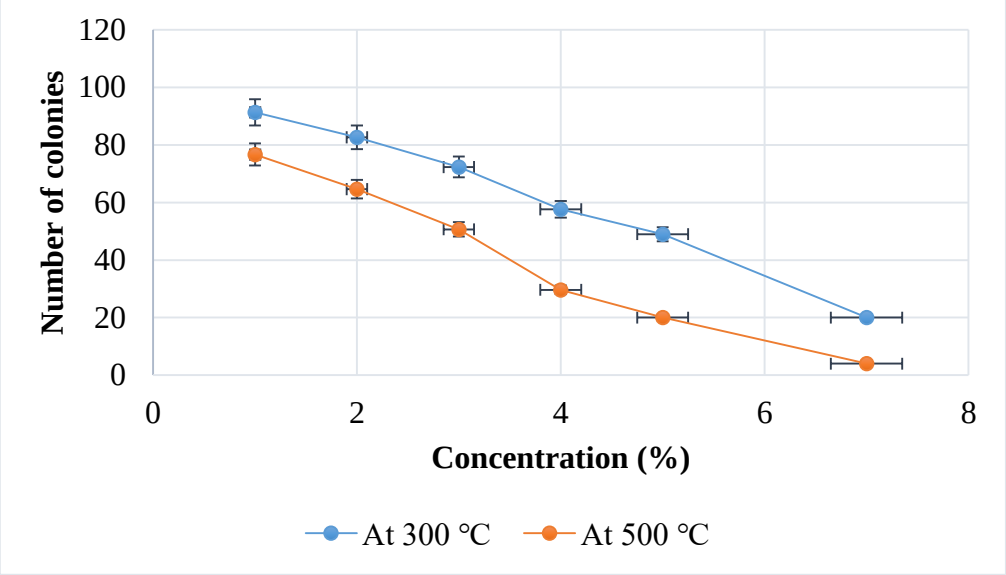


Figure 4.28: In vitro antibacterial activity of plant extract biochar composite.

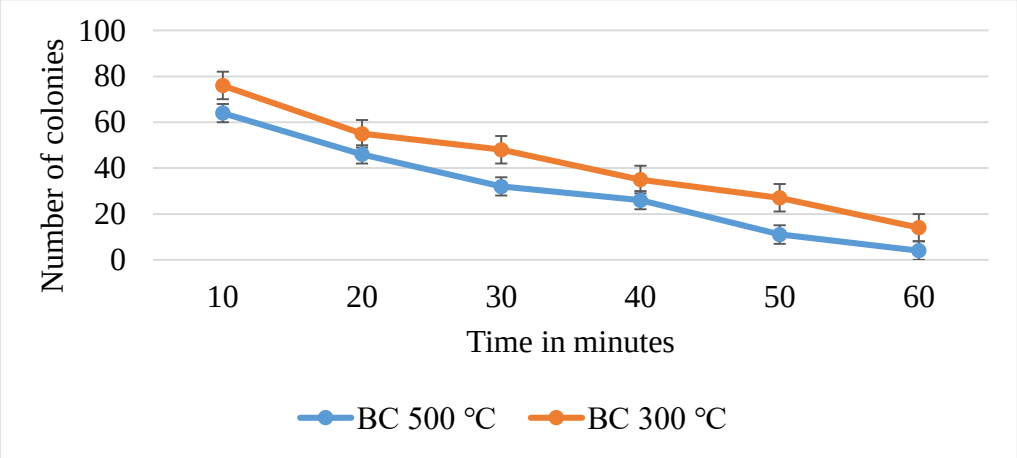


Figure 4.29: Effect of time on the antibacterial activity of biochar composites.

4.13 Effect of pH on the Growth of *R. solanacearum in vitro*

As shown in Figure 4.30, the highest growth of *R. solanacearum* was in the medium at a pH 6.0 (Refer Appendix 2). The medium with pH of 4.0, 5.0 and 8.0 significantly inhibited the growth of the bacteria. At a pH of 7.0 there was a moderate growth of the bacteria. This results are similar to that reported by (He et al., 2014). According to He et al., (2014) the

optimum pH for the growth of *R. solanacearum* is 6-7. In contrast, Li et al., (2017) reported that in acidic condition of pH 4.5, 5.0 and 5.5 the growth of *R. solanacearum* was favored. According to Chakraborty & Roy, (2016) lowering the pH to 4-5 in summer leads to eradication of the pathogen. Li et al., (2017) indicated that slightly acidic soil is the condition favorable for development of bacterium.

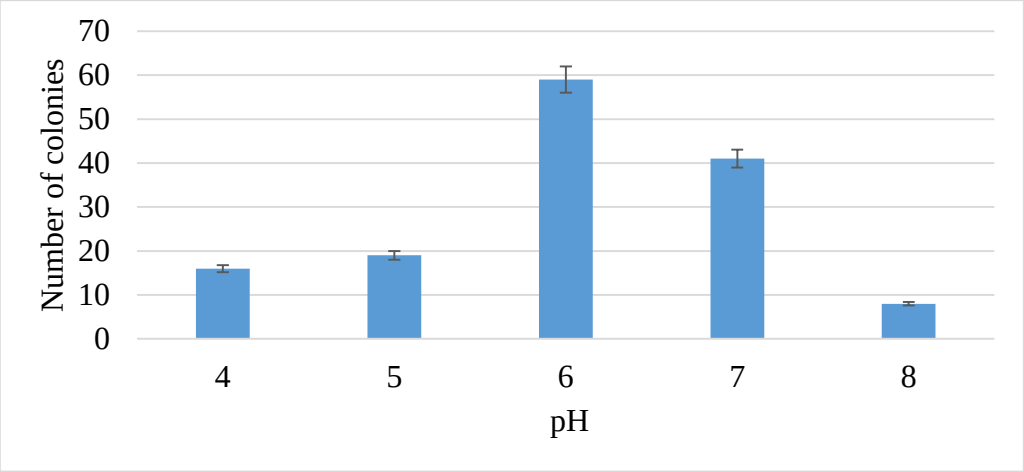


Figure 4.30: Effect of pH on the growth of *R. solanacearum*.

4.14 Effect of Plant Extract and Ampicillin on Disease Incidence

Bacterial wilt related symptoms were observed 4 days post inoculation in distilled water (negative control) treatment as seen in Figure 4.31. In contrast, these symptoms were not observed in plant extract and ampicillin treatment. This depicts that plant extract and ampicillin delayed the development of the pathogen. Even then, 8 days post inoculation (dpi) the disease incidence in plant extract (0.15 g) treatment was 20.0 ± 0.0% while no disease incidence was observed in ampicillin treatment. Twelve days post inoculation the percentage disease incidence was 20.0 ± 0.0% in ampicillin treatment and 40.0 ± 6.7% in plant extract treatment. Highest percentage diseases incidence for plant extract treatment was observed

16 days post-inoculation which was 100.0 ± 0.0%. This was significantly comparable (P< 0.05) to that of ampicillin which was 60.0 ± 0.0%. Twenty two days post-inoculation the disease incidence in ampicillin treatment was 80.0 ± 0.0%. Only one plant in each replicate survived in ampicillin treatment in the entire study. These results complemented that of *in vitro* studies since the activity of ampicillin was also slightly higher compared to that of the plant extract. Similar results have been previously reported by (K. A.M. Abo-Elyousr & Asran, 2009).

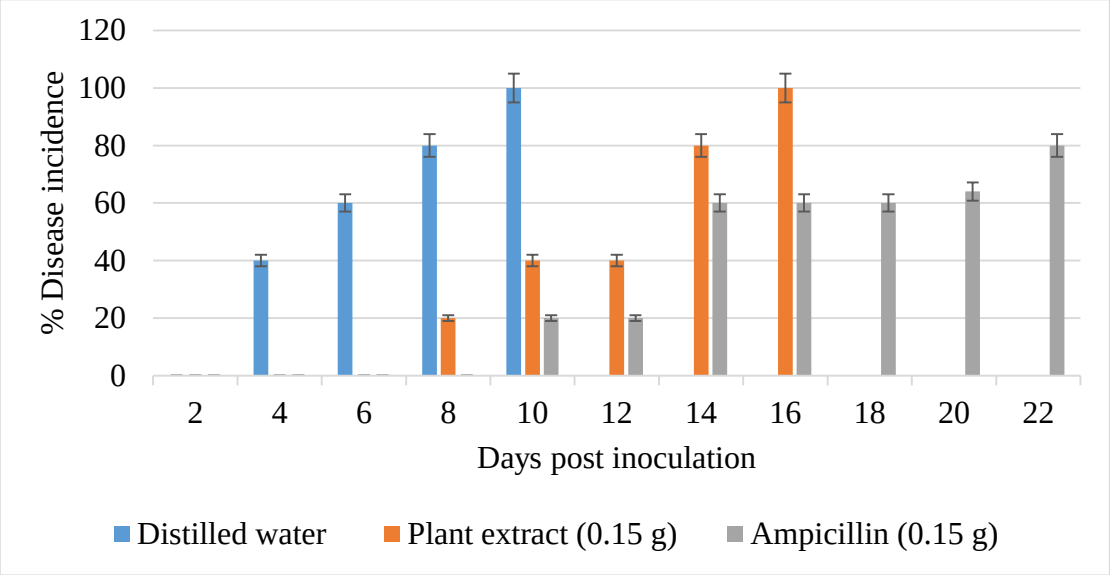


Figure 4.31: Suppressive impact of plant extract and that of ampicillin.

4.15 Effect of Biochar Treatment on Disease Incidence

Activated Biochar (Actd B 500 ℃ and Actd B 300 ℃) and plant extract biochar composite (BC 300 ℃ and BC 500 ℃) treatment significantly (P< 0.05) suppressed disease development and increased disease resistance in tomato plants.

4.15.1 Effect of Activated Biochar and Biochar Composite Prepared at 300 ºC on the Disease Incidence

Activated biochar (Actd B 300 ℃) and plant extract biochar composite (BC 300 ℃) significantly suppressed disease incidence compared to the control as seen in Figure 4.32

At a soil pH of 6.1 ± 0.2, the % disease incidence of Actd B 300 ℃, 1%, 2%, 3%, 4%, 5%, and 7% BC 300 ℃ was 80.0 ± 0.0%, 73.3 ± 0.0%, 64.5 ± 3.9%, 60.0 ± 0.0 %, 22.2 ± 3.9, 13.3 ± 6.7, and 0.0 ± 0.0% respectively, 30 days post inoculation. The highest disease incidence for 1% and 2% BC 300 ℃ was 80 ± 0.0% 32 days post inoculation. After 32 days no more diseases incidence was observed in Actd 300 ℃, 1% and 2% BC 300 ℃ treatment. Thirty four days post-inoculation the highest disease incidence was 80 ± 0.0%, 46.7 ± 6.7%, and 26.7 ± 0.0% for 3%, 4% and 5% BC 300 ℃ treatment respectively. No more plant death was observed after 34 days in the aforementioned treatment. For the 7% BC 300 ℃ treatment, the disease incidence was 6.7 ± 6.7% 36 days post inoculation. These depicts that 7% BC 300 ℃ significantly suppressed bacterial wilt to a greater extent. This is probably due to the synergistic effect of the plant extract and the biochar. Biochar (Actd B 300 ℃ and biochar composite) significantly increased the pH of the soil from 6.1 ± 0.2 to 6.6 ± 0.1. Presence of functional groups such as carbonyl and hydroxyl group might have contributed to the adsorption of root exudate thus disrupting the cross-talk between the pathogen and the plant. Presence of pores on the surface of the biochar (Figure 4.23 D) provide habitat for microorganism in the rhizosphere thus increasing competition leading to death of pathogens (Rawat et al., 2019).

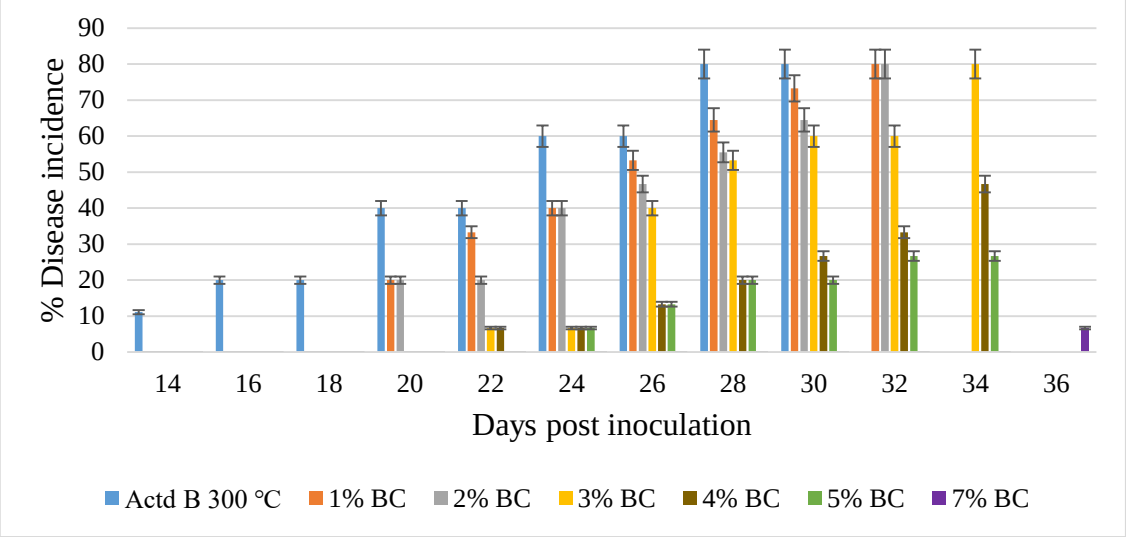


Figure 4.32: Effect of biochar (B 300 ℃) and biochar composite (BC 300 ℃) treatment on disease development at a pH of 6.1 ± 0.2.

4.15.2 Effect of Activated Biochar and Biochar Composite Prepared at 500 ℃ on the Disease Incidence

Activated biochar (Actd B 500 ℃) and biochar composite (BC 500 ℃) significantly suppressed bacterial wilt as seen in Figure 33 and 34. The disease incidence at soil pH of 6.1 ± 0.2 for Actd B 500 ℃, 1%, 2%, 3%, 4%, 5%, and 7% BC 500 ℃ was 66.7 ± 6.7%, 46.7 ± 0.0%, 46.7 ± 0.0%, 40 ± 0.0%, 13.3 ± 6.7%, 0.0 ± 0.0% and 0.0 ± 0.0% respectively, 30 dpi. Thirty four dpi the disease incidence for 1%, 2%, 3%, and 4% BC 500 ℃ was 64.5 ± 3.9%, 60.0 ± 0.0%, 46.7 ± 6.7%, and 20.0 ± 0.0% respectively. No more plant death was observed for the remaining days of the study. For 5% and 7% BC 500 ℃ treatment no disease incidence was observed in the entire study period (90 days). Further study was done on effect of BC 500 ℃ on bacterial wilt at a pH of 7.2 ± 0.2.

At a pH 7.2 ± 0.2, the highest % diseases incidence was 64.5 ± 3.9%, 60 ± 6.7%, 46.7 ± 6.7% and 22.2 ± 3.9% for Actd B 500 ℃, 1% BC, 2% BC and 3% BC 500℃ treatment respectively, 30 dpi. The percentage diseases incidence for 4%, 5% and 7% BC was 0.0 ± 0.0%, 30 days post-inoculation. No more plant death was observed after 30 days in all treatments. This results depicts that 4% BC 500 ℃ effectively reduced bacterial wilt to a greater extent and thus can be utilized as the optimal dose for application *in vivo* studies at pH of about 7.2 ± 0.2. Slightly acid soil is a condition favorable for development of the bacterium (S. Li et al., 2017). This may have contributed to higher percentage diseases incidence at pH of 6.1 ± 0.2 compared to that at pH of 7.2 ± 0.2.

Application of biochar into the soil significantly increases the pH of the soil. In this study the pH of the soil before addition of the biochar was 6.1 ± 0.2. Upon addition of the biochar Actd B 500 ℃ the pH increased to 7.2 ± 0.1. The soil pH at 7.2 ± 0.2 when treated with Actd B 500 ℃ and BC 500 ℃ the pH increased to 7.9 ± 0.1. Soil pH is one of the condition that affect survival of *R. solanacearum*. The bacteria is sensitive to high alkaline soils thus this is one of the factor that contributed to low diseases incidence (M. Zhang et al., 2019).

Activated charcoal has been reported to have notable ability to adsorb root exudates (Bais et al., 2005). Tomato root exudate contains compounds such as sugars, organic acids and amino acids (Astolfi et al., 2020) which has been reported to attract bacteria *R. solanacearum* toward the tomato plants (Yao & Allen, 2006). Biochar adsorb the root exudates disrupting the plant-pathogen cross talk which is a potential way of controlling bacterial wilt diseases in plants (Masiello et al., 2013.). Porous structure and large surface area are key physical properties of biochar that lead to increased adsorption (Gu et al., 2017). Activated biochar

prepared at 500 ℃ (Figure 23 C) is more porous compared to activated biochar prepared at 300 ℃ (Figure 23 D) thus increased adsorption sites.

