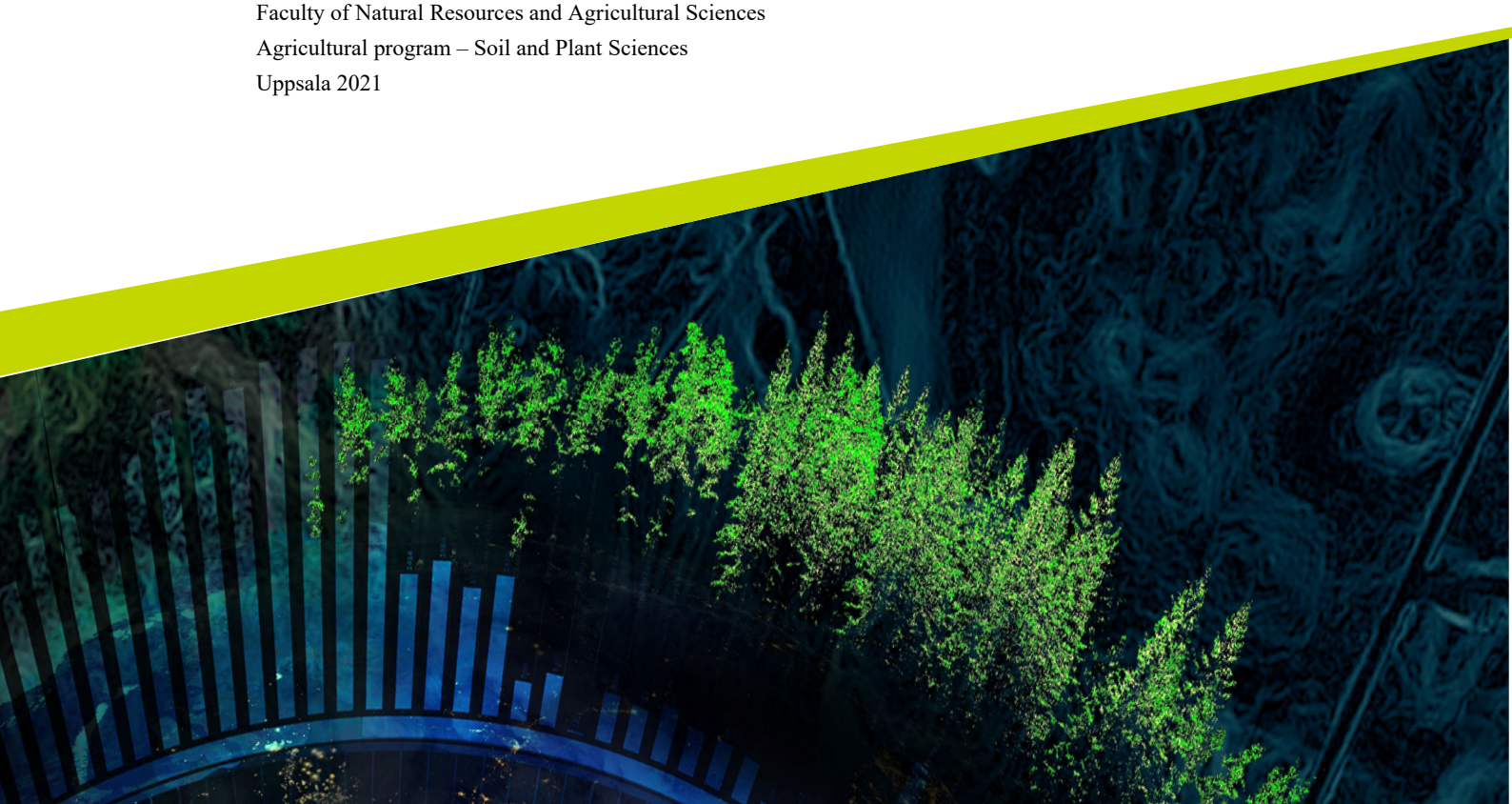
Plant products to control *Ralstonia solanacearum* causing bacterial wilt in tomatoes (*Solanum lycopersicum)* in Kenya

Växtmaterial för bekämpning av bakteriell vissnesjuka på tomat orsakad av Ralstonia solanacearum *i Kenya*

Anton Hampl

Independent project in Biology • 15 hp
Swedish University of Agricultural Sciences, SLU
Faculty of Natural Resources and Agricultural Sciences
Agricultural program – Soil and Plant Sciences
Uppsala 2021



Plant products to control *Ralstonia solanacearum* causing bacterial wilt in tomatoes (*Solanum lycopersicum*) in Kenya

Växtmaterial för bekämpning av bakteriell vissnesjuka på tomat orsakad av Ralstonia solanacearum *i Kenya*

Anton Hampl

Supervisor: Hanna Friberg, Department of Forest Mycology and Plant Pathology
Assistant supervisor: Dr. Esther Arunga, Agricultural Resource management, University of Embu
Examiner: Björn Andersson, Department of Forest Mycology and Plant Pathology

Credits: 15 credits
Level: First cycle, G2E
Course title: Independent project in Biology
Course code: EX0894
Programme/education: Agricultural Program – Soil and Plant Sciences
Course coordinating dept: Department of Forest Mycology and Plant Pathology

Place of publication: Uppsala
Year of publication: 2021
Online publication: https://stud.epsilon.slu.se/

Keywords: Kenya, Agriculture, *Solanum lycopersicum, Ralstonia solanacearum*, integrated pest management, phytobiocides

Swedish University of Agricultural Sciences
Faculty of Natural Resources and Agricultural Sciences
Department of Forest Mycology and Plant Pathology

Archiving and publishing

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. When you have approved, metadata and full text of your thesis will be visible and searchable online. When the document is uploaded it is archived as a digital file.

[X] YES, I hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work. https://www.slu.se/en/subweb/library/publish-and-analyse/register-and-publish/agreement-for-publishing/

[ ] NO, I do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.

Abstract

Plant pathogens are a significant contributor to yield losses around the globe. The plant pathogen *Ralstonia solanacearum* causes bacterial wilt, often resulting in yield loss in numerous vital crops such as tomatoes. In Kenya, this disease harms the income of the individual smallholder farmer, and to an extent might threaten this east African nation's food security. This study aimed to evaluate the effectiveness of using locally sourced plant products from *Lantana camara* (common lantana), *Allium sativum* (garlic), *Azadirachta indica* (Neem) and *Solanum incanum* (sodom apple) in the management of bacterial wilt in tomatoes (*Solanum lycopersicum*) in Kenya. The study was divided into two parts. In part one, the plant extracts were examined in a disc diffusion test to determine if plant extracts had an inhibitory effect on the growth of *R. solanacearum in vitro*. The second part was a greenhouse experiment conducted with tomato seedlings, where pulverised plant material to the soil substrate were added. The effectiveness of disease suppression of the different plant products was assessed using the AUDPC value (Area Under the Disease Progression Curve). No inhibitory effects from the plant extracts on the growth of *R. solanacearum* were observed in the *in vitro* experiment. The greenhouse experiment showed no significant difference between the treatments, which means that we cannot conclude that the species *L. camara*, *A. sativum*, *A. indica* and *S. incanum* have an inhibitory effect on the *in vitro* growth nor on the rate of disease progression of *R. solanacearum* in tomato. These experiments should be regarded as a pilot study. A more detailed analysis of the subject would be needed in order to draw a more reliable conclusion since this experiment encountered some setbacks.