Biochar has been proven to be a rich source of nutrients thus improves soil quality and increase soil nutrient thereby prompting plant growth (Rawat et al., 2019). Biochar is a rich source of calcium, carbon, phosphorous, nitrogen among other elements (S. Chen et al., 2020). Poor and unfertile soil are condition favorable for the development of bacterial wilt disease (Akkoc, 2019). Since biochar has been reported to improve soil and plant nutrition, soil quality as well as stimulate plant growth it has been utilized as a method of controlling bacterial wilt in many plant species belonging to family Solanaceae (Rawat et al., 2019).

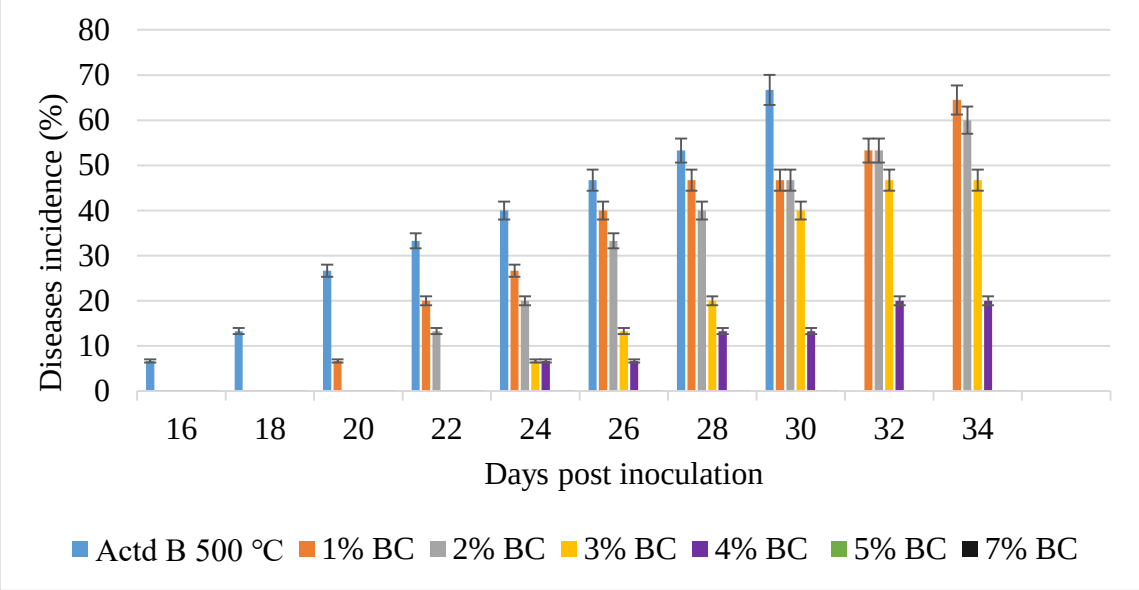


Figure 4.33: Effect of biochar (B 500 ℃) and biochar composite (BC 500 ℃) treatment on disease development at a pH of 6.1 ± 0.2.

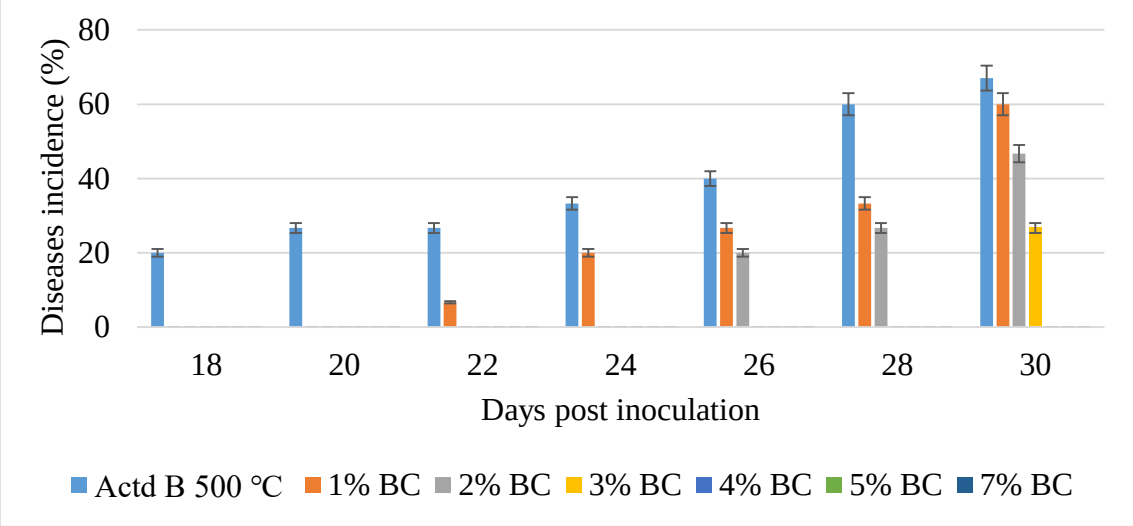


Figure 4.34: Effect of biochar (B 500 ℃) and biochar composite (BC 500 ℃) treatment on disease development at a pH of 7.3 ± 0.2.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Qualitative phytochemical screening of fruits extract of *S. incanum* showed the presence of alkaloids, tannins, glycosides, phenols, flavonoids, terpenoids, saponin, and steroids. GC-MS analysis identified a total of 15 compounds. Among the identified compounds, E-15-Heptadecenal, 2,4-Di-tert-butylphenol, 9-Octadecenamide, (Z)-, Thunbergol, n-Tetracosanol, and 1-Dodecene have been previously reported to have remarkable antibacterial activity. Presence of these compounds can be attributed to the observed antibacterial activity of the extract against *R. solanacearum* both *in vitro* and *in vivo* studies.

Isolated bacteria *R. solanacearum* were characterized by deep red color on the TZC medium and dirty white on nutrient agar medium. Avirulent colonies were dark red while virulent were white with pink center on TZC medium. Gram staining, KOH test, starch hydrolysis, gas test, catalase oxidase test, and ooze test confirmed the biochemical characteristic of the isolated bacteria.

Biochars derived from *S. incanum* fruits biomass showed differences in their physical, morphological and their functional groups. Biochar prepared at 300 ℃ had lower pH, ash content, and fixed carbon content and a higher volatile content. On the other hand, biochar prepared at 500 ℃ had a higher pH, ash content, and fixed carbon content while the volatile content was low. Biochar prepared at 500 ℃ was more porous as compared to that of 300 ℃. The intensity of the peaks differed as well as shifting as the pyrolysis temperature increased. New functional groups were observed on biochar composites spectra depicting

adsorption of the plant extract onto the biochars.

Activated biochar and plant extract biochar composites significantly reduced bacterial wilt disease incidence in tomato plants compared to ampicillin (positive control). Activated biochar prepared at 500 ℃ significantly suppressed bacterial wilt diseases to a greater extent compared to activated biochar prepared at 300 ℃. Biochar composites of the two biochars were more effective compared to activated biochars. Biochar composite for biochar prepared at 500 ℃ was more effective compared to biochar composite for biochar prepared at 300 ℃. This suggested that biochar composite for the biochar prepared at 500 ℃ is more effective in control of bacterial wilt. The antibacterial activity of the biochar composite could be therefore as a result of synergistic effect between the plant extract and the biochar.

5.2 Recommendation

i. Isolation of individual bioactive compounds and assessment of their antibacterial activity against *R. solanacearum*.

ii. Distinguish further and determine elemental composition of the biochar using instruments such as Transmission Electron Microscope (TEM), X-ray Diffraction (XRD), Total Organic Carbon (TOC) analyzer among others

iii. Identification and confirmation of the biovar and race of the bacterium using techniques such as polymerase chain reaction (PCR).

iv. Determine the effective cycle at which to apply the plant extract, biochar and biochar composite in attempt to control bacterial wilt.

REFERENCES

Abdelwahab, S. I., Zaman, F. Q., Mariod, A. A., Yaacob, M., Ahmed Abdelmageed, A. H., & Khamis, S. (2010). Chemical composition, antioxidant and antibacterial properties of the essential oils of Etlingera elatior and Cinnamomum pubescens Kochummen. *Journal of the Science of Food and Agriculture*, *90*(15), 2682–2688. https://doi.org/10.1002/jsfa.4140

Abo-Elyousr, K. A.M., & Asran, M. R. (2009). Antibacterial activity of certain plant extracts against bacterial wilt of tomato. *Archives of Phytopathology and Plant Protection*, *42*(6), 573–578. https://doi.org/10.1080/03235400701284740

Abo-Elyousr, Kamal A.M., Seleim, M. E. A., Abd-El-Moneem, K. M. H., & Saead, F. A. (2014). Integrated effect of Glomus mosseae and selected plant oils on the control of bacterial wilt disease of tomato. *Crop Protection*, *66*, 67–71. https://doi.org/10.1016/j.cropro.2014.07.022

Acemioğlu, B. (2019). Removal of a reactive dye using NaOH-activated biochar prepared from peanut shell by pyrolysis process. *International Journal of Coal Preparation and Utilization*, *0*(0), 1–23. https://doi.org/10.1080/19392699.2019.1644326

Adekiya, A. O., Agbede, T. M., Olayanju, A., Ejue, W. S., Adekanye, T. A., Adenusi, T. T., & Ayeni, J. F. (2020). Effect of Biochar on Soil Properties, Soil Loss, and Cocoyam Yield on a Tropical Sandy Loam Alfisol. *Scientific World Journal*, *2020*. https://doi.org/10.1155/2020/9391630

Ahmed, N. N., Islam, M. R., Hossain, M. A., Meah, M. B., & Hossain, M. M. (2013). Determination of Races and Biovars of Ralstonia solanacearum Causing Bacterial Wilt Disease of Potato. *Journal of Agricultural Science*, *5*(6), 86–93. https://doi.org/10.5539/jas.v5n6p86

Ahmed, N. U., Mahmud, N. U., Zaman, M. A. U., Ferdous, Z., & Halder, S. C. (2017). Effect of different salinity level on tomato (Lycopersicon esculentum) production under climate change condition in Bangladesh. *Annual Research and Review in Biology*,

13(3), 1–9. https://doi.org/10.9734/ARRB/2017/33613

Akkoç, B. Kirigo P. Mwaniki. (2019). Status of potato bacterial wilt in nakuru county (Kenya) and its management through crop rotation and soil amendments, *8*(5), 55.

Al Sinani, S. S., Eltayeb, E. A., Kamal, Y. T., Khan, M. S., & Ahmad, S. (2016). Variations in the cytotoxic glycoalkaloids solamargine and solasonine in different parts of the Solanum incanum plant during its growth and development in Oman . *Journal of Taibah University for Science*, *10*(6), 813–822. https://doi.org/10.1016/j.jtusci.2014.11.013

Almeida, L. C., Barbosa, A. S., Fricks, A. T., Freitas, L. S., Lima, Á. S., & Soares, C. M. F. (2017). Use of conventional or non-conventional treatments of biochar for lipase immobilization. *Process Biochemistry*, *61*, 124–129. https://doi.org/10.1016/j.procbio.2017.06.020

Aloo, B. N., Oluko, P., & Wanjohi, B. (2019). In vitro analysis of antimicrobial and phytochemical properties of crude extracts of selected plants against the tomato wilt disease. *African Crop Science Journal*, *27*(3), 479. https://doi.org/10.4314/acsj.v27i3.11

Aloyce, A., Ndakidemi, P. A., & Mbega, E. R. (2017). Identification and management challenges associated with Ralstonia solanacearum (Smith), causal agent of bacterial wilt disease of tomato in Sub-Saharan Africa. *Pakistan Journal of Biological Sciences*, *20*(11), 530–542. https://doi.org/10.3923/pjbs.2017.530.542

Álvarez, B., & Biosca, E. G. (2017). Bacteriophage-based bacterial wilt biocontrol for an environmentally sustainable agriculture. *Frontiers in Plant Science*, *8*(July), 1–7. https://doi.org/10.3389/fpls.2017.01218

Álvarez, B., Biosca, E. G., & López, M. M. (2010). On the life of Ralstonia solanacearum, a destructive bacterial plant pathogen. *Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, (January), 267–279. Retrieved from https://pdfs.semanticscholar.org/aa85/77e213e2977a0e4eb739795e3fea51187181.pdf

?_ga=2.156465595.1055873898.1504096691-1311319012.1504096691

Ambaye, T. G., Vaccari, M., van Hullebusch, E. D., Amrane, A., & Rtimi, S. (2020). Mechanisms and adsorption capacities of biochar for the removal of organic and inorganic pollutants from industrial wastewater. *International Journal of Environmental Science and Technology*. https://doi.org/10.1007/s13762-020-03060-w

An, S. Q., Potnis, N., Dow, M., Vorhölter, F. J., He, Y. Q., Becker, A., … Tang, J. L. (2019). Mechanistic insights into host adaptation, virulence and epidemiology of the phytopathogen Xanthomonas. *FEMS Microbiology Reviews*, *44*(1), 1–32. https://doi.org/10.1093/femsre/fuz024

Antimicrobial activity and phytochemical screening of Solanum incanum fruit extract against clinical samples of Staphylococcus aureus collecting from Nakuru Provincial General Hospital Laboratory , Kenya. (2017). *2*(September), 1–8.

Anwar, S. (2018). Pharmacological investigation of solanum incanum against P. falciparum, L. infantum, T. cruzi and T. brucei : A role of antioxidant effect and clinical overview. *Biomedical and Pharmacology Journal*, *11*(2), 653–660. https://doi.org/10.13005/bpj/1418

Arabia, W. S. (2017). *Systematics Implications of GC-MS Analysis of Secondary Metabolites in the Ethanol Extract of*. *57*(2), 1–15.

Arockiasamy, S. (2014). GC-MS analysis of chloroform extract of croton bonplandianum kalaivani . m . kuppuswamy1 , bhavana jonnalagadda2 and sumathy. (April).

Arrabal, C., Martínez García, F., Paz Arraiza, M., & Guerrero García, S. (2011). Chemical composition of essential oil of Senecio coincyi, an endemic species of the Central Iberian Peninsula. *Natural Product Communications*, *6*(1), 123–126. https://doi.org/10.1177/1934578x1100600129

Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total Phenolic content, Flavonoid content and antioxidant potential of wild vegetables from

western Nepal. *Plants*, *8*(4). https://doi.org/10.3390/plants8040096

Asaduzzaman, M., & Asao, T. (2018). Introductory Chapter: Quality Vegetable Production and Human Health Benefits. *Vegetables - Importance of Quality Vegetables to Human Health*, 1–6. https://doi.org/10.5772/intechopen.79430

Ascarrunz, S. D. M., Natsuaki, T., Honjo, H., & Fukui, R. (2011). Quick adaptation of Ralstonia solanacearum to copper stress to recover culturability and growth in water and soil. *Brazilian Journal of Microbiology*, *42*(2), 576–591. https://doi.org/10.1590/S1517-83822011000200023

Astolfi, S., Pii, Y., Mimmo, T., Lucini, L., Miras-Moreno, M. B., Coppa, E., … Cesco, S. (2020). Single and combined fe and s deficiency differentially modulate root exudate composition in tomato: A double strategy for fe acquisition? *International Journal of Molecular Sciences*, *21*(11), 1–20. https://doi.org/10.3390/ijms21114038

Ateka, J. M., Mbeche, R. M., & Muendo, K. M. (2021). Determinants of protected tomato production technologies among smallholder peri-urban producers in kiambu county, kenya. *Journal of Agriculture and Rural Development in the Tropics and Subtropics*, *122*(1), 43–52. https://doi.org/10.17170/kobra-202102113203

Atif, M. J., Ahanger, M. A., Amin, B., Ghani, M. I., Ali, M., & Cheng, Z. (2020). Mechanism of allium crops bulb enlargement in response to photoperiod: A review. *International Journal of Molecular Sciences*, *21*(4). https://doi.org/10.3390/ijms21041325

Ayana, G., & Fininsa, C. (2017). Effect of Crop Rotation on Tomato Bacterial Wilt (Ralstonia solanacearum) and Survival of the Pathogen in the Rhizospheres and Roots of Different Crops in Ethiopia. *International Journal of Phytopathology*, *5*(3), 81–88. https://doi.org/10.33687/phytopath.005.03.1932

Baba, S. A., & Malik, S. A. (2015). Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume . *Journal of Taibah University for Science*, *9*(4), 449–454. https://doi.org/10.1016/j.jtusci.2014.11.001

Babadoost, M. (2011). Important fungal diseases of tomato in the United States of America. *Acta Horticulturae*, *914*, 85–92. https://doi.org/10.17660/ActaHortic.2011.914.13

Bacterium, P., Xue, H., & Lozano-dur, R. (2020). *Insights into the Root Invasion by the Plant*. 1–9.

Baikar, S., & Malpathak, N. (2010). Secondary metabolites as DNA topoisomerase inhibitors: A new era towards designing of anticancer drugs. *Pharmacognosy Reviews*, *4*(7), 12–26. https://doi.org/10.4103/0973-7847.65320

Bais, H. P., Prithiviraj, B., Jha, A. K., Ausubel, F. M., & Vivanco, J. M. (2005). Mediation of pathogen resistance by exudation of antimicrobials from roots. *Nature*, *434*(7030), 217–221. https://doi.org/10.1038/nature03356

Baker, R. (2019). *Bactrocera dorsalis Pest Report to support ranking of EU candidate priority pests EFSA (European Food Safety Authority),*. (August). https://doi.org/10.5281/zenodo.2789712

Bakhtiari, A. R., Zakaria, M. P., Yaziz, M. I., Lajis, M. N. H. L., & Bi, X. (2014). E nvironment A sia. *EnvironmentAsia*, *7*(1), 104–111.

Banso, A., & Adeyemo, S. (2010). Phytochemical screening and antimicrobial assessment of *Abutilon mauritianum, Bacopa monnifera and Datura stramonium*. *Biokemistri*, *18*(1), 39–44. https://doi.org/10.4314/biokem.v18i1.56390

Bergougnoux, V. (2014). The history of tomato: From domestication to biopharming. *Biotechnology Advances*, *32*(1), 170–189. https://doi.org/10.1016/j.biotechadv.2013.11.003

Biosciences. (2012). Bacterial Gram Staining Bacterial Gram Staining. *A Geno Technology*, *253409*(August), 2–5.

Blair, M. W., Wu, X., & Bhandari, D. (2016). Metadata of the chapter that will be visualized in SpringerLink. https://doi.org/10.1007/978-3-319-26803-3

Boadu, A. (n.d.). A comparative chemistry of coa herbal medicine and herbal extracts of

Vernonia amygdalina (bitter leaf) and *Persea americana* (avocado)*University of kwazulu-natal*. (November 2018).

Borges, L. L., Alves, S. F., Sampaio, B. L., Conceição, E. C., Bara, M. T. F., & Paula, J. R. (2013). Environmental factors affecting the concentration of phenolic compounds in Myrcia tomentosa leaves. *Revista Brasileira de Farmacognosia*, *23*(2), 230–238. https://doi.org/10.1590/S0102-695X2013005000019

Bowles, T. M., Mooshammer, M., Socolar, Y., Calderón, F., Cavigelli, M. A., Culman, S. W., … Grandy, A. S. (2020). Long-Term Evidence Shows that Crop-Rotation Diversification Increases Agricultural Resilience to Adverse Growing Conditions in North America. *One Earth*, *2*(3), 284–293. https://doi.org/10.1016/j.oneear.2020.02.007

Boyaci, F., Unlu, A., & Abak, K. (2012). Screening for resistance to fusarium wilt of some cultivated eggplants and wild Solanum accessions. *Acta Horticulturae*, *935*, 23–27. https://doi.org/10.17660/ActaHortic.2012.935.2

Caldwell, D., Kim, B. S., & Iyer-Pascuzzi, A. S. (2017). Ralstonia solanacearum differentially colonizes roots of resistant and susceptible tomato plants. *Phytopathology*, *107*(5), 528–536. https://doi.org/10.1094/PHYTO-09-16-0353-R

Campos, H., & Ortiz, O. (2019). The potato crop: Its agricultural, nutritional and social contribution to humankind. In *The Potato Crop: Its Agricultural, Nutritional and Social Contribution to Humankind*. https://doi.org/10.1007/978-3-030-28683-5

Canyon Hydro, Summary, E., Of, F., Potential, T. H. E., Ferreres, X. R., Font, A. R., … Masuelli, M. (2013). We are IntechOpen , the world ’ s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. *Intech*, *32*(July), 137–144. Retrieved from http://www.intechopen.com/books/trends-in-telecommunications-technologies/gps-total-electron-content-tec- prediction-at-ionosphere-layer-over-the-equatorial-region%0AInTec%0Ahttp://www.asociatiamhc.ro/wp-content/uploads/2013/11/Guide-to-Hydropower.pdf

Cerda, R., Avelino, J., Gary, C., Tixier, P., Lechevallier, E., & Allinne, C. (2017). Primary and secondary yield losses caused by pests and diseases: Assessment and modeling in coffee. *PLoS ONE*, *12*(1), 1–17. https://doi.org/10.1371/journal.pone.0169133

Cerozi, B. da S., & Fitzsimmons, K. (2016). The effect of pH on phosphorus availability and speciation in an aquaponics nutrient solution. *Bioresource Technology*, *219*(September), 778–781. https://doi.org/10.1016/j.biortech.2016.08.079

Ch, G. Beta keto compounds, United State patent (2002). *(l. 1*(12).

Chakraborty, R., & Roy, T. S. (2016). Threats faced by brown rot of potato in Bangladesh. *Microbiology Research*, *7*(1). https://doi.org/10.4081/mr.2016.6258

Chaudhary, P., Sharma, A., Singh, B., & Nagpal, A. K. (2018). Bioactivities of phytochemicals present in tomato. *Journal of Food Science and Technology*, *55*(8), 2833–2849. https://doi.org/10.1007/s13197-018-3221-z

Chaudhry, Z., & Rashid, H. (2011). Isolation and characterization of Ralstonia solanacearum from infected tomato plants of Soan Skesar valley of Punjab. *Pakistan Journal of Botany*, *43*(6), 2979–2985.

Chen, J., Mao, S., Xu, Z., & Ding, W. (2019). Various antibacterial mechanisms of biosynthesized copper oxide nanoparticles against soilborne Ralstonia solanacearum. *RSC Advances*, *9*(7), 3788–3799. https://doi.org/10.1039/c8ra09186b

Chen, K., Khan, R. A. A., Cao, W., & Ling, M. (2020). Sustainable and ecofriendly approach of managing soil born bacterium ralstonia solanacearum (Smith) using dried powder of conyza canadensis. *Pathogens*, *9*(5), 1–18. https://doi.org/10.3390/pathogens9050327

Chen, S., Qi, G., Ma, G., & Zhao, X. (2020). Biochar amendment controlled bacterial wilt through changing soil chemical properties and microbial community. *Microbiological Research*, *231*(October 2019), 126373. https://doi.org/10.1016/j.micres.2019.126373

Chung, M. Y., Vrebalov, J., Alba, R., Lee, J., McQuinn, R., Chung, J. D., … Giovannoni, J. (2010). A tomato (Solanum lycopersicum) APETALA2/ERF gene, SlAP2a, is a

negative regulator of fruit ripening. *Plant Journal*, *64*(6), 936–947. https://doi.org/10.1111/j.1365-313X.2010.04384.x

Claoston, N., Samsuri, A. W., Ahmad Husni, M. H., & Mohd Amran, M. S. (2014). Effects of pyrolysis temperature on the physicochemical properties of empty fruit bunch and rice husk biochars. *Waste Management and Research*, *32*(4), 331–339. https://doi.org/10.1177/0734242X14525822

Coca, D. M., Ragassi, C. F., & Lopes, C. A. (2012). Utilização de esterco como medida auxiliar no controle da murcha bacteriana em genótipos de batata. *Revista Latinoamericana de La Papa*, *17*(1), 152–170.

Cooper, D. E. (2018). 'Growing your own.' *Plant Ethics*, 177–187. https://doi.org/10.4324/9781315114392-16

Correa, J., Postma, J. A., Watt, M., & Wojciechowski, T. (2019). Soil compaction and the architectural plasticity of root systems. *Journal of Experimental Botany*, *70*(21), 6019–6034. https://doi.org/10.1093/jxb/erz383

Cox, R. J., Peterson, H. L., Young, J., Cusik, C., & Espinoza, E. O. (2000). The forensic analysis of soil organic by FTIR. *Forensic Science International*, *108*(2), 107–116. https://doi.org/10.1016/S0379-0738(99)00203-0

Dai, Y., Zhang, P., Ito, K., Noda, K., & Senge, M. (2020). Clarification of the necessary meteorological conditions to control Ralstonia solanacearum via soil solarization. *Paddy and Water Environment*, *18*(4), 667–676. https://doi.org/10.1007/s10333-020-00809-4

Damicone, J. P., & Brandenberger, L. (2015). Diseases Caused by Bacteria , Viruses , and Nematodes. *Oklahoma Cooperative Extension Fact Sheets EPP-7626*, (Common Diseases of Tomatoes-Part II), 1–8. Retrieved from http://osufacts.okstate.edu

de Clercq, P., Mason, P. G., & Babendreier, D. (2011). Benefits and risks of exotic biological control agents. *BioControl*, *56*(4), 681–698. https://doi.org/10.1007/s10526-011-9372-

8

de Jesus Duarte, S., Glaser, B., & Cerri, C. E. P. (2019). Effect of biochar particle size on physical, hydrological and chemical properties of loamy and sandy tropical soils. *Agronomy*, *9*(4). https://doi.org/10.3390/agronomy9040165

Desta, W., Shumbahri, M., & Gebrehiwot, S. (2020). Application of Ficus carica L. And Solanum incanum L. Extracts in Coagulation of Milk. And Case of Traditional Practice in Ab'ala Area, Afar Regional State, Ethiopia. *Biochemistry Research International*, *2020*. https://doi.org/10.1155/2020/9874949

Dhaliwal, M.S. (2017). *Classification of Vegetable Crops In "Handbook of vegetable Crops".*

Dhaliwal, Major S. (2017). Root vegetables. (January).

Diaz, F., Freato, R., Yanaga, E., Hallevy, G., Minelle, Profiling and pathogenicity of *Ralstonia solanacearum* disease of tomato and it's control using *Senna didymobotrya* and *Moringa oleifera* plant extracts in Maseno (Kenya) (3), 43. https://doi.org/10.1017/CBO9781107415324.004

Dietzgen, R. G., Mann, K. S., & Johnson, K. N. (2016). Plant virus-insect vector interactions: Current and potential future research directions. *Viruses*, *8*(11), 1–21. https://doi.org/10.3390/v8110303

Din, N., Ahmad, M., Siddique, M., Ali, A., Naz, I., Ullah, N., & Ahmad, F. (2016). Phytobiocidal management of bacterial wilt of tomato caused by Ralstonia solanacearum (Smith) Yabuuchi. *Spanish Journal of Agricultural Research*, *14*(3). https://doi.org/10.5424/sjar/2016143-9012

Ding, W., Dong, X., Ime, I. M., Gao, B., & Ma, L. Q. (2014). Pyrolytic temperatures impact lead sorption mechanisms by bagasse biochars. *Chemosphere*, *105*, 68–74. https://doi.org/10.1016/j.chemosphere.2013.12.042

Domingues, R. R., Trugilho, P. F., Silva, C. A., De Melo, I. C. N. A., Melo, L. C. A.,

Magriotis, Z. M., & Sánchez-Monedero, M. A. (2017). Properties of biochar derived from wood and high-nutrient biomasses with the aim of agronomic and environmental benefits. *PLoS ONE*, *12*(5), 1–19. https://doi.org/10.1371/journal.pone.0176884

Dong, X., Li, G., Lin, Q., & Zhao, X. (2017). Quantity and quality changes of biochar aged for 5 years in soil under field conditions. *Catena*, *159*(August), 136–143. https://doi.org/10.1016/j.catena.2017.08.008

Elisha, I. L., Botha, F. S., McGaw, L. J., & Eloff, J. N. (2017). The antibacterial activity of extracts of nine plant species with good activity against Escherichia coli against five other bacteria and cytotoxicity of extracts. *BMC Complementary and Alternative Medicine*, *17*(1), 133. https://doi.org/10.1186/s12906-017-1645-z

Elnour, A. Y., Alghyamah, A. A., Shaikh, H. M., Poulose, A. M., Al-Zahrani, S. M., Anis, A., & Al-Wabel, M. I. (2019). Effect of pyrolysis temperature on biochar microstructural evolution, physicochemical characteristics, and its influence on biochar/polypropylene composites. *Applied Sciences (Switzerland)*, *9*(6), 7–9. https://doi.org/10.3390/app9061149

FAO. (2020). Food Security and Nutrition in the World. In IEEE Journal of Selected Topics in Applied Earth Observations and Remote Sensing.

Ferdeş, M., Dincă, M. N., Moiceanu, G., Zabava, B. Ş., & Paraschiv, G. (2020). Microorganisms and enzymes used in the biological pretreatment of the substrate to enhance biogas production: A review. *Sustainability (Switzerland)*, *12*(17). https://doi.org/10.3390/su12177205

Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P., & Forano, E. (2012). Microbial degradation of complex carbohydrates in the gut © 2012 Landes Bioscience . Do not distribute © 2012 Landes Bioscience . Do not distribute. *Gut Microbes*, (August), 289–306.

Gao, Y., Lu, Y., Lin, W., Tian, J., & Cai, K. (2019). Biochar suppresses bacterial wilt of tomato by improving soil chemical properties and shifting soil microbial community.

Microorganisms, *7*(12). https://doi.org/10.3390/microorganisms7120676

Garćia, R. O., Kerns, J. P., & Thiessen, L. (2019). Ralstonia solanacearum species complex: A quick diagnostic guide. *Plant Health Progress*, *20*(1), 7–13. https://doi.org/10.1094/PHP-04-18-0015-DG

Garrett, K. A., Dobson, A. D. M., Kroschel, J., Natarajan, B., Orlandini, S., Tonnang, H. E. Z., & Valdivia, C. (2013). The effects of climate variability and the color of weather time series on agricultural diseases and pests, and on decisions for their management. *Agricultural and Forest Meteorology*, *170*, 216–227. https://doi.org/10.1016/j.agrformet.2012.04.018

Geoffrey, S. K., Hillary, N. K., Antony, K. M., Mariam, M., & Mary, M. C. (2014). Challenges and Strategies to Improve Tomato Competitiveness along the Tomato Value Chain in Kenya. *International Journal of Business and Management*, *9*(9). https://doi.org/10.5539/ijbm.v9n9p205

Ghosal, M., & Mandal, P. (2012). Phytochemical screening and antioxidant activities of two selected "BIHI" fruits used as vegetables in Darjeeling Himalaya. *International Journal of Pharmacy and Pharmaceutical Sciences*, *4*(2), 567–574.

Gilardi, G., Gullino, M. L., & Garibaldi, A. (2013). Success and failures of biofumigation with Brassicas. *Acta Horticulturae*, *1005*, 383–388. https://doi.org/10.17660/ActaHortic.2013.1005.45

Gomaa, A. A., Samy, M. N., Desoukey, S. Y., & Kamel, M. S. (2021). GC-MS Analysis of Volatile Oil and Fatty Acids Composition of *Abutilon hirtum (Lam .) Sweet Leaves*. *4*, 119–123.

Gonzalo, M. J., Li, Y. C., Chen, K. Y., Gil, D., Montoro, T., Nájera, I., … Monforte, A. J. (2020). Genetic Control of Reproductive Traits in Tomatoes Under High Temperature. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.00326

Gopinath, S. C. B., Anbu, P., Arshad, M. K. M., Lakshmipriya, T., Voon, C. H., Hashim,

U., & Chinni, S. V. (2017). Biotechnological Processes in Microbial Amylase Production. *BioMed Research International*, *2017*. https://doi.org/10.1155/2017/1272193

Gu, Y., Hou, Y., Huang, D., Hao, Z., Wang, X., Wei, Z., … Friman, V. P. (2017). Application of biochar reduces Ralstonia solanacearum infection via effects on pathogen chemotaxis, swarming motility, and root exudate adsorption. *Plant and Soil*, *415*(1–2), 269–281. https://doi.org/10.1007/s11104-016-3159-8

Guodaar, L., & Studies, B. E. S. (2015). *Effects of Climate Variability on Tomato Crop Producyion in the Offinso North District of Ashanti Region*. 1–181.

Gutierrez, E. E. V. (2018). *An overview of recent studies of tomato (Solanum lycopersicum spp) from a social, biochemical and genetic perspective on quality parameters*. (December).

Hanssen, I. M., Lapidot, M., & Thomma, B. P. H. J. (2010). *Enfermedades Virales Tomate*. *23*(5), 539–548.

Hawkins. (1856). Cotton family. *Notes and Queries*, *s2-I*(15), 298–299. https://doi.org/10.1093/nq/s2-I.15.298-c

He, K., Yang, S. Y., Li, H., Wang, H., & Li, Z. L. (2014). Effects of calcium carbonate on the survival of Ralstonia solanacearum in soil and control of tobacco bacterial wilt. *European Journal of Plant Pathology*, *140*(4), 665–675. https://doi.org/10.1007/s10658-014-0496-4

Hernández, V., Hellín, P., Fenoll, J., Garrido, I., Cava, J., & Flores, P. (2015). Increasing Yield and Quality of Tomato Cultivated Under High Temperature Conditions Through the Use of Elicitors. *Procedia Environmental Sciences*, *29*(Agri), 184. https://doi.org/10.1016/j.proenv.2015.07.251

Hong, J. K., Jang, S. J., Lee, Y. H., Jo, Y. S., Yun, J. G., Jo, H., … Kim, H. J. (2018). Reduced bacterial wilt in tomato plants by bactericidal peroxyacetic acid mixture

treatment. *Plant Pathology Journal*, *34*(1), 78–84. https://doi.org/10.5423/PPJ.NT.06.2017.0131

Hu, Z., Zhang, L., Zhong, L., Zhou, Y., Xue, J., & Li, Y. (2019). Preparation of an antibacterial chitosan-coated biochar-nanosilver composite for drinking water purification. *Carbohydrate Polymers*, *219*(May), 290–297. https://doi.org/10.1016/j.carbpol.2019.05.017

Huang, W., Chen, J., & Zhang, J. (2018). Adsorption characteristics of methylene blue by biochar prepared using sheep, rabbit and pig manure. *Environmental Science and Pollution Research*, *25*(29), 29256–29266. https://doi.org/10.1007/s11356-018-2906-1

Ibrahim, M. H., Jaafar, H. Z. E., Rahmat, A., & Rahman, Z. A. (2011). Effects of nitrogen fertilization on synthesis of primary and secondary metabolites in three varieties of kacip fatimah (Labisia pumila blume). *International Journal of Molecular Sciences*, *12*(8), 5238–5254. https://doi.org/10.3390/ijms12085238

Iheagwam, F. N., Israel, E. N., Kayode, K. O., De Campos, O. C., Ogunlana, O. O., & Chinedu, S. N. (2019). GC-MS Analysis and Inhibitory Evaluation of Terminalia catappa Leaf Extracts on Major Enzymes Linked to Diabetes. *Evidence-Based Complementary and Alternative Medicine*, *2019*. https://doi.org/10.1155/2019/6316231

Imo, C., Abu, S. M., Emmanuel, O. P., & Yusuf, K. S. (2020). Phytochemical constituents and mineral composition of fruits of Solanum melongena and Cucumis sativus. *International Journal of Current Research in Biosciences and Plant Biology*, *7*(6), 26–36. https://doi.org/10.20546/ijcrbp.2020.706.003

Incanum, L. (2019). *In-Vitro Antioxidant Potentials and Free Radicals Scavenging Activity of Solanum*. *9*, 1292–1300.