Keywords: Kenya, Agriculture, *Solanum lycopersicum, Ralstonia solanacearum*, integrated pest management, phytobiocides

Sammanfattning

Växtpatogener ligger bakom en stor del av de skördeförluster vi observerar runtom i världen. Växtskadegöraren *Ralstonia solanacearum* orsakar växtsjukdomen bakteriell vissnesjuka, som i den östafrikanska nationen Kenya medför stora skördeförluster i betydande grödor som tomater. Denna skadegörare utgör därmed ett hot mot såväl den kenyanska nationens livsmedelsförsörjning som den enskilda kenyanska lantbrukarens ekonomi. Målet med denna studie var att undersöka om lokalt anskaffat växtmaterial från arterna *Lantana camara* (eldkrona), *Allium sativum* (vitlök), *Azadirachta indica* (nimträd) och *Solanum incanum* (sodomsäpple) kan användas som förebyggande insatsåtgärd mot vissnesjuka hos tomater (*Solanum lycopersicum*) i Kenya. Studien var uppdelad i två moment och genomfördes dels i laboratoriemiljö och dels i växthus på University of Embus campusområde. I den första delen testades extrakt från det lokalt förvärvade växtmaterialet i ett *in vitror* diffusionstest för att undersöka växtmaterialets inhiberande effekt på tillväxten av *R. solanacearum*. Den andra delen bestod av ett experiment på unga tomatplantor i växthus där pulveriserat material från växterna tillsattes i odlingssubstratet. Den sjukdomshämmande effekten av de olika behandlingarna av växtmaterial bestämdes som arean under sjukdomskurvan (AUDPC). Diffusionstestet *in vitro* påvisade ingen signifikant inhiberande effekt av växtextrakten på tillväxten av *R. solanacearum*. Tillsatsen av pulveriserat växtmaterial i odlingssubstratet gav inga signifikanta effekter på infektionsgraden av tomatplantorna.

Studien kunde inte påvisa någon effekt av vare sig extrakt eller växtmaterial från arterna *L. camara*, *A. sativum*, *A. indica* och *S. incanum,* varken på tillväxt *in vitro* eller på sjukdomsutvecklingen hos vissnesjuka i tomater. Dessa experiment bör betraktas som pilotstudier och en noggrannare utförd studie i ämnet skulle behöva utföras för att kunna dra säkrare slutsatser då experimentet stötte på motgångar.

Table of contents

List of Figures

List of tables

Abbreviations

AUDPC	Area Under the Disease Progress Curve
GDP	Gross Domestic Product
TCZ	2,3,5-Triphenyl Tetrazolium Chloride
ANOVA	Analysis of Variance
ppm	Parts Per Million
BCA	Biological Control Agent
w/v	Weight by Volume

1. Aim and hypothesis

1.1 Aim

This study consisted of two parts where the first part was a laboratory study and the second part a greenhouse experiment. The laboratory study aimed to investigate if water-extracts of dried plant material from the species common lantana (*Lantana camara*), garlic (*Allium sativum*), neem (*Azadirachta indica*), sodom apple (*Solanum incanum*) or water-extract from the mature fruit of sodom apple have an inhibitory effect on the growth of *Ralstonia solanacearum* grown in nutrient agar. The greenhouse experiment aimed to investigate if the disease progression of bacterial wilt in tomatoes (*Solanum lycopersicum*) caused by *R. solanacearum* can be reduced or inhibited with the amendment of dried plant material from the species common lantana, garlic, neem, sodom apple or a water-extract from the mature fruit of sodom apple into the soil media.

1.2 Hypothesis

The hypothesis was that water-extracts from dried plant material from the species common lantana, garlic, neem and sodom apple or a water-extract from the mature fruit of sodom apple would inhibit the growth of R. solanacearum in a disk diffusion test. This hypothesis is based on their reported high content of several varieties of steroid alkaloids, glyco-alkaloids, saponins, enzymes and antioxidants that have shown to possess anti-microbial properties. For the greenhouse experiment it was hypothesized that the amendment of water-extracts from dried plant material of the locally sourced common lantana, garlic, neem and sodom apple or a water-extract from the mature fruit of sodom apple into the soil media in which tomatoes are growing would retard or inhibit the disease progression of bacterial wilt.

2. Introduction

Our growing global population is putting increasing demands on the outputs of the world's agricultural systems. It is predicted that by 2050, agriculture needs to have increased its total production output by 70 % to meet the demands (Pretty *et al*., 2010). Some of the significant obstacles in reaching this goal are weeds, invertebrate pests and plant pathogens, as they cause a loss of an estimated 68% of the total potential production (Oerke and Dehne, 2004), where pathogens account for about one-third of that loss (Savary *et al*., 2019)

The gram-negative soil-borne β-proteobacterium *Ralstonia solanacearum* is a significant contributor towards global yield losses (Kelman, 1998). This pathogen, classified as one of the world's most critical phytopathogenic bacteria due to its severity, persistence, wide host range, and broad geographic distribution (Denny, 2006). It is the causal agent of bacterial wilt in cash crops such as tomato, eggplant, pepper and several ornamental plants as well as the cause of brown rot in potato, Moko disease in bananas and Granville wilt in tobacco. The yield losses can in tomatoes and potatoes range from 0 – 90%, from 10 to 30% in tobacco and 80–100% in bananas (Allen *et al*., 2005). There are four major monophyletic clusters of strains (phylotypes) within the species complex of *R. solanacearum*. These phylotypes, named I to IV respectively, are found in Asia, the Americas, Africa, and Oceania, and occasionally seen in some temperate regions around Europe (Guarischi-Sousa *et al*., 2016). *Ralstonia solanacearum* can survive in a wide range of different environments for long periods. It has been observed to survive in water for 40 years but is more commonly found in the debris of diseased plants, vegetative propagative organs and wild hosts. *Ralstonia solanacearum* causes wilting at high populations of around 10^8 – 10^{10} colony forming units (CFU) per gram of tissue and can spread through many means. The pathogens can spread via shedding roots of symptomatic and nonsymptomatic plants during a severe infection. Another common way for the bacteria to proliferate is via the bacterial ooze it produces inside the host. It then propagates to the surrounding environment via contaminated farming equipment, insect vectors, seeds, irrigation and contaminated soil. When the bacteria enter the plant, it moves into the xylem where it reproduces (Hikichi *et al*., 2007). The initial symptoms on tomatoes are wilting of the youngest leaves during warm and sunny days while the recovery under cooler temperatures during

the night are delayed (Nakaho *et al*., 2000). As the disease develops, this wilting will spread throughout the plant, and the entire plant may wilt. Another visual symptom is the development of brown spots on the plant stems. The bacterial infection clogs the plant's vascular tissue entirely during favourable conditions, resulting in wilting and death.

Tomato (*Solanum lycopersicum*) is an extensively cultivated vegetable in Kenya (Ochilo *et al*., 2019). The crop constitutes around 7% of the nation's total horticultural produce and 14% of all vegetables produced (Geoffrey *et al*., 2014). Nearly all households eat tomatoes, making it a staple source of vitamin A, C and lycopene (Asante, 2013). The annual output of tomatoes is around 570 000 tonnes in Kenya, making it one of the leading tomato producing nations in sub-Saharan Africa (FAOSTAT, 2021). Over the years, tomato production in Kenya has intensified. However, yields have remained low due to abiotic factors such as erratic rainfall and high temperatures combined with biotic factors such as insects, fungi, bacteria and viruses (Anastacia *et al*., 2011). Heavy reliance and purposeless use of agrochemical products among smallholder farmers are consequences from controlling these pests. This dependence on pesticides leads to environmental impacts and poses a health hazard to farmers and consumers (Asante, 2013).

Figure 1. Map of Kenya (cdc.gov, 2019)

Kenya, a nation that received its independence from the United Kingdom by the year 1963, is located in the centre of the African continent's eastern subregion (CIA, 2019). Somalia borders the country to the east, Ethiopia to the north, South Sudan to the northwest, Uganda to the west, Tanzania to the south and the Indian Ocean to the southeast (CIA, 2019). Kenya, named after its highest mountain, Mount Kenya, houses a great variation in its landscapes and types of climate. The topography varies from plateaus situated at high altitudes to vast plains in the lowlands. The Kenyan climate is equally diverse with tropical- and monsoon rainforests in the west and southwest highlands, hot and dry arid steppes and deserts in the north and east, tropical savannah along the coast and even polar tundra climate in the higher areas around Mount Kenya (CIA, 2019). Kenya's climate is affected by the El Nino Southern Oscillation and the Inter-Tropical Convergence Zone (World Bank Group, 2021). During a year Kenya receives two periods of wet seasons. The first rainy season usually begins in around March and subsides at the end of May. The second one begins around late September and shows a decreasing trend in December. Kenya's arid and very arid areas constitute about 65% of the country's land area and receive an annual average rainfall of between 200 and

600mm (KLA, 2015). The annual temperatures range from 23°C to 34°C with the higher range of temperature occurring in the arid regions. The semi-arid areas are located on a higher altitude of around 900m to 1800m and experience an average annual rainfall of 500 to 1000mm while being slightly colder in the average temperature. These highlands are thus better suited for farming with its higher rate of precipitation.

Agriculture is Kenya's second most significant contributor to its gross domestic product next to the service sector, accounting for approximately 33% (CIA, 2019). About 75% of Kenya's population of roughly 53 million work at least part-time in the agricultural sector, livestock and pastoral activities included. Most of the farmland is located in the western, central and southern parts of the country and makes up about a tenth of the total land area. Ranching, pastoralism, and wildlife conservation are standard production practices in the arid regions as crop failures have a higher prevalence in these areas, whilst the semi-arid provinces support both crop cultivation and livestock rearing (KLA, 2015). The central productive agricultural regions are located in the highlands, where most of the food crops like maize, cassava, tomatoes, kale, cabbage and potatoes are cultivated (CIA, 2019). The principal cash crops cultivated in these regions are coffee, tea and horticultural produce such as ornamental flowers. More than 75% of the agricultural output comes from small-scale, rain-fed farming or livestock production (CIA, 2019). Most of these small-holder subsistence farmers are only capable of producing enough produce for one family. The farms generally have a mixed production with a few livestock and cultivation of subsistence crops on around 0.5 - 2.5 ha of land, where tomatoes are an essential subsistence and cash crop (DAO, 1999). Since these smallholder farmers form the basis of Kenya's agricultural sector, an improvement in these farmers' production methods would strengthen the agricultural industry and the nation. However, the intensified production has brought with it a higher use of agrochemicals such as pesticides and biocides, leading to environmental issues with degrading quality of freshwater from these residues (CIA, 2019).

The use of biological control agents (BCAs) entails the utilisation of living organisms to restrict the effect or quantity of a harmful insects, pathogens or plants by making the problematic organisms less abundant or reducing the damage level they would otherwise cause (Jonsson, 2020). The use of BCAs in controlling the spread of *R. solanacearum* in crops has sparked scientists' interest in recent years. BCAs offer an alternative pest-control method that has reduced risks of causing environmental harm and pollution of drinking water compared to the use of agrochemicals (Yuliar *et al*., 2015). There is a wide variation in the mechanisms that BCAs employ to suppress *R. solanacearum* in soil. They can, for example, reduce the amount of *R. solanacearum* by parasitising or releasing antibiotic

compounds. Promising observations with successful disease suppression have been shown on *R. solanacearum* by applying avirulent strains of and *Pseudomonas* spp. outcompeting the virulent types by competing for nutrients and space (Ramesh and Phadke, 2012). Several other bacterial species such as *Bacillus thuringiensis* (Zhou *et al*., 2008) and *Ralstonia pickettii* (Wei *et al*., 2013*)* have been observed to outcompete *R. solanacearum*. The hypothesised mechanisms behind these interactions are induced systemic resistance in the host plant, antibiosis, and the production of certain enzymes that can degrade the pathogen's cell wall and siderophores. Reported drawbacks on the use of BCAs are inconsistent colonisation, narrow range of diseases that they control, plant hosts that they associate with or that the degree of suppression is sometimes too low to be commercially acceptable (Yuliar *et al*., 2015). There are still difficulties in producing, storing, and subsequently applying BCAs at a cost-effective rate.

The use of a combination of different cropping practices, such as rotating crops (Guo *et al*., 2004) and applying BCAs (Lemessa and Zeller, 2007), have been shown to substantially affect the prevalence of bacterial wilt in tomatoes grown under field conditions. Cropping practices to counter bacterial wilt that is both effective and low cost are lacking for small-scale farmers in sub-Saharan and tropical regions around Africa, where the hot and humid climate greatly benefits *R. solanacearum*.

The use of organic soil amendments has been shown to provide suppressing effects on plant pathogens in several types of crops (Coventry *et al*., 2005) (Lazarovits *et al*., 1999). It is less costly to acquire for the everyday Kenyan farmer in comparison with BCAs. *Lantana camara, Allium sativum, Azadirachta indica* and *Solanum incanum* are commonly occurring crops or weeds in the region of East Africa. These plants contain secondary metabolites, many of which have been shown to possess anti-microbial properties, which may suppress pathogens (Naz *et al*., 2015) (Alemu *et al*., 2013). *Lantana camara* (common lantana) is an invasive weed species and has a significant economic impact in many countries worldwide (Ghisalberti, 2000). It was introduced to East Africa from South America as an ornamental herb, and its vigorous growth makes the plant very efficient at out-competing native species. Being slightly poisonous the leaves and stems are not attractive to grazers (Deena and Thoppil, 2000), but the seeds that are encapsulated in small black berries spread wide and fast via rodents and birds. *Allium sativum* (garlic) is one of the world's most studied and highly regarded medicinal herb (Goncagul and Ayaz, 2010). It is a common ingredient in most kitchens around the world for its flavour. These properties are caused by countless biologically active substances such as enzymes and sulphur-rich compounds.

Azadirachta indica (neem) is another plant renowned for its medicinal properties, mostly due to its rich content of antioxidants (Zong, Cao and Wang, 2012). The

Neem tree originates from an area that today makes up India and has spread out and become naturalised in many other parts of the world. Seeds, bark and leaves from the neem tree have shown promising results in suppressing fungi and other microbes in both agricultural and medicinal practices (Alzohairy, 2016). The leaves contain various biologically active compounds such as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol and nimbolide.

Solanum incanum (sodom apple) is a shrubby herb that is a widespread weed in Kenya. The mature yellow fruits of the plant can be used as a natural remedy for wounds and inflammatory conditions, due to its high content of several varieties of steroid alkaloids, glycoalkaloids, saponins and antioxidants, that have shown to possess anti-microbial properties (Mwonjoria *et al*., 2014).

Due to the economic importance of tomatoes to farmers in Kenya, the severity of bacterial wilt infections and the lack of liquidity and resources among these communities (Rao and Qaim, 2011), our objective was to test the hypothesis that water extracts or whole plant material from locally sourced weeds and herbs that are either of low cost or free of charge such as *L. camara, A. sativum, A. indica and S. incanum* would (1) inhibit the growth of *R. solanacearum* in an *in vitro* disc diffusion test, to find an inexpensive, environmentally friendly component for an integrated pest management program. (2) have a significant effect on the suppression of bacterial wilt in tomatoes in an *in vivo* environment, as well as in a Kenyan greenhouse environment.

3. Materials & methods

3.1. Preparation of the plant extracts

3.1.1. Fieldwork methodology

Fresh leaves of *L. camara*, *S. incanum* and mature fruits of *S. incanum* were collected from the campus ground at University of Embu. Fresh neem leaves were collected from a neem tree located on a property nearby. *A. sativum* was obtained by buying fresh garlic powder from a local market in Embu town.