Indhumathi, T., & Mohandass, S. (2014). Efficacy of Ethanolic extract of Solanum incanum fruit extract for its antimicrobial activity. *Int.J.Curr.Microbiol.App.Sci*, *3*(6), 939–949. Retrieved from http://www.ijcmas.com

Jasim, H., Hussein, A. O., Hameed, I. H., & Kareem, M. A. (2015). Characterization of alkaloid constitution and evaluation of antimicrobial activity of solanum nigrum using gas chromatography mass spectrometry (GC-MS). *Journal of Pharmacognosy and Phytotherapy*, *7*(4), 57–73. https://doi.org/10.5897/JPP2015.0346

Jia, M., Wang, F., Bian, Y., Jin, X., Song, Y., Kengara, F. O., … Jiang, X. (2013). Effects of pH and metal ions on oxytetracycline sorption to maize-straw-derived biochar. *Bioresource Technology*, *136*, 87–93. https://doi.org/10.1016/j.biortech.2013.02.098

Jia, Y., Shi, S., Liu, J., Su, S., Liang, Q., Zeng, X., & Li, T. (2018). Study of the effect of pyrolysis temperature on the Cd2+ adsorption characteristics of biochar. *Applied Sciences (Switzerland)*, *8*(7). https://doi.org/10.3390/app8071019

Jiang, G., Wei, Z., Xu, J., Chen, H., Zhang, Y., She, X., … Liao, B. (2017). Bacterial wilt in China: History, current status, and future perspectives. *Frontiers in Plant Science*, *8*(September), 1–10. https://doi.org/10.3389/fpls.2017.01549

Jibat, M., Terefe, H., & Derso, E. (2018). Integrated management of bacterial wilt (Ralstonia solanacearum) of ginger (Zingiber officinale) in Southwestern Ethiopia. *Archives of Phytopathology and Plant Protection*, *51*(15–16), 834–851. https://doi.org/10.1080/03235408.2018.1504374

Jindo, K., Mizumoto, H., Sawada, Y., Sanchez-Monedero, M. A., & Sonoki, T. (2014). Physical and chemical characterization of biochars derived from different agricultural residues. *Biogeosciences*, *11*(23), 6613–6621. https://doi.org/10.5194/bg-11-6613-2014

Kabir, M. S. N., Ali, M., Lee, W. H., Cho, S. I., & Chung, S. O. (2020). Physicochemical quality changes in tomatoes during delayed cooling and storage in a controlled chamber. *Agriculture (Switzerland)*, *10*(6), 1–12. https://doi.org/10.3390/agriculture10060196

Kago, E. K., Kinyua, Z. M., Maingi, J. M., & Okemo, P. O. (2019). Effect of Field Treatment with Selected Soil Amendments on Bacterial Wilt Incidences in Tomatoes, Capsicum

and Potatoes. *Journal of Experimental Agriculture International*, *41*(1), 1–12. https://doi.org/10.9734/jeai/2019/v41i130387

Kalimuthu, A., Chinnadurai, V., Juliet, Y., & Prabakaran, R. (2016). GCMS Analysis of Leaf and Salt Stress Callus of Eggplant (Solanum melongena L.). *British Journal of Pharmaceutical Research*, *14*(6), 1–11. https://doi.org/10.9734/bjpr/2016/30425

Kambura, K. C. (2020). *E*valuation of Bacillus and Trichoderma species for biological control of bacterial wilt caused by *Ralstonia solanacearum* in tomato.

Kanaan, H., Medina, S., Krassnovsky, A., & Raviv, M. (2015). Survival of Macrophomina phaseolina s.l. and Verticillium dahliae during solarization as affected by composts of various maturities. *Crop Protection*, *76*, 108–113. https://doi.org/10.1016/j.cropro.2015.07.006

Kaunda, J. S., & Zhang, Y. J. (2020). Chemical constituents from the fruits of Solanum incanum L. *Biochemical Systematics and Ecology*, *90*(February), 104031. https://doi.org/10.1016/j.bse.2020.104031

Khalifa, W., & Thabet, M. (2015). Biochar Amendment Enhances Tomato Resistance to Some Soil Borne Diseases. *Middle East Journal of Agriculture Research*, 1088–1100.

Khasabulli, B. D., Musyimi, D. M., Miruka, D. M., Opande, G. T., & Jeruto, P. (2017). Isolation and Characterisation of Ralstonia Solanacearum Strains of Tomato Wilt Disease from Maseno, Kenya. *Journal of Asian Scientific Research*, *7*(9), 404–420. https://doi.org/10.18488/journal.2.2017.79.404.420

Kipngeno, C. D., Mshimba, S. M., Gilbert, C., & O, A. J. (2014). Antimicrobial activity and phytochemical investigation of crude extracts of the fruits of Solanum incanum (Solananceae) and Dovyalis abbysinica (Flacourtiaceae). *Science Journal of Microbiology*, 1–4. https://doi.org/10.7237/sjmb/193

Kouki, M., & Manetas, Y. (2002). Resource availability affects differentially the levels of gallotannins and condensed tannins in Ceratonia siliqua. *Biochemical Systematics and*

Ecology, *30*(7), 631–639. https://doi.org/10.1016/S0305-1978(01)00142-9

Kumar, D., Saleem Dar, M., Khan, K., Kumar Choudhary, D., Srinagar, R., Mohammad Saleem Dar, I., … Ahmad Khan, K. (2018). Ralstonia solanacearum: A wide spread and global bacterial plant wilt pathogen. *Journal of Pharmacognosy and Phytochemistry JPP*, *7*(72), 85–90.

Kumar, K., Henry, D. C., & Sivakumar, K. (2019). *Bioprofiling of phytochemicals and phytonutritional potentials of Solanum incanum L* . *128*(May), 328–347.

Kumar, V., Bhatnagar, A. K., & Srivastava, J. N. (2011). *Antibacterial activity of crude extracts of Spirulina platensis and its structural elucidation of bioactive compound*. *5*(32), 7043–7048. https://doi.org/10.5897/JMPR11.1175

Kusch, P., Deininger, S., Specht, S., Maniako, R., Haubrich, S., Pommerening, T., … Kaiser, A. (2011). *In Vitro and In Vivo Antimalarial Activity Assays of Seeds from Balanites aegyptiaca : Compounds of the Extract Show Growth Inhibition and Activity against Plasmodial Aminopeptidase*. *2011*. https://doi.org/10.1155/2011/368692

Laird, D. A., Fleming, P., Davis, D. D., Horton, R., Wang, B., & Karlen, D. L. (2010). Impact of biochar amendments on the quality of a typical Midwestern agricultural soil. *Geoderma*, *158*(3–4), 443–449. https://doi.org/10.1016/j.geoderma.2010.05.013

Lawal, A. A., Hassan, M. A., Ahmad Farid, M. A., Tengku Yasim-Anuar, T. A., Samsudin, M. H., Mohd Yusoff, M. Z., … Shirai, Y. (2021). Adsorption mechanism and effectiveness of phenol and tannic acid removal by biochar produced from oil palm frond using steam pyrolysis. *Environmental Pollution*, *269*. https://doi.org/10.1016/j.envpol.2020.116197

Lee, J. E., & Park, Y. K. (2020). Applications of modified biochar-based materials for the removal of environment pollutants: A mini review. *Sustainability (Switzerland)*, *12*(15). https://doi.org/10.3390/su12156112

Lee, Y. H., Choi, C. W., Kim, S. H., Yun, J. G., Chang, S. W., Kim, Y. S., & Hong, J. K.

(2012). Chemical pesticides and plant essential oils for disease control of tomato bacterial wilt. *Plant Pathology Journal*, *28*(1), 32–39. https://doi.org/10.5423/PPJ.OA.10.2011.0200

Li, H., Liu, L., Cui, J., Cui, J., Wang, F., & Zhang, F. (2020). High-efficiency adsorption and regeneration of methylene blue and aniline onto activated carbon from waste edible fungus residue and its possible mechanism. *RSC Advances*, *10*(24), 14262–14273. https://doi.org/10.1039/d0ra01245a

Li, S., Liu, Y., Wang, J., Yang, L., Zhang, S., Xu, C., & Ding, W. (2017). Soil acidification aggravates the occurrence of bacterial wilt in south China. *Frontiers in Microbiology*, *8*(APR), 1–12. https://doi.org/10.3389/fmicb.2017.00703

Liang, H., Chen, L., Liu, G., & Zheng, H. (2016). *Surface morphology properties of biochars produced from different feedstocks*. (Iccte), 1205–1208. https://doi.org/10.2991/iccte-16.2016.210

Lin, W. C., Lu, C. F., Wu, J. W., Cheng, M. L., Lin, Y. M., Yang, N. S., … Cheng, C. P. (2004). Transgenic tomato plants expressing the Arabidopsis NPR1 gene display enhanced resistance to a spectrum of fungal and bacterial diseases. *Transgenic Research*, *13*(6), 567–581. https://doi.org/10.1007/s11248-004-2375-9

Lin, W., Lin, M., Zhou, H., Wu, H., Li, Z., & Lin, W. (2019). The effects of chemical and organic fertilizer usage on rhizosphere soil in tea orchards. *PLoS ONE*, *14*(5), 1–16. https://doi.org/10.1371/journal.pone.0217018

Liu, L., Deng, G., & Shi, X. (2020). Adsorption characteristics and mechanism of p-nitrophenol by pine sawdust biochar samples produced at different pyrolysis temperatures. *Scientific Reports*, *10*(1), 1–11. https://doi.org/10.1038/s41598-020-62059-y

Liu, N., Charrua, A. B., Weng, C. H., Yuan, X., & Ding, F. (2015). Characterization of biochars derived from agriculture wastes and their adsorptive removal of atrazine from aqueous solution: A comparative study. *Bioresource Technology*, *198*, 55–62.

https://doi.org/10.1016/j.biortech.2015.08.129

Lu, Y., Rao, S., Huang, F., Cai, Y., Wang, G., & Cai, K. (2016). Effects of Biochar Amendment on Tomato Bacterial Wilt Resistance and Soil Microbial Amount and Activity. *International Journal of Agronomy*, *2016*. https://doi.org/10.1155/2016/2938282

Madzimure, J., Nyahangare, E. T., Hamudikuwanda, H., Hove, T., Belmain, S. R., Stevenson, P. C., & Mvumi, B. M. (2013). Efficacy of Strychnos spinosa (Lam.) and Solanum incanum L. aqueous fruit extracts against cattle ticks. *Tropical Animal Health and Production*, *45*(6), 1341–1347. https://doi.org/10.1007/s11250-013-0367-6

Mainasara, M. M., Aliero, B. L., Aliero, A. A., & Yakubu, M. (2012). *Phytochemical and Antibacterial Properties of Root and Leaf Extracts of Calotropis procera*. *20*(1), 1–6.

Malik, A., Arooj, M., Butt, T. T., Zahid, S., Zahid, F., Jafar, T. H., … Mirza, M. U. (2018). In silico and in vivo characterization of cabralealactone, solasodin and salvadorin in a rat model: Potential anti-inflammatory agents. *Drug Design, Development and Therapy*, *12*, 1431–1443. https://doi.org/10.2147/DDDT.S154169

Mamphogoro, T. P., Babalola, O. O., & Aiyegoro, O. A. (2020). Sustainable management strategies for bacterial wilt of sweet peppers (Capsicum annuum) and other Solanaceous crops. *Journal of Applied Microbiology*, *129*(3), 496–508. https://doi.org/10.1111/jam.14653

Manase, M. J., Mitaine-Offer, A. C., Pertuit, D., Miyamoto, T., Tanaka, C., Delemasure, S., … Lacaille-Dubois, M. A. (2012). Solanum incanum and S. heteracanthum as sources of biologically active steroid glycosides: Confirmation of their synonymy. *Fitoterapia*, *83*(6), 1115–1119. https://doi.org/10.1016/j.fitote.2012.04.024

Mao, J., Zhang, K., & Chen, B. (2019). Linking hydrophobicity of biochar to the water repellency and water holding capacity of biochar-amended soil *. *Environmental Pollution*, *253*, 779–789. https://doi.org/10.1016/j.envpol.2019.07.051

Markandeya, Shukla, S. P., & Kisku, G. C. (2015). Linear and non-linear kinetic modeling for adsorption of disperse dye in batch process. *Research Journal of Environmental Toxicology*, *9*(6), 320–331. https://doi.org/10.3923/rjet.2015.320.331

Mati, B. M. (2014). What you need to know about waterlogging in agricultural lands. *Waterlogging in Agricultural Lands*, *1*, 1–10.

Mazher, M., Malik, N. Z., Riaz, M., Hussain, A., Ali, Y., & Noshad, Q. Q. (2016). Phytochemistry and Antibacterial Assay of Fruit, Leaf and Stem Extracts of Solanum nigrum L. in Different Solvent. *International Journal of Biosciences (IJB)*, *9*(6), 129–136. https://doi.org/10.12692/ijb/9.6.129-136

Meng, F. (2013). Ralstonia Solanacearum Species Complex and Bacterial Wilt Disease. *Journal of Bacteriology & Parasitology*, *04*(02), 2–5. https://doi.org/10.4172/2155-9597.1000e119

Meng, F., Babujee, L., Jacobs, J. M., & Allen, C. (2015). Comparative transcriptome analysis reveals cool virulence factors of Ralstonia solanacearum race 3 biovar 2. *PLoS ONE*, *10*(10), 1–22. https://doi.org/10.1371/journal.pone.0139090

Mohamed Jaabir, M. S., Vigneshwaran, R., Ehtisham Ul Hassan, T. M. D., & Senthil Kumar, S. (2010). Study on the antimicrobial activity of ethanolic extract of the fruits of solanum torvum and its phytochemical analysis by GC-MS. *Biomedical and Pharmacology Journal*, *3*(1), 117–121.

Mohd Hasan, M. H., Bachmann, R. T., Loh, S. K., Manroshan, S., & Ong, S. K. (2019). Effect of Pyrolysis Temperature and Time on Properties of Palm Kernel Shell-Based Biochar. *IOP Conference Series: Materials Science and Engineering*, *548*(1). https://doi.org/10.1088/1757-899X/548/1/012020

Mongalo, N. I., Dikhoba, P. M., Soyingbe, S. O., & Makhafola, T. J. (2018). activity and cytotoxicity of South African medicinal plants against mycotoxigenic fungi. *Heliyon*, (September), e00973. https://doi.org/10.1016/j.heliyon.2018.e00973

Monteiro, J. M., Albuquerque, U. P., Lins Neto, E. M. F., Araújo, E. L., Albuquerque, M. M., & Amorim, E. L. C. (2006). The effects of seasonal climate changes in the caatinga on tannin levels in Myracrodruon urundeuva (Engl.) Fr. All. and Anadenanthera colubrina (Vell.) Brenan. *Revista Brasileira de Farmacognosia*, *16*(3), 338–344. https://doi.org/10.1590/s0102-695x2006000300010

Moranga, L. (2016). Analysis of Factors Influencing Tomato Farmers' Willingness To Adopt Innovative Timing Approaches for Management of Climate Change Effects in Taita Taveta County, Kenya Lawrence Ongwae Moranga a Thesis Submitted in Partial Fulfillment of the Requirements. 97.

Mukome, F. N. D., Zhang, X., Silva, L. C. R., Six, J., & Parikh, S. J. (2013). Use of chemical and physical characteristics to investigate trends in biochar feedstocks. *Journal of Agricultural and Food Chemistry*, *61*(9), 2196–2204. https://doi.org/10.1021/jf3049142

Muñoz, C., Quilodrán, C., & Navia, R. (2014). Evaluation of biochar-plant extracts complexes on soil nitrogen dynamics. *Journal of Biobased Materials and Bioenergy*, *8*(3), 377–385. https://doi.org/10.1166/jbmb.2014.1448

Musyoka, T. M. (2014). *A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science (Biostatistics) in the School of Pure and Applied Sciences of Kenyatta University*. *1*(9), 1–84.