3.1.2. Laboratory work

All the collected leaves were brought to the laboratory and dried under a fan for about two days, whereafter they were ground to a fine powder using a blender. Most of the powdered material was set aside for use in the greenhouse experiment, and the rest was divided and placed in 50 mL Falcon tubes were one part dry plant matter was mixed with ten parts sterile water. The mix of plant material and water was then incubated at approximately 24 °C for 48 hours. The garlic powder went through the same procedure with 48 hours of soaking. The fresh fruits of *S. incanum* were washed and mixed at a 10% weight to volume (w/v) with water and then allowed to extract for 48 hours. This extraction was used for both the disc diffusion tests and the greenhouse experiment.

The mixtures were strained after the 48 hours through a 0,2 µm membrane filter to ensure a sterile extract, and then refrigerated.

The second batch of extracts was prepared using the same procedure, with an increased concentration of 1:5 dried plant material to water ratio (20% w/v). This was done to test whether the increased concentration would result in an increased inhibition zone in the disc-diffusion test.

3.2. Preparation of the bacterial culture

3.2.1. Fieldwork methodology

Bacterial cultures were prepared from a crop of tomatoes just starting to reach the maturated stage of fruiting, located near the University of Embu. A bacterial ooze test was performed to confirm the presence of bacteria in the crop. Sterilised scalpels and containers were used to transport the infected plant tissue to the laboratory. The plants that showed characteristic symptoms such as wilting and brown spots on the stem were further examined. Part of the symptom-affected plants was removed with a scalpel, tested for ooze and then brought to the lab.

3.2.2. Laboratory work

The infected plant tissue was divided further into smaller portions and washed in distilled water. The tissue was sterilised for three minutes in a 1% sodium hypochlorite solution and then washed with distilled water. The plant material was homogenized in a sterilised mortar with 2 mL of 0.5 M potassium phosphate buffer added. This was then streaked on a modified TZC (per litre: 10 g of peptone, 1 g of casamino acids, 10 g of dextrose, 18 g of agar oxide and 1 mL of filtered and autoclaved 1% aqueous solution of 2,3,5-triphenyl tetrazolium chloride) medium (Kelman, 1954). TZC is a semi-selective media for *R. solanacearum*. The isolated bacteria were incubated at 28°C for 48 hours.

Dr Esther Arunga supplied a second source of *R. solanacearum* isolates, to ensure that in the scenario of no visible growth of the sampled specimens from the sourced tomato plants we would have a guaranteed source of *R. solanacearum*.

Both samples were later subcultured to obtain pure colonies with the single colony isolation-technique. The bacteria were picked from the sourced sample with a sterile loop and then streaked out onto an autoclaved Petri dish containing a suitable growth media. The goal was to achieve a spread so that individual colonies are formed, which was done by sterilising the loop with a flame and spreading out the initial streak in a successive cycle over the growth media (Thomas *et al*., 2015). This was done three times over with incubation at 28°C for two days each, this time on the modified TZC medium. The two isolated cultures showed characteristic morphological traits of a virulent *R. solanacearum* strain with a thick, fast-growing biofilm that was cream-white with pink/red pigments (Kelman, 1954). The isolated cultures supplied by my supervisor Dr Arunga were selected due to faster-growing colonies.

Purified *R. solanacearum* cultures were scraped off with a plastic scraper and suspended in a nutrient broth solution (per litre: 25 g Nutrient broth and distilled water). This suspension was allowed to grow while stirring with a magnetic stirrer for 48 hours at 24°C and then further diluted until a concentration of 1.5 x 10^8 CFU/mL was reached. The concentration was estimated by sampling the suspension with a photo-spectrometer and achieving an optical density of 0.3 at 600 nm (Lemessa and Zeller, 2007). The suspension

from the purified cultures was later used for both the disc diffusion tests and the greenhouse experiment.

3.3. Disc diffusion test

Water extract from the dried plant material at 10% and 20% (w/v) respectively was tested to inhibit the growth of *R. solanacearum in vitro* by the Agar disc-diffusion method (Heatley, 1944). Paper plugs were soaked with 20 µl of 10% (w/v) plant-water extract and dried overnight at 28°C. Two control treatments were also prepared: one with streptomycin at a concentration of 200 part per million (ppm) and one with distilled water. 100 µl of the bacterial suspension at a concentration of $1.5x10^8$ CFU/mL was applied to Petri dishes filled with nutrient agar and spread evenly with a plastic spreader. Seven paper buttons were placed on each plate, five for each treatment and two of the controls. The plates were incubated overnight at 28°C, and the inhibition zone was measured with a standard plastic ruler. Each treatment had 19 replicates and this experiment was performed twice. A third experiment with an increased concentration of plant-water extract at 20% (w/v) was conducted to see if the increased concentration would yield an increased inhibition zone.

3.4. Greenhouse experiment

3.4.1. Preparation and conduct of the experiment

An *in vivo* experiment was conducted in a greenhouse on the campus ground at the University of Embu to study the effects of the four different plant powders and the fruit mixture of *S. incanum* on the development of bacterial wilt in tomatoes. Three-week-old tomato seedlings of the cultivar Rambo F1 were washed in sterilised water and transplanted into 17 cm diameter plastic pots. Each pot contained one kilogram of soil (two parts silt to one part sand) disinfected by partial sterilisation with aerated steam at 100°C for one hour for three consecutive days. Before transplanting, each pot received a treatment of 40 g of dried plant material or 40 mL of fruit extract for the *S. incanum* fruit treatment. For comparison, two control treatments were used: One with 50 mL of the standard antibiotic streptomycin at a concentration of 200 parts per million (ppm) applied in the soil twice, first at day one and once more at day 14. The other control treatment was 50 mL of distilled water that was applied simultaneously as the streptomycin during day 1 and 14. The transplanted tomatoes were then inoculated with the bacterium by cutting one of the lower leaves with a scissor dipped in the bacterial suspension containing *R. solanacearum* at a concentration of 1.5x10^8 CFU/mL. The greenhouse experiment

was performed under a completely randomised design protocol with four replicates. All plants were watered as needed. Tissue from assumed infected plants was sampled and processed as described earlier by culturing agar to confirm *R. solanacearum* by morphological traits.

Disease severity was assessed at day 1, 7, 9, 11 and 22 and scored by visual observation, visually determining the disease severity using a scale from 1 to 4 according to how much the leaves had wilted (1: No visible symptom, 2: The wilting of a leaf to half the foliage, 3: Half to most of the foliage wilted, 4: Whole foliage wilted and a plant dead;Wei *et al*., 2013).

3.5. Data analysis

Disease progression in the greenhouse experiment was determined by assessing the levels of disease in different treatments. The disease severity (1-4) was used to calculate the AUDPC-value for each treatment, which was first processed via log-transformation to conform to normality. Analysis of variance (ANOVA) was performed to test whether the differences between the disease progress in the treatments were significant. All statistical analyses were done using R (R Core Team, 2017). Data from the disc diffusion test was collected and presented using Microsoft Excel.

4. Results

4.1. Disc diffusion tests

Water extracts at 10% and 20% (w/v) from *Lantana camara*, *A. sativum, A. indica* and *S. incanum* gave no significant inhibitory effect ($p > 0.05$) on the growth of *R. solanacearum*. All three experiments resulted in inhibition of growth only from the control treatment with streptomycin. The zone of inhibition caused by the streptomycin varied throughout all three experiments. Because no treatment other than the control of streptomycin had an inhibitory effect on the growth of *R. solanacearum,* the results of these tests are not presented or analysed for significant interactions (Table A1, Table A2, Table A3).

4.2. Greenhouse experiment

None of the treatments with *L. camara*, *A. sativum*, *A. indica* or *S. incanum* showed any significant effect ($p > 0.05$) in the control of bacterial wilt infection compared with the control treatment with no application (Fig. 2). All the plants treated with *A. sativum* died right after transplantation and most of the plants treated with *A. indica,* indicating phytotoxic activity.