Mvumi, C. (2019). Efficacy of Bitter Apple (Solanum incanum) in Relation to Contact Time for Controlling Cabbage Aphids (Brevicoryne brassicae). *Asian Journal of Crop Science*, *11*(2), 71–76. https://doi.org/10.3923/ajcs.2019.71.76

Mwangi, F. N. (2015). Evaluation of Botanical Pesticides and Coloured Sticky Insect Traps for Management of Insect Pests (Thrips, Whiteflies and Aphids) in French Beans (Phaseolus Vulgaris L.). *University of Nairobi (Digital Repository)*.

Mwangi, M. W., Narla, J. W. K. R. D., & Muiru, G. M. K. W. M. (2015). Tomato Management Practices and Diseases Occurrence in Mwea West Sub County. *Journal*

of Natural Sciences Research, *5*(20), 119–124.

Mwaura, L., Anjarwalla, P., Ofori, D. A., Stevenson, P. C., Smith, P., & Jamnadass, R. (2015). *Pesticidal plant leaflet : Solanum incanum L.* 2.

Nabiswa, A. (2020). Tomato production , distribution and marketing in kenya , a baseline survey of Kirinyaga district report . (July).

Namisy, A., Chen, J. R., Prohens, J., Metwally, E., Elmahrouk, M., & Rakha, M. (2019). Screening cultivated eggplant and wild relatives for resistance to bacterial wilt (Ralstonia solanacearum). *Agriculture (Switzerland)*, *9*(7), 1–12. https://doi.org/10.3390/agriculture9070157

Nandi, A., Yan, L. J., Jana, C. K., & Das, N. (2019). Role of Catalase in Oxidative Stress- And Age-Associated Degenerative Diseases. *Oxidative Medicine and Cellular Longevity*, *2019*. https://doi.org/10.1155/2019/9613090

Naumoska, K., Jug, U., Metličar, V., & Vovk, I. (2020). Oleamide, a bioactive compound, unwittingly introduced into the human body through some plastic food/beverages and medicine containers. *Foods*, *9*(5). https://doi.org/10.3390/foods9050549

Nawaz, M. A., Zakharenko, A. M., Zemchenko, I. V., Haider, M. S., Ali, M. A., Imtiaz, M., … Golokhvast, K. S. (2019). Phytolith Formation in Plants: From Soil to Cell. *Plants*, *8*(8), 249. https://doi.org/10.3390/plants8080249

Ngongeh, L. A., Idika, I. K., & Ibrahim Shehu, A. R. (2014). Climate change/global warming and its impacts on parasitology/entomology. *Open Parasitology Journal*, *5*(1), 1–11. https://doi.org/10.2174/1874421401405010001

Nuylert, A., Kuwahara, Y., Hongpattarakere, T., & Asano, Y. (2018). Identification of saturated and unsaturated 1-methoxyalkanes from the Thai millipede Orthomorpha communis as potential “Raincoat Compounds.” *Scientific Reports*, *8*(1), 1–9. https://doi.org/10.1038/s41598-018-30156-8

Nuzzo, A., Buurman, P., Cozzolino, V., Spaccini, R., & Piccolo, A. (2020). Infrared spectra

of soil organic matter under a primary vegetation sequence. *Chemical and Biological Technologies in Agriculture*, *7*(1), 1–12. https://doi.org/10.1186/s40538-019-0172-1

Nwanna, E. E., Ibukun, E. O., Oboh, G., Ademosun, A. O., Boligon, A. A., & Athayde, M. (2014). HPLC-DAD analysis and In-Vitro property of polyphenols extracts from (Solanum Aethiopium) fruits on α-amylase, α-glucosidase and angiotensin-1-converting enzyme activities. *International Journal of Biomedical Science*, *10*(4), 272–281.

Ochilo, W. N., Nyamasyo, G. N., Kilalo, D., & Otieno, W. (2019). Characteristics and production constraints of smallholder tomato production in Kenya Characteristics and production constraints of smallholder tomato production in Kenya. *Scientific African*, *2*(March), e00014. https://doi.org/10.1016/j.sciaf.2018.e00014

Ochilo, W. N., Nyamasyo, G. N., Kilalo, D., Otieno, W., Otipa, M., Chege, F., … Lingeera, E. K. (2019). Ecological limits and management practices of major arthropod pests of tomato in Kenya. *Journal of Agricultural Science and Practice*, *4*(2), 29–42. https://doi.org/10.31248/jasp2019.124

Of, R., Agricultureanimal, O. F., Rural, R., Support, S., Plan, P. M., & Cropsrssp-, F. O. R. T. (2012). *E2896 v2*.

Ofori, D. A., Anjarwalla, P., Jamnadass, R., Steveson, P. C., & Smith, P. (2013). Pesticidal plant leaflet. *Pesticidal Plant Leaflet*, 6–7.

Oljira, T., & Berta, S. (2020). Isolation and characterization of wilt-causing pathogens of local growing pepper (capsicum annuum L.) in Gurage Zone, Ethiopia. *International Journal of Agronomy*, *2020*. https://doi.org/10.1155/2020/6638683

Ong, S. N., Taheri, S., Othman, R. Y., & Teo, C. H. (2020). Viral disease of tomato crops (Solanum lycopesicum L.): an overview. *Journal of Plant Diseases and Protection*, *127*(6), 725–739. https://doi.org/10.1007/s41348-020-00330-0

Orczyk, M., Wojciechowski, K., & Brezesinski, G. (2020). Journal of Colloid and Interface

Science The influence of steroidal and triterpenoid saponins on monolayer models of the outer leaflets of human erythrocytes , E . coli and S . cerevisiae cell membranes. *Journal of Colloid And Interface Science*, *563*, 207–217. https://doi.org/10.1016/j.jcis.2019.12.014

Oromia, C. (n.d.). Fresh and industrial tomato.

Osoyo, A. (2021). Report Name : Tomato Annual.

Otipa, M., & Gitonga, C. (n.d.). Tomato Production Manual 2016.

Oyinloye, O. E. (n.d.). In vitro antimicrobial , anti-oxidant properties and GC-MS analysis of the crude methanolic extract and fractions of Solanum dasyphyllum Schumach and Thonn . leaves. 1–12.

Ozores-hampton, M., Kiran, F., & Mcavoy, G. (2012). Blossom Drop , Reduced Fruit Set , and Post-Pollination Disorders in Tomato 1 Potential Causes of Blossom Drop. *Univ. Florida, Inst. Food Agr. Sci., Electronic Data Info. Source.*, *HS1195*(9), 1–6.

Pal, K. K. and B. M. G. (2006). Biological control of plant pathogens. The Plant Health Instructor. doi: 10.1094. *The Plant Health Instructor*, 1–25. https://doi.org/10.1094/PHI-A-2006-1117-02.Biological

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science*, *5*. https://doi.org/10.1017/jns.2016.41

Panth, M., Hassler, S. C., & Baysal-Gurel, F. (2020). Methods for management of soilborne diseases in crop production. *Agriculture (Switzerland)*, *10*(1). https://doi.org/10.3390/agriculture10010016

Park, M. G., Choi, J., Hong, Y. S., Park, C. G., Kim, B. G., Lee, S. Y., … Cha, W. (2020). Negative effect of methyl bromide fumigation work on the central nervous system. *PLoS ONE*, *15*(8 August), 1–18. https://doi.org/10.1371/journal.pone.0236694

Passari, A. K., Upadhyaya, K., Singh, G., Abdel-Azeem, A. M., Thankappan, S., Uthandi, S., … Singh, B. P. (2019). Enhancement of disease resistance, growth potential, and

photosynthesis in tomato (Solanum lycopersicum) by inoculation with an endophytic actinobacterium, Streptomyces thermocarboxydus strain BPSAC147. *PLoS ONE*, *14*(7), 1–20. https://doi.org/10.1371/journal.pone.0219014

Pathology, P. (2019). *S*oil-borne antagonists for biological control of bacterial wilt disease caused by *Ralstonia solanacearum* in tomato and pepper author (s): M . T. Nguyen and S . L . Ranamukhaarachchi published by : springer stable url : https://www.jstor.org/stable/4199. 92(2), 395–405.

Pavan Kumar, S., Raja Babu, K., & Srinivasulu, A. (2018). Symptomology of major fungal diseases on tomato and its management. *~ 1817 ~ Journal of Pharmacognosy and Phytochemistry*, *7*(6), 1817–1821.

Pawaskar, J., Joshi, M. S., Navathe, S., Agale, R. C., & Sawant Konkan Krishi Vidyapeeth, B. (2014). Physiological and Biochemical Characters of Ralstonia solanacearum. *International Journal of Research in Agricultural Sciences*, *1*(6), 2348–3997. Retrieved from https://www.researchgate.net/publication/273059476

Payum, T. (2020). Proximate composition and GC-MS analysis of ethanol extract of Solanum spirale Roxb. *Current Botany*, *11*, 200–204. https://doi.org/10.25081/cb.2020.v11.6394

Persley, D. (2012). *Integrated Viral Disease Management in Vegetable Crops*. 154 pp. Retrieved from http://ausveg.com.au/intranet/technical-insights/docs/120029_VG07128_pdf_file_3719.pdf

Phuyal, N., Jha, P. K., Raturi, P. P., & Rajbhandary, S. (2020). In Vitro Antibacterial Activities of Methanolic Extracts of Fruits, Seeds, and Bark of Zanthoxylum armatum DC. *Journal of Tropical Medicine*, *2020*. https://doi.org/10.1155/2020/2803063

Plazas, M., Vilanova, S., Gramazio, P., Rodríguez-Burruezo, A., Fita, A., Herraiz, F. J., … Prohens, J. (2016). Interspecific hybridization between eggplant and wild relatives from different genepools. *Journal of the American Society for Horticultural Science*, *141*(1), 34–44. https://doi.org/10.21273/jashs.141.1.34

Polkade, A. V., Mantri, S. S., Patwekar, U. J., & Jangid, K. (2016). Quorum sensing: An under-explored phenomenon in the phylum Actinobacteria. *Frontiers in Microbiology*, *7*(FEB), 1–13. https://doi.org/10.3389/fmicb.2016.00131

Poojary, M. M., Vishnumurthy, K. A., & Vasudeva Adhikari, A. (2015). Extraction, characterization and biological studies of phytochemicals from Mammea suriga. *Journal of Pharmaceutical Analysis*, *5*(3), 182–189. https://doi.org/10.1016/j.jpha.2015.01.002

Prepared by SHEP PLUS. Tomato production (n.d.).

Qureshi, Z., Khan, T., Shah, A. J., & Wahid, F. (2019). Solanum incanum extract enhances wound healing and tissue regeneration in burn mice model. *Bangladesh Journal of Pharmacology*, *14*(2), 101–106. https://doi.org/10.3329/bjp.v14i2.40098

Rakha, M., Namisy, A., Chen, J.-R., El-Mahrouk, M. E., Metwally, E., Taha, N., … Taher, D. (2020). Development of Interspecific Hybrids between a Cultivated Eggplant Resistant to Bacterial Wilt (Ralstonia solanacearum) and Eggplant Wild Relatives for the Development of Rootstocks. *Plants*, *9*(10), 1405. https://doi.org/10.3390/plants9101405

Ralte, L. (n.d.). Traditionally used edible Solanaceae plants of *Mizoram* , India have high antioxidant and antimicrobial potential for effective phytopharmaceutical and nutraceutical formulations. 1–17.

Rawat, J., Saxena, J., & Sanwal, P. (2019). Biochar: A Sustainable Approach for Improving Plant Growth and Soil Properties. *Biochar - An Imperative Amendment for Soil and the Environment*. https://doi.org/10.5772/intechopen.82151

Raza, A., Razzaq, A., Mehmood, S. S., Zou, X., Zhang, X., Lv, Y., & Xu, J. (2019). Impact of climate change on crops adaptation and strategies to tackle its outcome: A review. *Plants*, *8*(2). https://doi.org/10.3390/plants8020034

Razmavar, S., Abdulla, M. A., Ismail, S. B., & Hassandarvish, P. (2014). Antibacterial

activity of leaf extracts of Baeckea frutescens against methicillin-resistant Staphylococcus aureus. *BioMed Research International*, *2014*. https://doi.org/10.1155/2014/521287

Review, M. I. A., Gull, P., Pateiro, M., & Munekata, P. E. S. (2020). *Tomato as Potential Source of Natural Additives for*.

Reyes-Escobar, J., Zagal, E., Sandoval, M., Navia, R., & Muñoz, C. (2015). Development of a biochar-plant-extract-based nitrification inhibitor and its application in field conditions. *Sustainability (Switzerland)*, *7*(10), 13585–13596. https://doi.org/10.3390/su71013585

Risticevic, S. (2012). Solid phase microextraction coupled to comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry for metabolite profiling of apples : Potential of non-invasive in vivo sampling assay in characterization of metabolome. *University of Waterloo, PhD Thesis*.

Ronsse, F., van Hecke, S., Dickinson, D., & Prins, W. (2013). Production and characterization of slow pyrolysis biochar: Influence of feedstock type and pyrolysis conditions. *GCB Bioenergy*, *5*(2), 104–115. https://doi.org/10.1111/gcbb.12018

Rotenberg, D., Jacobson, A. L., Schneweis, D. J., & Whitfield, A. E. (2015). Thrips transmission of tospoviruses. *Current Opinion in Virology*, *15*, 80–89. https://doi.org/10.1016/j.coviro.2015.08.003

Roy, R., Tiwari, M., Donelli, G., & Tiwari, V. (2018). Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. *Virulence*, *9*(1), 522–554. https://doi.org/10.1080/21505594.2017.1313372

RSA. (2015). Report of a Study on Fresh Vegetables Market in Kenya Desk Review By Research Solutions Africa (RSA) Ltd December , 2015. 1–42.

Saad, M. J., Hua, C. C., Misran, S., Zakaria, S., Sajab, M. S., & Abdul Rahman, M. H. (2020). Rice husk activated carbon with naoh activation: Physical and chemical

properties. *Sains Malaysiana*, *49*(9), 2261–2267. https://doi.org/10.17576/jsm-2020-4909-23

Sahle, T., & Okbatinsae, G. (2017). Phytochemical investigation and antimicrobial activity of the fruit extract of Solanum incanum grown in Eritrea. *Ornamental and Medicinal Plants*, *1*(1), 15–25.

Sakhiya, A. K., Anand, A., & Kaushal, P. (2020). Production, activation, and applications of biochar in recent times. In *Biochar* (Vol. 2). https://doi.org/10.1007/s42773-020-00047-1

Sakthivel, K., Gautam, R. K., Kumar, K., Dam Roy, S., Kumar, A., Devendrakumar, C., … Vinatzer, B. A. (2016). Diversity of ralstonia solanacearum strains on the Andaman Islands in India. *Plant Disease*, *100*(4), 732–738. https://doi.org/10.1094/PDIS-03-15-0258-RE

Sakulnarmrat, K., Dalar, A., Sukru Bengu, A., & Konczak, I. (2018). Phytochemical composition and health-enhancing properties of Oryza sativa L. leaf tea. *Integrative Food, Nutrition and Metabolism*, *5*(6), 1–11. https://doi.org/10.15761/ifnm.1000234

Salah, A. I., Ali, H. A. M., & Imad, H. H. (2015). Spectral analysis and anti-bacterial activity of methanolic fruit extract of Citrullus colocynthis using gas chromatography-mass spectrometry. *African Journal of Biotechnology*, *14*(46), 3131–3158. https://doi.org/10.5897/ajb2015.14957

Sande, D., Mullen, J., Wetzstein, M., & Houston, J. (2011). Environmental impacts from pesticide use: A case study of soil fumigation in Florida tomato production. *International Journal of Environmental Research and Public Health*, *8*(12), 4649–4661. https://doi.org/10.3390/ijerph8124649

Sang, M. K., & Kim, K. D. (2012). The volatile-producing Flavobacterium johnsoniae strain GSE09 shows biocontrol activity against Phytophthora capsici in pepper. 383–398. https://doi.org/10.1111/j.1365-2672.2012.05330.x

Sanoubar, R., & Barbanti, L. (2017). Fungal diseases on tomato plant under greenhouse condition. *European Journal of Biological Research*, *7*(4), 299–308. https://doi.org/10.5281/zenodo.1011161

Santiago, T. R., Grabowski, C., Rossato, M., Romeiro, R. S., & Mizubuti, E. S. G. (2015). Biological control of eucalyptus bacterial wilt with rhizobacteria. *Biological Control*, *80*, 14–22. https://doi.org/10.1016/j.biocontrol.2014.09.007

Satyal, P., Maharjan, S., & Setzer, W. N. (2015). Volatile Constituents from the Leaves, Fruits (Berries), Stems and Roots of Solanum xanthocarpum from Nepal. *Natural Product Communications*, *10*(2), 361–364. https://doi.org/10.1177/1934578x1501000239

Sbhatu, D. B., & Abraha, H. B. (2020). Preliminary Antimicrobial Profile of Solanum incanum L.: A Common Medicinal Plant. *Evidence-Based Complementary and Alternative Medicine*, *2020*. https://doi.org/10.1155/2020/3647065

Seed, P., & Techniques, E. (2014). *Popular Kheti*. *1*(1), 162–163.

Senthil, J., Rameashkannan, M. V., & Mani, P. (2016). Phytochemical Profiling of Ethanolic Leaves Extract of Ipomoea sepiaria (Koenig Ex . Roxb). *International Journal of Innovative Research in Science, Engineering and Technology*, *5*(3), 3140–3147. https://doi.org/10.15680/IJIRSET.2016.0503063

Shaaban, A., Se, S. M., Mitan, N. M. M., & Dimin, M. F. (2013). Characterization of biochar derived from rubber wood sawdust through slow pyrolysis on surface porosities and functional groups. *Procedia Engineering*, *68*, 365–371. https://doi.org/10.1016/j.proeng.2013.12.193

Shah, K. K., Modi, B., Pandey, H. P., Subedi, A., Aryal, G., Pandey, M., & Shrestha, J. (2021). Diversified Crop Rotation: An Approach for Sustainable Agriculture Production. *Advances in Agriculture*, *2021*. https://doi.org/10.1155/2021/8924087

Shamshiri, R. R., Jones, J. W., Thorp, K. R., Ahmad, D., Man, H. C., & Taheri, S. (2018).

Review of optimum temperature, humidity, and vapour pressure deficit for microclimate evaluation and control in greenhouse cultivation of tomato: A review. *International Agrophysics*, *32*(2), 287–302. https://doi.org/10.1515/intag-2017-0005

Sharma, D., & Singh, Y. (2019). Characterization of *Ralstonia solanacearum* isolates using biochemical , cultural , molecular methods and pathogenicity tests. *8*(4), 2884–2889.

She, X., Yu, L., Lan, G., Tang, Y., & He, Z. (2017). Identification and genetic characterization of ralstonia solanacearum species complex isolates from cucurbita maxima in China. *Frontiers in Plant Science*, *8*(October). https://doi.org/10.3389/fpls.2017.01794

Sherzod, R., Yang, E. Y., Cho, M. C., Chae, S. Y., Kim, J. H., Nam, C. W., & Chae, W. B. (2019). Traits affecting low temperature tolerance in tomato and its application to breeding program. *Plant Breeding and Biotechnology*, *7*(4), 350–359. https://doi.org/10.9787/PBB.2019.7.4.350

Shettar, A. K., Sateesh, M. K., Kaliwal, B. B., & Vedamurthy, A. B. (2017). In vitro antidiabetic activities and GC-MS phytochemical analysis of Ximenia americana extracts. *South African Journal of Botany*, *111*, 202–211. https://doi.org/10.1016/j.sajb.2017.03.014

Sing'ombe, O. (2019). Study of Specific Growth Inhibitors of *Ralstonia solanacearum*. (March). Retrieved from http://ousar.lib.okayama-u.ac.jp/files/public/5/56829/20190627135510841036/K0005988_fulltext.pdf

Singh, D., Yadav, D. K., Sinha, S., & Choudhary, G. (2014). Effect of temperature, cultivars, injury of root and inoculums load of Ralstonia solanacearum to cause bacterial wilt of tomato. *Archives of Phytopathology and Plant Protection*, *47*(13), 1574–1583. https://doi.org/10.1080/03235408.2013.851332

Singh, V. K., Singh, A. K., & Kumar, A. (2017a). Disease management of tomato through PGPB: current trends and future perspective. *3 Biotech*, *7*(4). https://doi.org/10.1007/s13205-017-0896-1

Singh, V. K., Singh, A. K., & Kumar, A. (2017b). Disease management of tomato through PGPB: current trends and future perspective. *3 Biotech*, *7*(4), 1–10. https://doi.org/10.1007/s13205-017-0896-1

Ślesak, I., Ślesak, H., Zimak-Piekarczyk, P., & Rozpądek, P. (2016). Enzymatic Antioxidant Systems in Early Anaerobes: Theoretical Considerations. *Astrobiology*, *16*(5), 348–358. https://doi.org/10.1089/ast.2015.1328

Sobiesiak, M. (2017). Chemical Structure of Phenols and Its Consequence for Sorption Processes. *Phenolic Compounds - Natural Sources, Importance and Applications*. https://doi.org/10.5772/66537

States, F. (2020). Crop production manual. In *Crop production manual*. https://doi.org/10.4060/ca7556en

Sun, J., He, F., Pan, Y., & Zhang, Z. (2017). Effects of pyrolysis temperature and residence time on physicochemical properties of different biochar types. *Acta Agriculturae Scandinavica Section B: Soil and Plant Science*, *67*(1), 12–22. https://doi.org/10.1080/09064710.2016.1214745

Tabe-Ojong, M. P., Molua, E. L., Nzie, J. R. M., & Fuh, G. L. (2020). Production and supply of tomato in Cameroon: Examination of the comparative effect of price and non-price factors. *Scientific African*, *10*, e00574. https://doi.org/10.1016/j.sciaf.2020.e00574

Tancos, M. A., Chalupowicz, L., Barash, I., Manulis-Sasson, S., & Smart, C. D. (2013). Tomato fruit and seed colonization by Clavibacter michiganensis subsp. michiganensis through external and internal routes. *Applied and Environmental Microbiology*, *79*(22), 6948–6957. https://doi.org/10.1128/AEM.02495-13

Tayibi, S., Monlau, F., Fayoud, N. E., Abdeljaoued, E., Hannache, H., Zeroual, Y., … Barakat, A. (2021). Production and Dry Mechanochemical Activation of Biochars Derived from Moroccan Red Macroalgae Residue and Olive Pomace Biomass for Treating Wastewater: Thermodynamic, Isotherm, and Kinetic Studies. *ACS Omega*, *6*(1), 159–171. https://doi.org/10.1021/acsomega.0c04020

Thairu, Y., Usman, Y., & Nasir, I. (2014). Laboratory perspective of gram staining and its significance in investigations of infectious diseases. *Sub-Saharan African Journal of Medicine*, *1*(4), 168. https://doi.org/10.4103/2384-5147.144725

The interactions between biotic and abiotic factors that influence the sustainability of tomato production in South Africa. (2016).

Tomczyk, A., Sokołowska, Z., & Boguta, P. (2020). Biochar physicochemical properties: pyrolysis temperature and feedstock kind effects. *Reviews in Environmental Science and Biotechnology*, *19*(1), 191–215. https://doi.org/10.1007/s11157-020-09523-3

Tomlinson, D. L., Elphinstone, J. G., Soliman, M. Y., Hanafy, M. S., Shoala, T. M., El-Fatah, H. A., … Janse, J. D. (2009). Recovery of Ralstonia solanacearum from canal water in traditional potato-growing areas of Egypt but not from designated Pest-Free Areas (PFAs). *European Journal of Plant Pathology*, *125*(4), 589–601. https://doi.org/10.1007/s10658-009-9508-1

Tondo, M. L., de Pedro-Jové, R., Vandecaveye, A., Piskulic, L., Orellano, E. G., & Valls, M. (2020). KatE From the Bacterial Plant Pathogen Ralstonia solanacearum Is a Monofunctional Catalase Controlled by HrpG That Plays a Major Role in Bacterial Survival to Hydrogen Peroxide. *Frontiers in Plant Science*, *11*(July), 1–12. https://doi.org/10.3389/fpls.2020.01156

Vambe, M. (2018). Pharmacological and phytochemical evaluation of seven plants used for microbial-related ailments in South African traditional medicine.

Villela, lucia maria aversa. (2013). Efficacy of plant extracts and extracting agents against *Colletotrichum gloeosporoides* of pawpaw fruits. *Journal of Chemical Information and Modeling*, *53*(9), 1689–1699.

Vu, N. T., & Oh, C. S. (2020). Bacteriophage usage for bacterial disease management and diagnosis in plants. *Plant Pathology Journal*, *36*(3), 204–217. https://doi.org/10.5423/PPJ.RW.04.2020.0074

Vu, T. T., Kim, H., Tran, V. K., Vu, H. D., Hoang, T. X., Han, J. W., … Kim, J. C. (2017). Antibacterial activity of tannins isolated from Sapium baccatum extract and use for control of tomato bacterial wilt. *PLoS ONE*, *12*(7), 1–12. https://doi.org/10.1371/journal.pone.0181499

Wang, G., Cao, F., Chang, L., Guo, X., & Wang, J. (2014). Temperature has more effects than soil moisture on biosynthesis of flavonoids in Ginkgo (Ginkgo biloba L.) leaves. *New Forests*, *45*(6), 797–812. https://doi.org/10.1007/s11056-014-9437-5

Wang, H., Luo, Y., Dai, H., & Mei, W. (2013). Antibacterial activity against Ralstonia solanacearum of phenolic constituents isolated from dragon's blood. *Natural Product Communications*, *8*(3), 337–338. https://doi.org/10.1177/1934578x1300800316

Wang, S., Gao, B., Zimmerman, A. R., Li, Y., Ma, L., Harris, W. G., & Migliaccio, K. W. (2015). Physicochemical and sorptive properties of biochars derived from woody and herbaceous biomass. *Chemosphere*, *134*, 257–262. https://doi.org/10.1016/j.chemosphere.2015.04.062

Welbaum, G. E. (2017). Vegetable history, nomenclature, and classification. *Vegetable Production and Practices*, 1–15. https://doi.org/10.1079/9781780645346.0001

Woolf, D. (2010). E, AJ; Street-Perrott, FA; Lehmann, J.; Josheph, S. *Sustainable Biochar to Mitigate Global Climate Change. Nat. Commun*, *1*(56), 1.

Xiao, G., Xu, T., Faheem, M., Xi, Y., Zhou, T., Moryani, H. T., … Du, J. (2021). Evolution of singlet oxygen by activating peroxydisulfate and peroxymonosulfate: A review. *International Journal of Environmental Research and Public Health*, *18*(7). https://doi.org/10.3390/ijerph18073344

Xie, Y., Yang, W., Tang, F., Chen, X., & Ren, L. (2014). Antibacterial Activities of Flavonoids: Structure-Activity Relationship and Mechanism. *Current Medicinal Chemistry*, *22*(1), 132–149. https://doi.org/10.2174/09298673216661409161134443

Xu, J., Wolters-Arts, M., Mariani, C., Huber, H., & Rieu, I. (2017). Heat stress affects

vegetative and reproductive performance and trait correlations in tomato (Solanum lycopersicum). *Euphytica*, *213*(7), 1–12. https://doi.org/10.1007/s10681-017-1949-6

Yang, X., Zhang, S., Ju, M., & Liu, L. (2019). Preparation and modification of biochar materials and their application in soil remediation. *Applied Sciences (Switzerland)*, *9*(7). https://doi.org/10.3390/app9071365

Yao, J., & Allen, C. (2006). *Chemotaxis Is Required for Virulence and Competitive Fitness of the Bacterial Wilt Pathogen Ralstonia solanacearum*. *188*(10), 3697–3708. https://doi.org/10.1128/JB.188.10.3697

Yuan, J. H., Xu, R. K., & Zhang, H. (2011). The forms of alkalis in the biochar produced from crop residues at different temperatures. *Bioresource Technology*, *102*(3), 3488–3497. https://doi.org/10.1016/j.biortech.2010.11.018

Yuliar, Asi Nion, Y., & Toyota, K. (2015). Recent trends in control methods for bacterial wilt diseases caused by Ralstonia solanacearum. *Microbes and Environments*, *30*(1), 1–11. https://doi.org/10.1264/jsme2.ME14144

Zeist, A. R., de Resende, J. T. V., Pozzebon, B. C., Gabriel, A., da Silva, A. A., & Zeist, R. A. (2019). Combination of solarization, biofumigation and grafting techniques for the management of bacterial wilt in tomato. *Horticultura Brasileira*, *37*(3), 260–265. https://doi.org/10.1590/s0102-053620190302

Zeng, Y., Xue, Y., Long, L., & Yan, J. (2019). Novel Crayfish Shell Biochar Nanocomposites Loaded with Ag-TiO 2 Nanoparticles Exhibit Robust Antibacterial Activity. *Water, Air, and Soil Pollution*, *230*(2), 1–9. https://doi.org/10.1007/s11270-019-4104-2

Zhang, J., Liu, J., & Liu, R. (2015). Effects of pyrolysis temperature and heating time on biochar obtained from the pyrolysis of straw and lignosulfonate. *Bioresource Technology*, *176*, 288–291. https://doi.org/10.1016/j.biortech.2014.11.011

Zhang, M., Riaz, M., Zhang, L., El-Desouki, Z., & Jiang, C. (2019). Biochar induces changes

to basic soil properties and bacterial communities of different soils to varying degrees at 25 mm rainfall: More effective on acidic soils. *Frontiers in Microbiology*, *10*(JUN). https://doi.org/10.3389/fmicb.2019.01321

Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products : a comprehensive review. *Chinese Medicine*, 1–26. https://doi.org/10.1186/s13020-018-0177-x

Zhang, S., Liu, X., Jiang, Q., Shen, G., & Ding, W. (2017). Legacy effects of continuous chloropicrin-fumigation for 3-years on soil microbial community composition and metabolic activity. *AMB Express*, *7*(1), 1–11. https://doi.org/10.1186/s13568-017-0475-1

Zhao, F., Wang, P., Lucardi, R. D., Su, Z., & Li, S. (2020). Natural sources and bioactivities of 2,4-di-tert-butylphenol and its analogs. *Toxins*, *12*(1), 1–26. https://doi.org/10.3390/toxins12010035

Zhao, S. X., Ta, N., & Wang, X. D. (2017). Effect of temperature on the structural and physicochemical properties of biochar with apple tree branches as feedstock material. *Energies*, *10*(9). https://doi.org/10.3390/en10091293

Zhu, K., Fu, H., Zhang, J., Lv, X., Tang, J., & Xu, X. (2012). Studies on removal of NH4+-N from aqueous solution by using the activated carbons derived from rice husk. *Biomass and Bioenergy*, *43*, 18–25. https://doi.org/10.1016/j.biombioe.2012.04.005

Zinaye, B. (2020). A Review on Phytochemical Medicinal Plants Against Pathogenic Bacteria in South East Ethiopia. *Advances in Life Science and Technology*, *78*, 35–42. https://doi.org/10.7176/alst/78-05

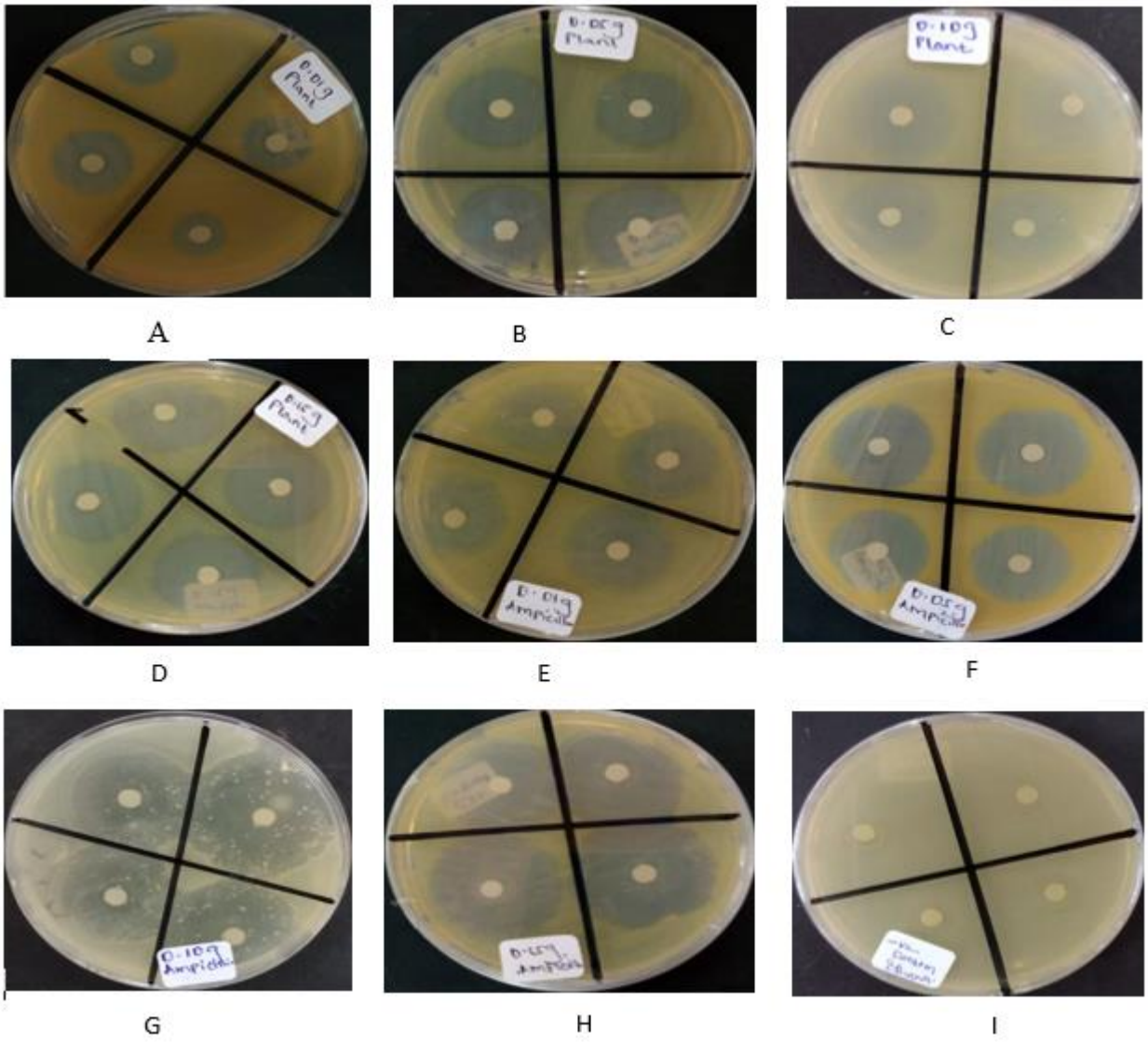
Zlatić, N., Jakovljević, D., & Stanković, M. (2019). Temporal, plant part, and interpopulation variability of secondary metabolites and antioxidant activity of Inula helenium l. *Plants*, *8*(6). https://doi.org/10.3390/plants8060179