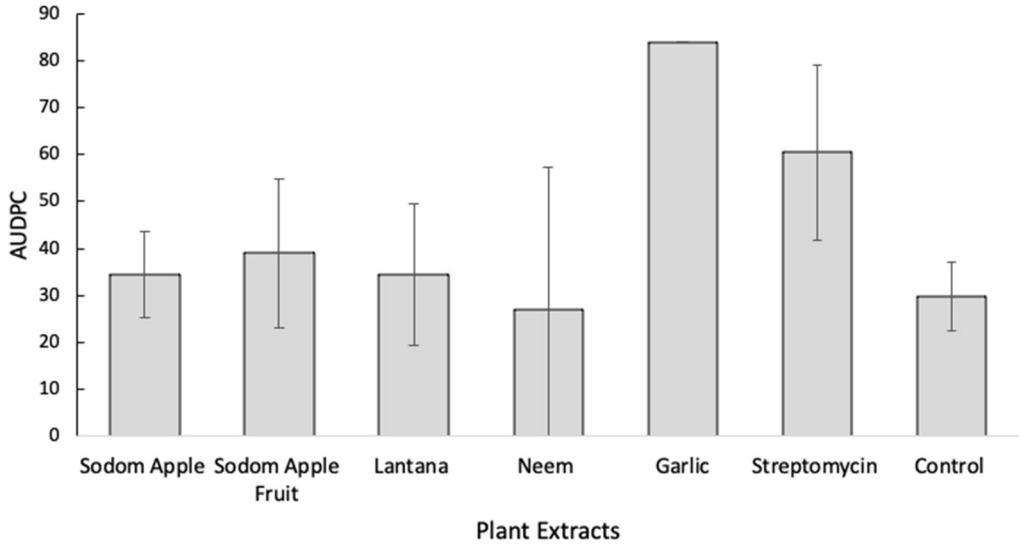


Figure 2. Area under disease progress curve (AUDPC) for bacterial wilt of tomato in treatments with plant extracts (mean ± SD, n=*4).*

4.3. Confirmation of infection

During the first week, many plants showed early bacterial wilt symptoms such as wilting of the upper leaves. Later, brown spots started to occur on the stems of the tomato plants. At day 17, after initial inoculation, tissues from supposedly infected plants were sampled and further processed in the laboratory. After two days, the expected growth of *R. solanacearum* with its pink/red hue, fluidal shape and vigorous speed of development visually confirmed the infection (Fig. 3).

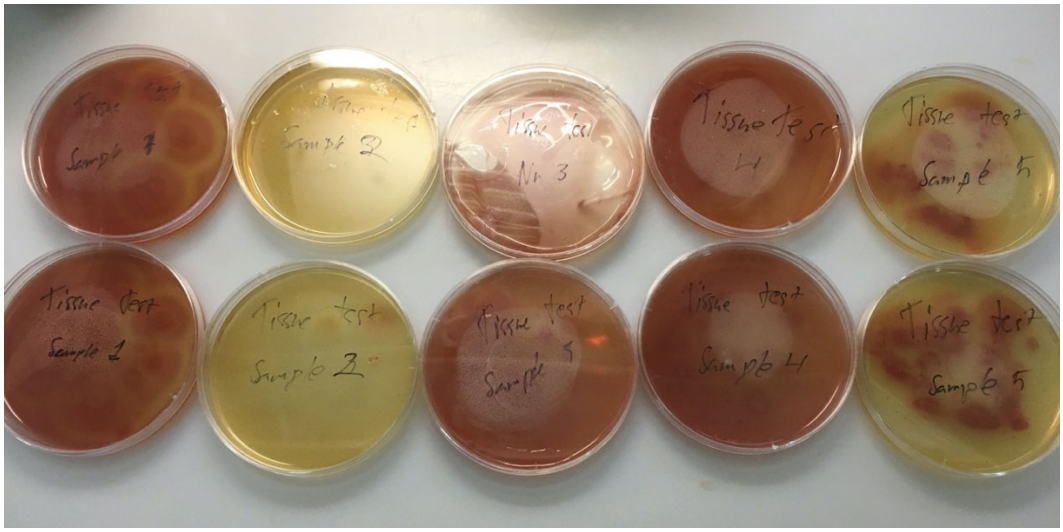


Figure 3. Positive samples from a tissue test of re-isolated bacteria from the infected plants.

5. Discussion

Shrubs and herbs can be regarded as a significant supply of anti-microbial compounds, making them a cheap and readily available source of sustainable pesticides for farmers (Dubey *et al*., 2010). Anti-bacterial (De Lima *et al*., 2006), anti-fungal and even anti-viral properties are attributed to a plethora of plants. By applying material from these plants as soil amendments in either fresh form, dried form or as an aqueous solution, the number of chemical inputs could be reduced and, in this way, lowering farmers costs as well as reducing the environmental impact from food production (Pimentel D. *et al*., 1993).

This study was conducted to test if plant material from local plants can suppress bacterial wilt caused by *R. solanacearum* in greenhouse tomatoes as an inexpensive, environmentally friendly and cost-effective alternative to chemical amendments in Kenyan tomato production. It was expected, based on earlier experiments (Din *et al*., 2016), that in a disc diffusion experiment aqueous extracts of dried leaves from *L. camara*, *A. sativum*, *A. indica*, *S. incanum* and the mature fruits of *S. incanum* would have a significant inhibitory effect on the rate of growth of *R. solanacearum*, compared to a control. Furthermore, we expected that the application of dried plant material in the form of leaves from *L. camara*, *A. sativum*, *A. indica* and *S. incanum*, as well as a mixture of water and the mature fruits of *S. incanum*, would significantly decrease the degree of disease in greenhouse tomatoes in comparison to no treatment.

Based on similar and earlier conducted experiments (Din *et al*., 2016), we expected to see inhibited growth of *R. solanacearum* from the extracts during the disc diffusion tests, but this never occurred. The lack of *in vitro* growth inhibition from these extracts may be explained by an insufficient concentration of anti-microbial secondary metabolites in the plant material. The gathered plant material might simply not contain any secondary metabolites with anti-microbial properties. Regarding the dried garlic powder which we extracted at a relatively high concentration of 10% (w/v), Shafiur Rahman *et al*. found in 2006 that drying the garlic can have a significant effect on retaining the active components responsible for the anti-microbial effects of garlic. Ajoene is an organosulfuric compound found in garlic that possesses broad-spectrum anti-microbial properties (Hussein *et al*., 2017). This compound would be at risk of being reduced when the garlic is dried

and might explain we did not observe any inhibition from the garlic treatment. The first rainy season started late in Embu this year and had not yet begun when harvesting of the plant material was carried out. These dry conditions might have affected the plants' metabolic composition with differing concentrations of compounds such as glycoalkaloids and saponins (Reddy *et al*., 2004), therefore possibly affecting the inhibitory effect the extracts might have had on the growth of *R. solanacearum*.

Flavonoids are phenolic compounds are produces in plants such as the neem tree to respond to pathogenic infections (Xu *et al*., 2001). These compounds have an inhibitory function by forming a complex with the extracellular proteins produced by microbes, while also binding to the proteins of bacteria and inhibiting the microbial synthesis of amino acids. Saponins are another group of compounds known to possess anti-microbial properties and can be found in both the leaves and the fruit of *S. incanum*. These saponins can inhibit bacterial growth by reacting with sterols in bacteria membranes (Wang *et al*., 2000). Another broad group of antimicrobial compounds are the alkaloids. Some have shown to inhibit vital enzymes and the DNA-synthesis in bacteria (Kong *et al*., 2009).

One method suggested in the literature to improve the extraction process's efficacy is to replace water with organic solvents such as ethyl acetate to increase the extracted amounts of antimicrobial compounds (Zhang *et al*., 2018). It has been used in comparison to water-extraction with garlic bulbs in an *in vitro* test of growth inhibition of *R. solanacearum* similar to ours (Jeyaseelan *et al*., 2010). They found the organic solvent solution to be twice as potent as the water extraction, potentially indicating that more antimicrobial compounds were soluble with organic solvents. The use of organic solvents could decrease the need for extraction material, but with plant material such as garlic or neem, the price for solvents would most likely be less attractive for the more resource-poor farmers of Kenya.

In the greenhouse experiment, the treatments that were evaluated for their suppressing effects on bacterial wilt did not show any result, i.e., the value of each treatments AUDPC did not significantly differ from the control. None of the plants transplanted into the pots with soil amended with garlic powder survived the initial two days, indicating that the garlic powder might possess phytotoxic properties at the concentration used. In contrast to this, Din *et al*. (2016) reported that *A. sativum* have inhibitory effects on *R. solanacearum in vitro*, without any phytotoxic properties on tomato plants. The same non-phytotoxic traits were found in a similar experiment were Ahmad *et al*., (2017) screened for phytobiocidal effects of garlic on *Alternaria solani*, causing early blight in tomatoes. Instead of using fresh green, leaves as was utilised by Din *et al*., (2016), we used dried garlic powder out of convenience and accessibility. Using powdered garlic cloves the concentration of biologically active substances may have reached a point where these rather

beneficial compounds turned phytotoxic (Goncagul and Ayaz, 2010), or as discussed earlier, reduced to negligible amounts (Shafiur Rahman *et al*., 2006).

Cutting the lower leaves of a plant with contaminated scissors is one of the most effective ways of ensuring reliable inoculation of *R. solanacearum* (Mohan *et al*., 2002). Still, this method might have narrowed down the potential mechanisms of suppressive effect from the plant material. By not directly adding both the plant treatments (40g/kg soil) and the inoculum of *R. solanacearum* into the pot, we did not test the plant material's direct toxic effect on the pathogen in soil. Although we tested for the effect of the plant matter extracts in an *in vitro* setting, the *in vivo* environment might not have generated any differing results. A second way the plant compounds might work is by stimulating microbes in the soil that are antagonistic to *R. solanacearum* (Quimby *et al*., 2002). Seeing as how we did not sterilise the dried plant matter, we cannot rule out that we might have introduced microbes in the soil. One plausible mechanism of action in this experimental setup might have been that the compounds in the dried material and pressed fruits could induce innate defence mechanisms in the tomato plants, either directly or indirectly via soil-borne microbes (Cook and Baker, 1983). Also, we did not test for any enzymatic or genetic markers (Lafitte *et al*., 2004) associated with the induction of the tomato plants defence mechanisms. Such a test might have allowed us to observe better if the different treatments affected the tomato defence regulation (Han *et al*., 2009).

The plants treated with streptomycin (200 ppm) showed a significantly higher AUDPC-value (Fig. 1) than the control treatment with zero amendments added. We motivated the use of streptomycin as it would be absorbed by the plant roots (Kumar *et al*., 2005) and function as an efficient bactericide against an infection of *R. solanacearum*. Instead, we saw an increased degree of disease in these plants. It might be that tomatoes can't or poorly absorbs the streptomycin. Antibiotics such as sulfamethoxazole have shown phytotoxic effects in plants (Liu *et al*., 2009) so it could be that the streptomycin had a phytotoxic effect on the tomato plants. It could also be that the assessed disease progression was wilting caused by stress from low water availability in combination with intense sunlight. Still, another study would have to be conducted to examine this hypothesis.

Choosing to work only with morphological characterisation was used to save both time and resources. Several strong indicators of the presence of *R. solanacearum* were observed, such as a thick, fast-growing biofilm that was coloured cream-white with pink/red pigments (Kelman, 1954). Conducting biochemical characterisation of the chosen bacterium might have given us more certainty, but since the project was under a tight schedule compromises in the experiment's execution had to be made. The isolate chosen for both experiments was a confirmed strain of *R. solanacearum* whilst the strain sourced from the greenhouse was not confirmed by PCR-testing.

Choosing to assess the disease progression by visually determining the disease severity based on the degree of drooping and wilting of leaves in an ordinal data scale might have affected the data analysis outcome. The data was processed using an ANOVA, a parametrical analysis method that is not standard practice in processing ordinal data sets. In discussing this with my supervisor Hanna Friberg, she advised that with a sufficient number of observations per replicate, us having five, would be enough for the data to behave continuous and therefore be appropriate to use with an ANOVA. In assessing the AUDPC of a disease progression, the conventional approach uses ratio data such as the percentage of leaf area infected (Jeger and Viljanen-Rollinson, 2001). One alternative way to approach the analysis differently would have been with a one-way ANOVA on ranks, also known as the Kruskal–Wallis H test (Chan and Walmsley, 1997).

The plants used in this experiment have earlier shown potential for disease prevention (Din *et al*., 2016) (Alemu *et al*., 2013), and as an attractive component in an integrated pest management program for resource-poor small-scale farmers against *R. solanacearum.* The majority of the plants used grow year-round and can be acquired for free, garlic being an exception but an easily cultivated crop. Using dried plant-matter or extracts of fruits from plants like *Lantana camara*, *Allium sativum*, *Azadirachta indica* and *Solanum incanum* did not significantly suppress bacterial wilt in tomatoes, compared to the use of antibiotics or with no inputs at all. The result did not vary much from treatment to treatment, and the possibility that the extracts do not possess any suppressing effect on bacterial wilt in tomatoes is conceivable. However, there is still much room for improvement for examining the impact of the plants mentioned above on suppressing *R. solanacearum* in tomatoes.

6. References

Ahmad, F., Raziq, F., Ullah, N., Khan, H. and Din, N., 2017. In vitro and in vivo bioassay of phytobiocidal effect of plant extracts on Alternaria solani causing agent of early blight disease in tomato. Archives of Phytopathology and Plant Protection, 50(11-12), pp.568-583.

Akhtar M, Malik A. (2000): Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes. A review. Bioresour Technol. 2000; 74:35–47.

Alemu, D., Lemessa, F., Wakjira, M. and Berecha, G., 2013. Antibacterial Activity of Some Invasive Alien Species Extracts Against Tomato (Lycopersicon esculentum Mill) Bacterial Wilt Caused by Ralstonia solanacearum (Smith). Plant Pathology Journal, 12(2), p.61-70.

Allen, C., Prior, P. and Hayward, A.C., 2005. Bacterial wilt disease and the Ralstonia solanacearum species complex. American Phytopathological Society (APS Press).

Alzohairy, M. (2016). Therapeutics Role of Azadirachta indica (Neem) and Their Active Constituents in Diseases Prevention and Treatment. *Evidence-Based Complementary and Alternative Medicine*, 2016, p.1-11.

Anastacia, O., Masinde, A., Kwambai, T.K., Wambani, N.H., 2011, Evaluation of tomato (lycopersicon esculentum l.) variety tolerance to foliar diseases at kenya agricultural research institute centre-kitale in north west kenya, Afr. J. Plant Sci. 5 (11) pp. 676–681.

Asante, B.O., Osei, M.K., Dankyi, A.A., Berchie, J.N., Mochiah, M.B., Lamptey, J.N.L., Haleegoah, J., Osei, K., Bolfrey-Arku, G., 2013. Producer characteristics and determinants of technical efficiency of tomato-based production systems in Ghana. Journal of Development and Agricultural Economics, 5(3), pp.92-103.

CIA, Central Intelligence Agency, www.cia.gov 1. The world factbook, Kenya, 2019-04-23 https://www.cia.gov/library/publications/the-world-factbook/geos/ke.html

Climateknowledgeportal.worldbank.org. 2021. World Bank Climate Change Knowledge Portal. Available at: https://climateknowledgeportal.worldbank.org/country/kenya/climate-data-historical [Accessed 8 February 2021].