Zolfi Bavariani, M., Ronaghi, A., & Ghasemi, R. (2019). Influence of Pyrolysis

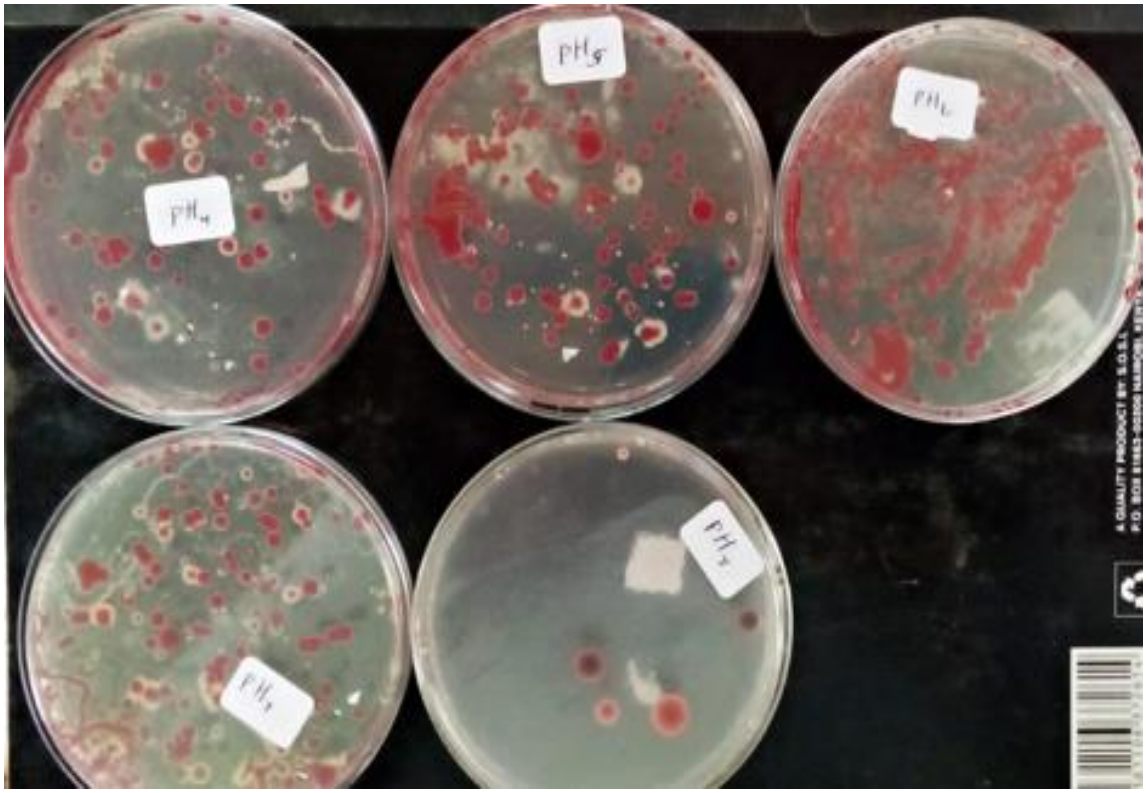
Temperatures on FTIR Analysis, Nutrient Bioavailability, and Agricultural use of Poultry Manure Biochars. *Communications in Soil Science and Plant Analysis*, *50*(4), 402–411. https://doi.org/10.1080/00103624.2018.1563101

APPENDIX

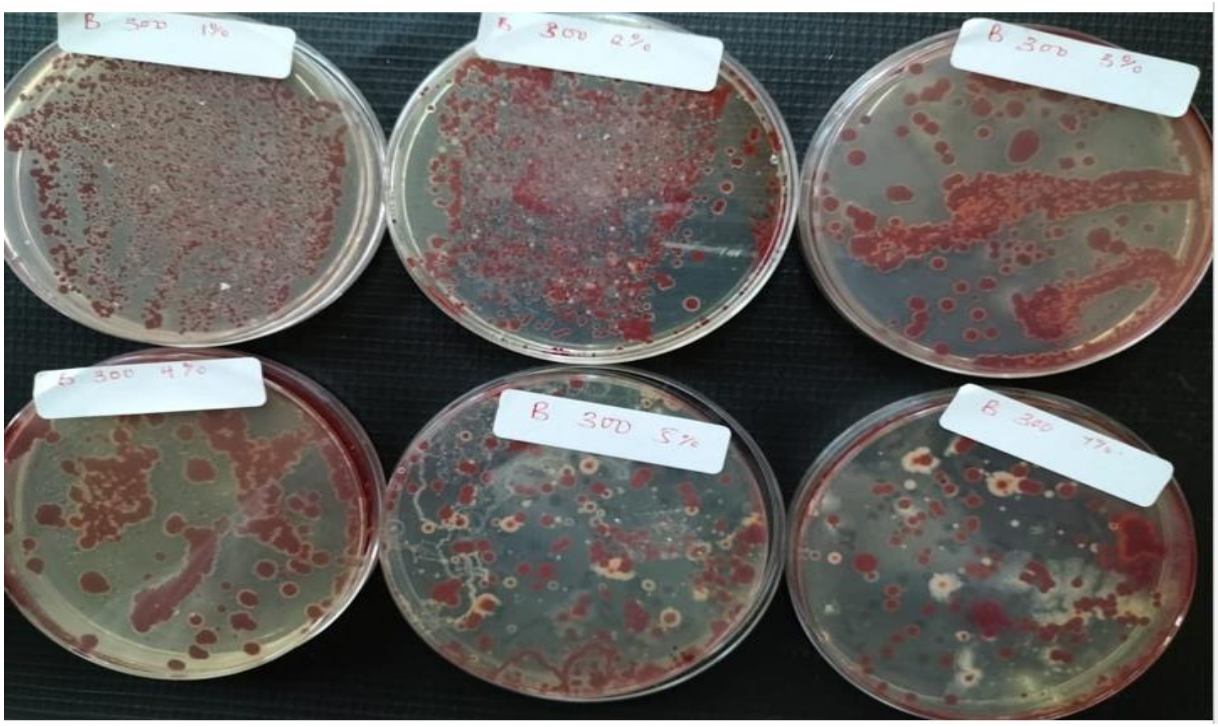
Appendix 1: Clear zone around the agar disk; A, B, C and D are plant extract treatment at a concentration of 0.01, 0.05, 0.10, and 0.15 g/10 ml. E, F, G, and H are ampicillin treatment at the aforementioned concentrations. I is negative control

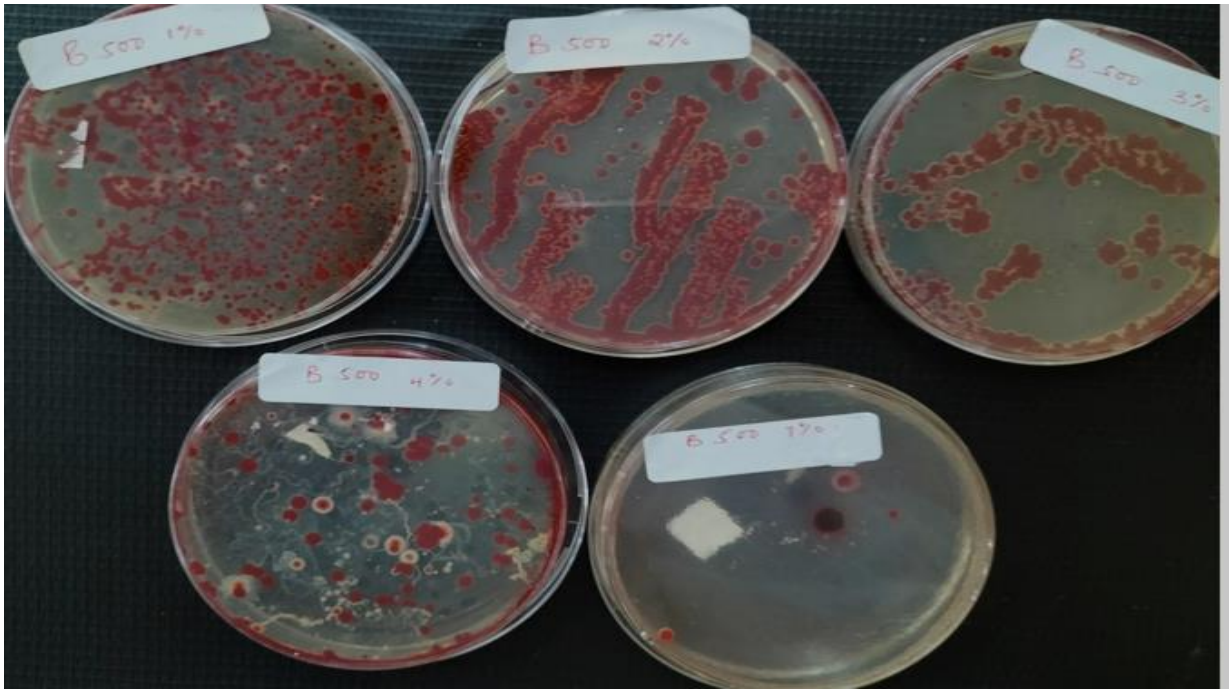


Appendix 2: Effect of pH on the growth of *Ralstonia solanacearum*

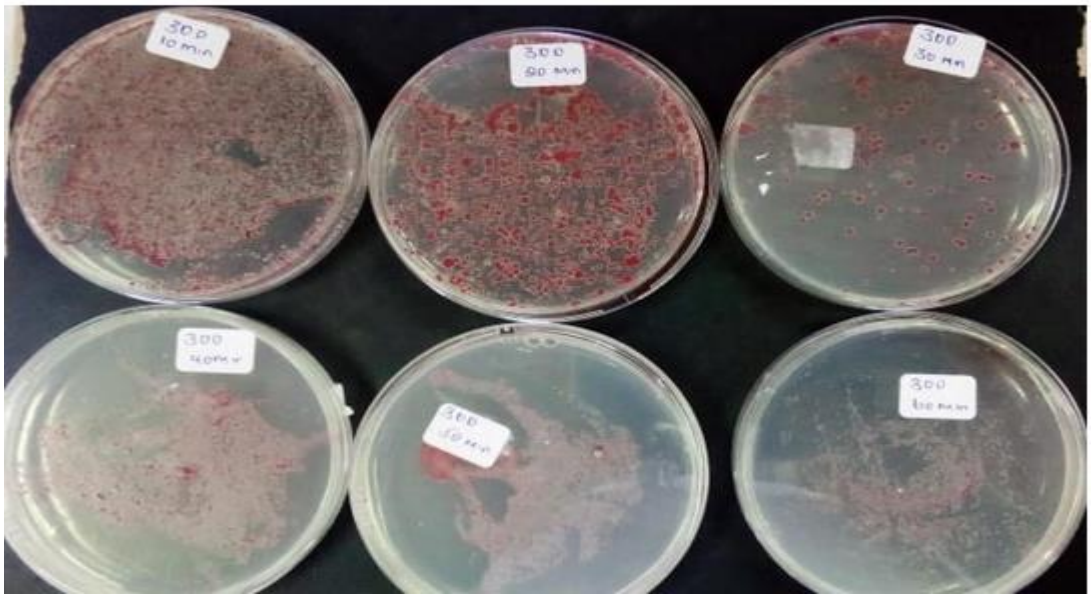


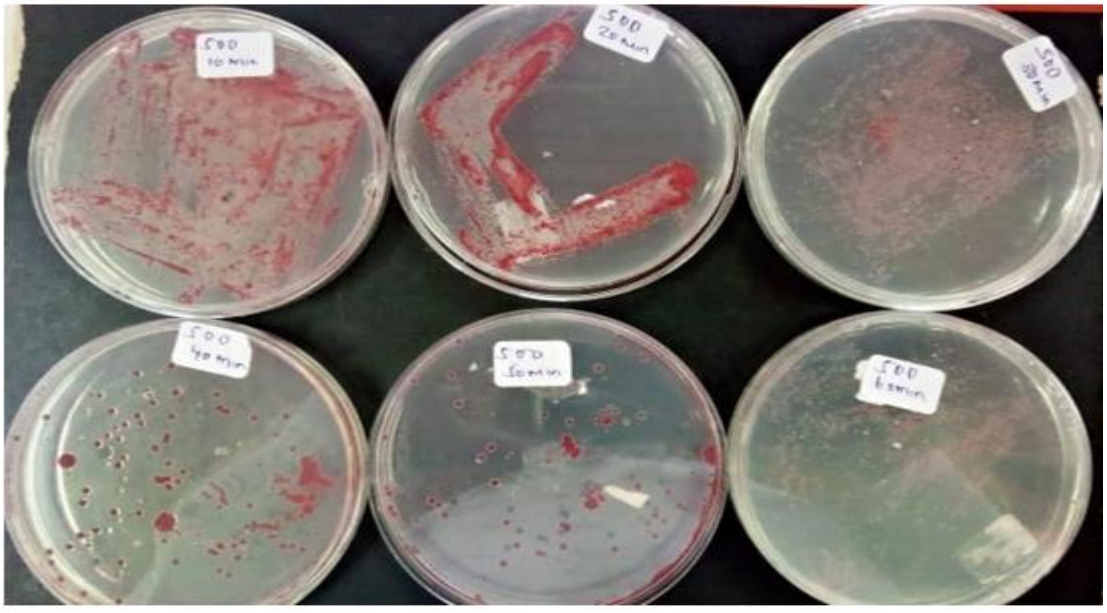
Appendix 3: *In vitro* antibacterial activity of biochar composite at different concentrations





Appendix 4: Effect of time on the antibacterial activity of the biochar composites





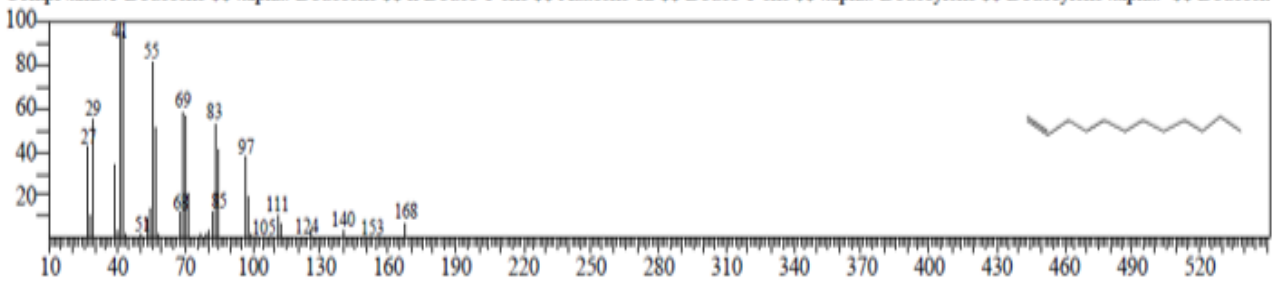
Appendix 5: Wilting tomato plants

Appendix 6: Dead tomato plants

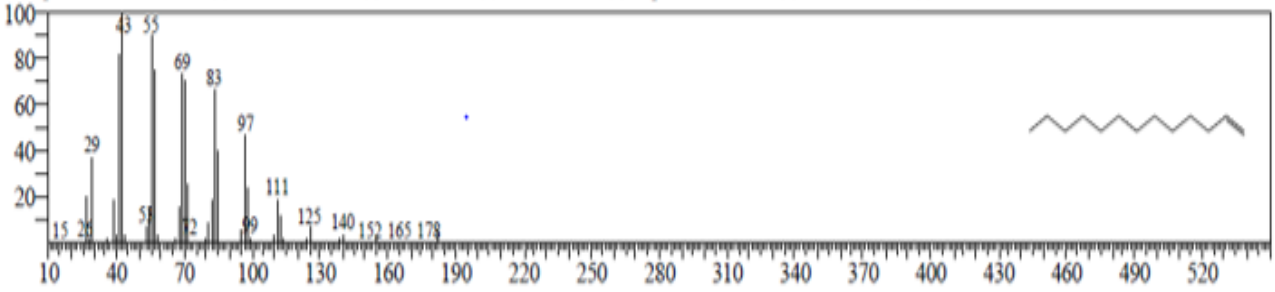
Appendix 7: Non-affected tomato plants

Appendix 8: Mass spectra of compounds identified in *S. incanum*

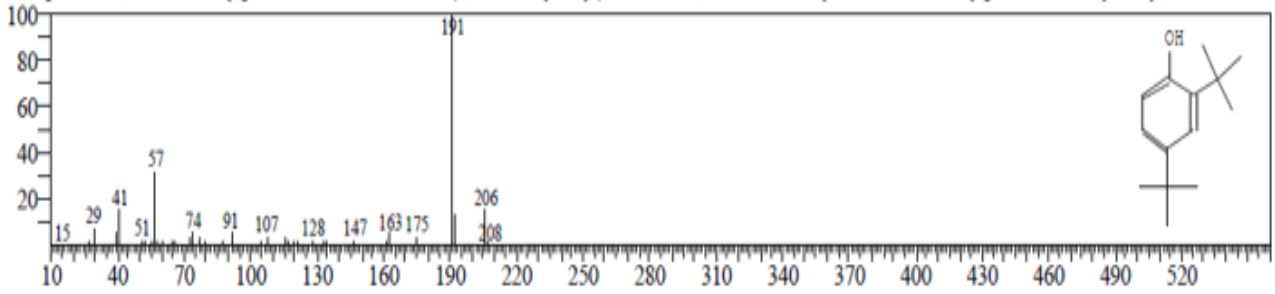
Hit#:1 Entry:25881 Library:NIST14.lib
SI:92 Formula:C12H24 CAS:112-41-4 MolWeight:168 RetIndex:1204
CompName:1-Dodecene $$ .alpha.-Dodecene $$ n-Dodec-1-ene $$ Adecene 12 $$ Dodec-1-ene $$ .alpha.-Dodecylene $$ Dodecylene .alpha.- $$ Dodecen