Chan, Y. and Walmsley, R., 1997. Learning and Understanding the Kruskal-Wallis One-Way Analysis-of-Variance-by-Ranks Test for Differences Among Three or More Independent Groups. Physical Therapy, 77(12), p.1755-1761.

Cook, R.J. and Baker, K.F., 1983. The nature and practice of biological control of plant pathogens. American Phytopathological Society.

Coventry, E., Noble, R., Mead, A. and Whipps, J., 2005. Suppression of Allium white rot (Sclerotium cepivorum) in different soils using vegetable wastes. European Journal of Plant Pathology, 111(2), p.101-112.

Denny T., "Plant Pathogenic Ralstonia species" in GNANAMANICKAM, S. S. (2006). Plant-associated bacteria. Dordrecht, Springer. p 1-62

DAO (1999-2005). Annual reports 1999-2005, District Ministry of Agriculture, Trans Nzoia, West Pokot, and Nandi districts. Annual Reports. Ministry of Agriculture, Kenya, p. 1-28.

Deena, M. and Thoppil, J. (2000). Antimicrobial activity of the essential oil of Lantana camara. Fitoterapia, 71(4), p.453-455.

De Lima, M. R. F., de Souza Luna, J., Dos Santos, A. F., De Andrade, M. C. C., Sant'Ana, A. E. G., Genet, J. P., ... & Moreau, N. (2006). Anti-bacterial activity of some Brazilian medicinal plants. Journal of ethnopharmacology, 105(1-2), 137-147.

Din, N., Ahmad, M., Siddique, M., Ali, A., Naz, I., Ullah, N. and Ahmad, F. (2016). Phytobiocidal management of bacterial wilt of tomato caused by Ralstonia solanacearum (Smith) Yabuuchi. *Spanish Journal of Agricultural Research*, 14(3), p.1006.

Dubey, N. K., Shukla, R., Kumar, A., Singh, P., & Prakash, B. (2010). Prospects of botanical pesticides in sustainable agriculture. Current Science, 98(4), p. 479-480.

Erhoeven, J., Arheimer, B., Yin, C. and Hefting, M. (2006). Regional and global concerns over wetlands and water quality. *Trends in Ecology & Evolution*, 21(2), p.96-103.

FAO (2019). *Kenya at a glance | FAO in Kenya | Food and Agriculture Organization of the United Nations*. Fao.org. Tillgänglig via: http://www.fao.org/kenya/fao-in-kenya/kenya-at-a-glance/en/ [13 maj 2019].

Fao.org. 2021. FAOSTAT. Available at: <http://www.fao.org/faostat/en/#data/QC> [Accessed 7 February 2021].

Friberg, H. Researcher/Lecturer, SLU, Zoom-meeting 2021-02-09

Geoffrey, S.K., Hillary, N.K., Kibe, M.A., Mariam, M. and 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),

Guo, J.H., H.Y. Qi, Y.H. Guo, H.L. Ge, L.Y Gong, L.X. Zhang and P.H. Sun, 2004. Biocontrol of tomato wilt by plant growth-promoting rhizobacteria. Biol. Control, 29, p.66-72.

Goncagul, G. and Ayaz, E. (2010). Antimicrobial Effect of Garlic (Allium sativum). *Recent Patents on Anti-Infective Drug Discovery*, 5(1), p.91-93.

Guarischi-Sousa, Rodrigo; Puigvert, Marina; Coll, Núria S.; Siri, María Inés; Pianzzola, María Julia; Valls, Marc; Setubal, João C. (2016). "Complete genome sequence of the potato pathogen Ralstonia solanacearum UY031". Standards in Genomic Sciences. 11 (1): 7.

Han, Y., Wang, Y., Bi, J., Yang, X., Huang, Y., Zhao, X., Hu, Y. and Cai, Q., 2009. Constitutive and Induced Activities of Defense-Related Enzymes in Aphid-Resistant and Aphid-Susceptible Cultivars of Wheat. Journal of Chemical Ecology, 35(2), p.176-182.

Heatley, N. (1944). A method for the assay of penicillin. *Biochemical Journal*, 38(1), p.61-65.

Hikichi, Y., Yoshimochi, T., Tsujimoto, S., Shinohara, R., Nakaho, K., Kanda, A., Kiba, A. and Ohnishi, K. (2007). Global regulation of pathogenicity mechanism of Ralstonia solanacearum. *Plant Biotechnology*, 24(1), p.149-154.

Hindi NKK. 2013. In vitro antibacterial activity of aquatic garlic extract, apple vinegar and apple vinegar - garlic extract combination. Am J Phytomed Clin Therapeutics 1: p.42-51

Hussein, H., Hameed, I. and Hadi, M., 2017. A Review: Anti-microbial, Anti-inflammatory effect and Cardiovascular effects of Garlic: Allium sativum. Research Journal of Pharmacy and Technology, 10(11), p.40-69.

Jeger, M. and Viljanen-Rollinson, S., 2001. The use of the area under the disease-progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. Theoretical and Applied Genetics, 102(1), p.32-40.

Jonsson, M., 2020. What is biological control? | slu.se. [online] SLU.SE. Available at: <https://www.slu.se/en/Collaborative-Centres-and-Projects/centre-for-biological-control-cbc/about-biological-control/> [Accessed 8 February 2021].

Kelman, A. (1954) The Relationship of Pathogenicity of Pseudomonas solanacearum to Colony Appearance in a Tetrazolium Medium. Phytopathology, 44, p. 693-695.

Kelman A. (1998) One Hundred and One Years of Research on Bacterial Wilt. In: Prior P., Allen C., Elphinstone J. (eds) Bacterial Wilt Disease. Springer, Berlin, Heidelberg

Kong, W., Zhao, Y., Xiao, X., Jin, C., Liu, Y. And Li, Z., 2009. Comparison of Anti-bacterial Activity of Four Kinds of Alkaloids in Rhizoma Coptidis Based on Microcalorimetry. Chinese Journal of Chemistry, 27(6), p.1186-1190.

Kumar, K., Gupta, S., Baidoo, S., Chander, Y. and Rosen, C., 2005. Antibiotic Uptake by Plants from Soil Fertilized with Animal Manure. Journal of Environmental Quality, 34(6), p.2082-2085.

LAND USE IN KENYA The case for a national land use policy. 3rd ed., 2015. Nakuru: Kenya Land Alliance. Available at: <http://www.kenyalandalliance.or.ke/wp-content/uploads/2015/03/kla_land_use_in_kenya_case_for_policy.pdf> [Accessed 8 February 2021].

Lafitte, H., Price, A. and Courtois, B., 2004. Yield response to water deficit in an upland rice mapping population: associations among traits and genetic markers. Theoretical and Applied Genetics, 109(6), p.1237-1246.

Lazarovits, G., Conn, K. and Potter, J., 1999. Reduction of potato scab, verticillium wilt, and nematodes by soymeal and meat and bone meal in two Ontario potato fields. Canadian Journal of Plant Pathology, 21(4), p.345-353.

Lemessa, F. and W. Zeller, 2007. Isolation and characterization of *Ralstonia Solanacearum* strains from solanaceae crops in Ethiopia. J. Basic Microbiol., 47, p. 40-49.

Liu, F., Ying, G., Tao, R., Zhao, J., Yang, J. and Zhao, L., 2009. Effects of six selected antibiotics on plant growth and soil microbial and enzymatic activities. Environmental Pollution, 157(5), p.1636-1642.

Mohan, S., Fallahi, E. and Bijman, V., 2002. Evaluation of apple varieties for susceptibility to erwinia amylovora by artificial inoculation under field conditions. Acta Horticulturae, (590), p.373-375.

Mwonjoria, J., Ngeranwa, J., Kariuki, H., Githinji, C., Sagini, M. and Wambugu, S. (2014). Ethno medicinal, phytochemical and pharmacological aspects of solanum incanum (lin.). *International Journal of Pharmacology and Toxicology*, 2(2).

Nakaho, K., Hibino, H. and Miyagawa, H. (2000). Possible Mechanisms Limiting Movement of Ralstonia solanacearum in Resistant Tomato Tissues. Journal of Phytopathology, 148(3), p.181-190.

Naz, I., Saifullah, Palomares-Rius, J., Khan, S., Ali, S., & Ahmad, M. *et al*. (2015). Control of Southern root knot nematode Meloidogyne incognita (Kofoid and White) Chitwood on tomato using green manure of Fumaria parviflora Lam (Fumariaceae). *Crop Protection*, *67*, p.121-129.

Ochilo, W., Nyamasyo, G., Kilalo, D., Otieno, W., Otipa, M., Chege, F., Karanja, T. and Lingeera, E., 2019. Characteristics and production constraints of smallholder tomato production in Kenya. Scientific African, 2,

Oerke, E. and Dehne, H. (2004). Safeguarding production—losses in major crops and the role of crop protection. *Crop Protection*, 23(4), p.275-285.

Pimentel, D., McLaughlin, L., Zepp, A., Lakitan, B., Kraus, T., Kleinman, P., ... & Selig, G. (1993). Environmental and economic effects of reducing pesticide use in agriculture. Agriculture, Ecosystems & Environment, 46(1-4), 273-288.

Pretty, J., Sutherland, W., Ashby, J., Auburn, J., Baulcombe, D., Bell, M., Bentley, J., Bickersteth, S., Brown, K., Burke, J., Campbell, H., Chen, K., Crowley, E., Crute, I., Dobbelaere, D., Edwards-Jones, G., Funes-Monzote, F., Godfray, H., Griffon, M., Gypmantisiri, P., Haddad, L., Halavatau, S., Herren, H., Holderness, M., Izac, A., Jones, M., Koohafkan, P., Lal, R., Lang, T., McNeely, J., Mueller, A., Nisbett, N., Noble, A., Pingali, P., Pinto, Y., Rabbinge, R., Ravindranath, N., Rola, A., Roling, N., Sage, C., Settle, W., Sha, J., Shiming, L., Simons, T., Smith, P.,

Strzepeck, K., Swaine, H., Terry, E., Tomich, T., Toulmin, C., Trigo, E., Twomlow, S., Vis, J., Wilson, J. and Pilgrim, S., 2010. The top 100 questions of importance to the future of global agriculture. International Journal of Agricultural Sustainability, 8(4), p.219-236.

Rao, E. and Qaim, M. (2011). Supermarkets, Farm Household Income, and Poverty: Insights from Kenya. *World Development*, 39(5), p.784-796.

R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ramesh R, Phadke GS., 2012. Rhizosphere and endophytic bacteria for the suppression of eggplant wilt caused by Ralstonia solanacearum. Crop Prot. 2012;37: p.35–41.

Reddy, A., Chaitanya, K. and Vivekanandan, M., 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. Journal of Plant Physiology, 161(11), p.1189-1202.

Savary, S., Willocquet, L., Pethybridge, S., Esker, P., McRoberts, N. and Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution*, 3(3), p.430-439.

Thomas, P., Sekhar, A., Upreti, R., Mujawar, M. and Pasha, S., 2015. Optimization of single plate-serial dilution spotting (SP-SDS) with sample anchoring as an assured method for bacterial and yeast CFU enumeration and single colony isolation from diverse samples. *Biotechnology Reports*, 8, p.45-55.

Wai, K.P.P., Lee, J., Mo, HS. *et al*., 2013. Sources of resistance to bacterial wilt and restorer-of-fertility genotype for cytoplasmic male sterility in Capsicum pepper. Horticulture, Environment, and Biotechnology. 54, p.266–271

Wang, Y., McAllister, T., Yanke, L. and Cheeke, P., 2000. Effect of steroidal saponin from Yucca schidigera extract on ruminal microbes. Journal of Applied Microbiology, 88(5), p.887-896.

Wei Z, Huang J, Tan S, Mei X, Shen Q, Xu Y., 2013. The congeneric strain Ralstonia pickettii QL-A6 of Ralstonia solanacearum as an effective biocontrol agent for bacterial wilt of tomato. Biological Control. 2013;65: p.278–285.

Wwwnc.cdc.gov. (2019). *Health Information for Travelers to Kenya - Traveler view | Travelers' Health | CDC*. Available at: https://wwwnc.cdc.gov/travel/destinations/traveler/none/kenya

Xu, H. X., & Lee, S. F. (2001). Activity of plant flavonoids against antibiotic-resistant bacteria. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 15(1), 39-43.

Yuliar, Nion, Y. A., & Toyota, K. (2015). Recent trends in control methods for bacterial wilt diseases caused by Ralstonia solanacearum. Microbes and environments, 30(1), 1–11.

Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products: a comprehensive review. Chinese medicine, 13, 20.

Zhou Y, Choi YL, Sun M, Yu Z., 2008. Novel roles of Bacillus thuringiensis to control plant diseases. Applied Microbiology and Biotechnology. 2008 Sep; 80(4). p.563-72.

Zong A., Cao H., Wang F. Anticancer polysaccharides from natural resources: a review of recent research. Carbohydrate Polymers. 2012;90(4), p.1395–1410.

7. Acknowledgements

I would like to thank my supervisors, Hanna Friberg and Esther Arunga, for their advice, help and guidance during this study. After that, I would like to direct my uttermost gratitude towards Mattias Jonsson, Jamleck Muturi and Justin Nyaga for the support and help during the work. And at last, thank you, Mr Anthony Kimani and Brenda Odongo, for contributing with great spirit and support in the laboratory. I am thankful for all the people handling the administrative work in both universities and all the kind and welcoming students and workers at the University of Embu.

This project was made possible via funding from the Linnaeus-Palme partnership, from which I received a scholarship that was essential for making this project come together.

A big thank you to Nadja Kvick Nastaj, Sanna Bergkvist, Ida Johansson and Kim Rock for the company and support during this project, and my family and friends back home for the support during the stay.

Uppsala, February 10th, 2021

Anton Hampl

8. Appendix

Table A1. Results from the first disc-diffusion tests on the 1st of May 2019

Streptomycin	Diameter (cm)
1	1,8
2	1,8
3	1,7
4	1,8
5	1,8
6	1,7
7	1,6
8	1,8
9	1,8
10	1,8
11	1,9
12	1,7
13	1,9
14	1,6
15	1,8
16	1,8
17	1,8
18	1,9
19	1,9
Average	1,78

*Only the result of streptomycin is presented due to no other treatment showing any inhibitory effect. For the same reason the testing for significance was excluded

Table A2. Results from the second trial with the disc diffusion tests on the 18th of May, 2019

Plate	Diameter (cm)
1	1,4
2	1,2
3	1,3
4	1,3
5	1,2
6	1,1
7	0,9
8	1,2
9	1,2
10	1,4
11	1,1
12	1,1
13	1,2
14	1,2
15	1,3
16	1,2
17	1,1
18	1,1
19	1,4
Average	1,21

*Only the result of streptomycin is presented due to no other treatment showing any inhibitory effect. For the same reason the testing for significance was excluded

Table A3. Results from the third trial with the disc-diffusion tests on the 28th of May, 2019

Plate	Diameter (cm)
1	2,3
2	2,4
3	2,2
4	2,4
5	2,1
6	2,3
7	2,4
8	2
9	2,8
10	2,1
11	2,4
12	2,3
13	2,2
14	2,9
15	3,2
16	3
17	2,3
18	1,8
19	2,2
Average	2,4

*Only the result of streptomycin is presented due to no other treatment showing any inhibitory effect. For the same reason the testing for significance was excluded