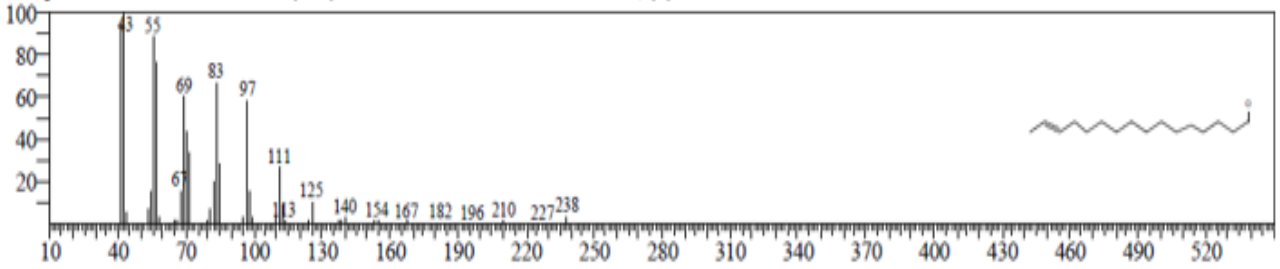
Hit#:1 Entry:34798 Library:NIST14.lib
SI:96 Formula:C13H26 CAS:2437-56-1 MolWeight:182 RetIndex:1304
CompName:1-Tridecene $$ n-Tridec-1-ene $$ 1-C13H26 $$ Tridecene-1 $$ .alpha.-Tridecene $$ Tridec-1-ene $$



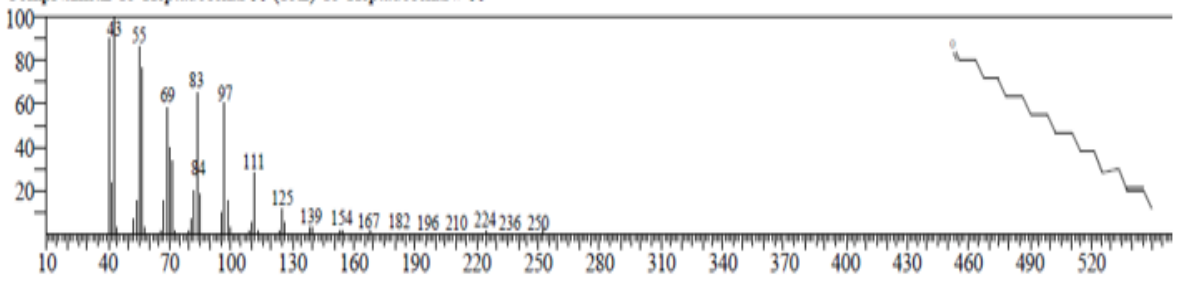
Hit#:1 Entry:51535 Library:NIST14.lib
SI:95 Formula:C14H22O CAS:96-76-4 MolWeight:206 RetIndex:1555
CompName:2,4-Di-tert-butylphenol $$ Phenol, 2,4-bis(1,1-dimethylethyl)- $$ Phenol, 2,4-di-tert-butyl- $$ 2,4-di-t-Butylphenol $$ 1-Hydroxy-2,4-di-tert-bu



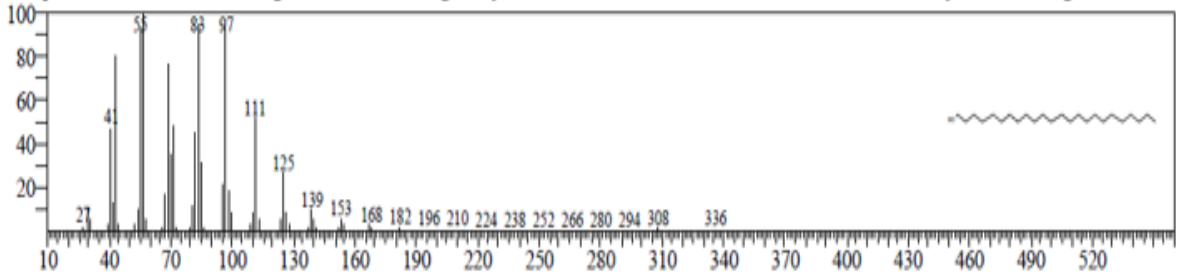
Hit#:1 Entry:77344 Library:NIST14.lib
SI:97 Formula:C16H30O CAS:330207-53-9 MolWeight:238 RetIndex:1808
CompName:E-14-Hexadecenal $$ (14E)-14-Hexadecenal $$ 14-Hexadecenal, (E)- $$



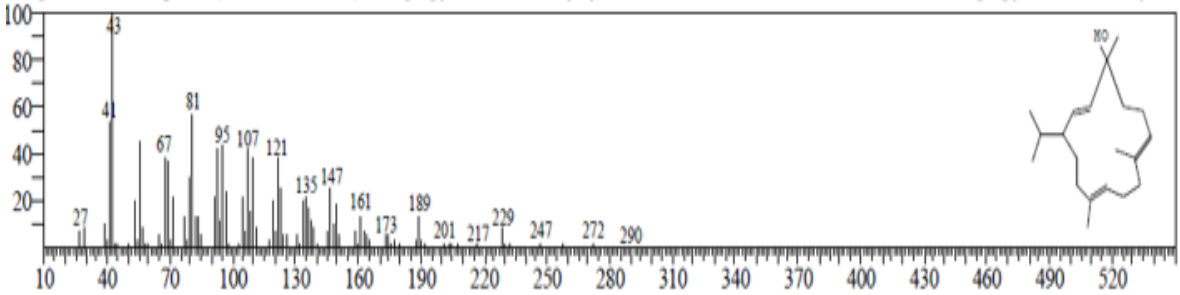
Hit#:1 Entry:89019 Library:NIST14.lib
SI:97 Formula:C17H32O CAS:0-00-0 MolWeight:252 RetIndex:1907
CompName:E-15-Heptadecenal $$ (15E)-15-Heptadecenal # $$



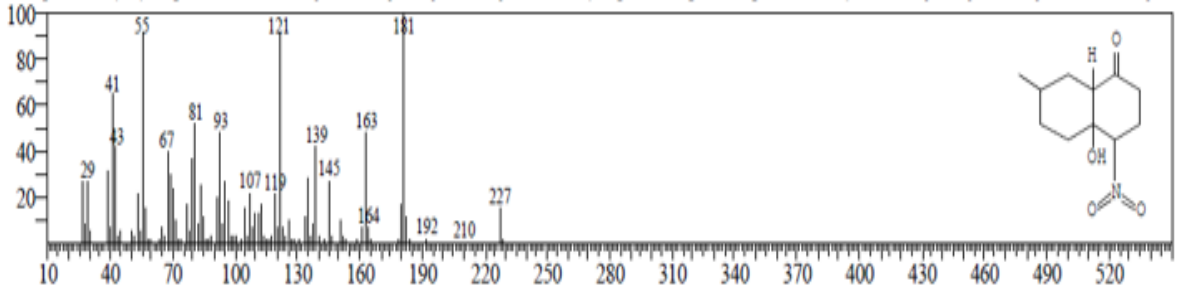
Hit#:1 Entry:178097 Library:NIST14.lib
SI:96 Formula:C24H50O CAS:506-51-4 MolWeight:354 RetIndex:2650
CompName:n-Tetracosanol-1 $$ Lignoceric alcohol $$ Lignoceryl alcohol $$ 1-Tetracosanol $$ Tetracosan-1-ol $$ Tetracosyl alcohol $$ Lignocerol $$ Te



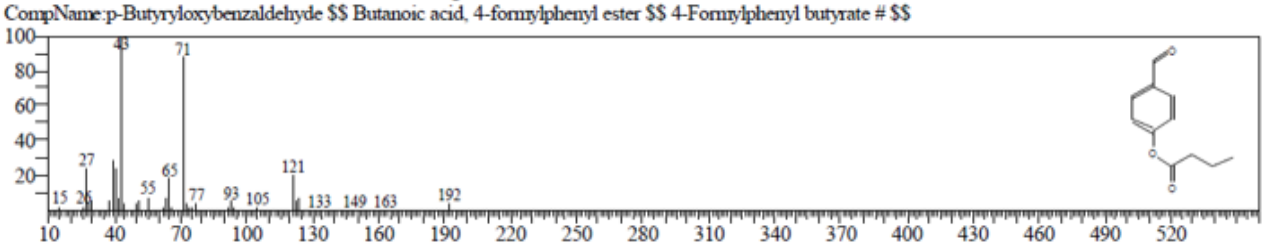
Hit#:1 Entry:122493 Library:NIST14.lib
SI:56 Formula:C20H34O CAS:25269-17-4 MolWeight:290 RetIndex:2211
CompName:Thunbergol $$ (1R,2E,4S,7E,11E)-4-Isopropyl-1,7,11-trimethylcyclotetradeca-2,7,11-trienol $$ Isocembrol $$ 4-Isopropyl-1,7,11-trimethyl-2,



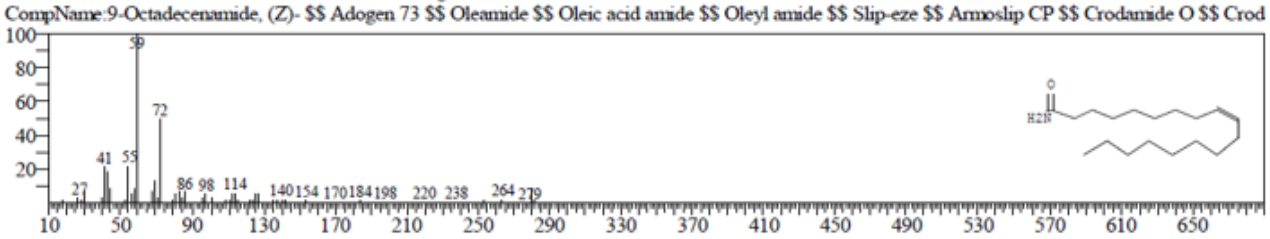
Hit#:1 Entry:68096 Library:NIST14.lib
SI:71 Formula:C11H17NO4 CAS:79880-68-5 MolWeight:227 RetIndex:1827
CompName:1(2H)-Naphthalenone, octahydro-4a-hydroxy-7-methyl-4-nitro-, (4.alpha.,4a.alpha.,7.alpha.,8a.beta.)- $$ 4a-Hydroxy-7-methyl-4-nitrooctahydr



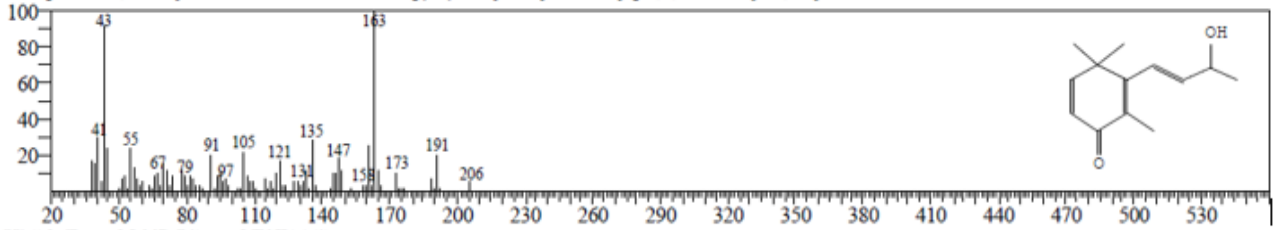
Hit#:2 Entry:40787 Library:NIST14.lib
SI:61 Formula:C11H12O3 CAS:50262-49-2 MolWeight:192 RetIndex:1561
CompName:p-Butyryloxybenzaldehyde $$ Butanoic acid, 4-formylphenyl ester $$ 4-Formylphenyl butyrate # $$



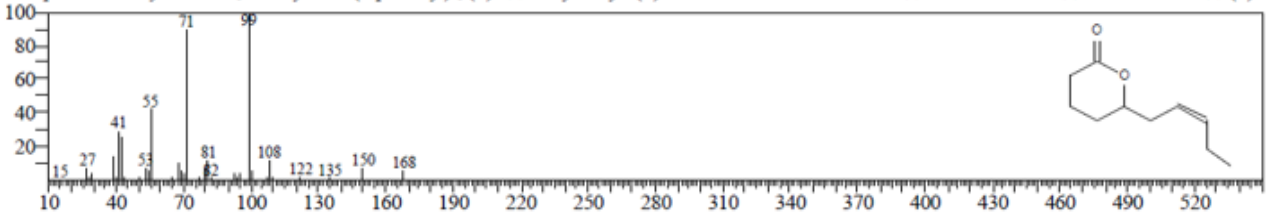
Hit#:1 Entry:114035 Library:NIST14.lib
SI:88 Formula:C18H35NO CAS:301-02-0 MolWeight:281 RetIndex:2228
CompName:9-Octadecenamide, (Z)- $$ Adogen 73 $$ Oleamide $$ Oleic acid amide $$ Oleyl amide $$ Slip-eze $$ Armoslip CP $$ Crodamide O $$ Crod



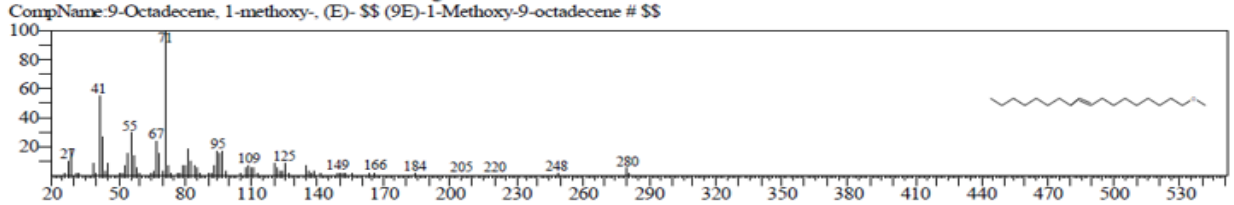
Hit#:1 Entry:51411 Library:NIST14.lib
SI:62 Formula:C13H18O2 CAS:0-00-0 MolWeight:206 RetIndex:1637
CompName:2,3-Dehydro-4-oxo-.beta.-ionol $$ 3-[(1E)-3-Hydroxy-1-butenyl]-2,4,4-trimethyl-2,5-cyclohexadien-1-one # $$



Hit#:2 Entry:25667 Library:NIST14.lib
SI:66 Formula:C10H16O2 CAS:25524-95-2 MolWeight:168 RetIndex:1412
CompName:2H-Pyran-2-one, tetrahydro-6-(2-pentenyl)-, (Z)- $$ 5-Hydroxy-7(Z)-decenoic acid-.delta.-lactone $$ Jasmine lactone $$ Jasminlactone $$ (Z)-



Hit#:3 Entry:115187 Library:NIST14.lib
SI:69 Formula:C19H38O CAS:56847-01-9 MolWeight:282 RetIndex:1994
CompName:9-Octadecene, 1-methoxy-, (E)- $$ (9E)-1-Methoxy-9-octadecene # $$



Appendix 9: Analysis of variance for antibacterial activity

In vitro antibacterial activity of plant extract and ampicillin

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	74.59375	3	24.86458	3.083979	0.021525	6.591382
Within Groups	32.25	4	8.0625			
Total	106.8438	7				

In vivo antibacterial activity of plant extract and ampicillin

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6711.111	4	1677.778	5.634328	0.031428	5.192168
Within Groups	1488.889	5	297.7778			
Total	8200	9				

In vivo antibacterial activity of biochar composite (BC 500 ℃+ P.E)

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	12217.28	8	1527.16	3.756073	0.014666	2.115223
Within Groups	21955.56	54	406.5844			
Total	34172.84	62				

In vivo antibacterial activity of biochar composite (BC 300 ℃+ P.E)

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6330.159	6	1055.026	3.88694	0.040912	2.572712
Within Groups	5700	21	271.4286			
Total	12030.16	27				

In vitro antibacterial activity of biochar composites (BC 300 ℃+ P.E and BC 500 ℃+ P.E)

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7116.407	5	1423.281	5.910728	0.02576	4.387374
Within Groups	1444.778	6	240.7963			
Total	8561.185	11				