GENETIC DIVERSITY, CROSS PATHOGENICITY AND CONTROL OF BACTERIAL BLIGHT OF COWPEA USING *BACILLUS* SP. AND SELECTED BOTANICALS

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DECLARATION

Declaration by the Candidate

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DEDICATION

To my parents who have committed to do whatever it takes to ensure that my siblings and I attain quality education.

To my cousins and siblings who have been really supportive of me.

To my wife, Mercy Chepkirui and daughter Patience Jerono Kirarei for their patience, love and understanding for all the time I put into my research.

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To my family, who silently endured my absence, my frustrations, and my endless preoccupation with research- your sacrifices are woven into every page of this thesis. Without your prayers, love, and patience, this work would not exist. On the other hand, I am happy to have graduated. On the other, I am a bit sad to still be unemployed at 33. My hair is gone and I cannot afford a transplant. The funny thing is, my supervisor is now encouraging me to continue with a postdoc. I'm tired. Hair gone. Lastly, to all PhD students who are silently battling exhaustion, loneliness, and self-doubt. May you find peace in knowing that you are not alone.

ABSTRACT

Cowpea (*Vigna unguiculata* (L.) Walp.) is an economically important crop cultivated both for domestic and commercial purposes. It is one of the most resilient crops suited to arid and semi-arid areas. Cowpea production is affected by several diseases caused by phytopathogens. Bacterial blight caused by *Xanthomonas axonopodis* pv. *vignicola* has been reported in many cowpea producing areas causing reduction in both quality and quantity of the harvestable leaves and grains. However, in Kenya, the disease occurrence status has not been exhaustively documented, similar to the causal pathogen characterization and management using biological control agents and botanicals. This research conducted field surveys in farms from six counties representing different zones to analyse the disease occurrence levels. The identification of the pathogen was done by morphological and biochemical features as well as by analysis of 16S rDNA and its genetic diversity was determined using inter-simple sequence repeat markers. The pathogen cross pathogenicity was tested by inoculating other legumes (beans, soya bean, green gram, garden peas and lentils) with the most virulent isolate. Dual culture and inverted plate techniques were used to test bio-efficacy of the biological control agents and botanical extracts. *Xanthomonas axonopodis* pv. *vignicola* was confirmed as the causal pathogen in 48 farms out of 80 farms. The mean disease incidence was 44.89% across the sampled sites, being highest in Kakamega County (50.49%) and the least in Uasin Gishu County (33.57%). *Xanthomonas axonopodis* pv. *vignicola* isolates displayed slight variance in morphological and cultural characteristics on nutrient agar. The biochemical tests and analysis by blasting of sequence from 16S rDNA region confirmed *Xanthomonas axonopodis* pv. *vignicola* as the causal agent. The polymorphic information content ranged from 0.2384 to 0.4486, indicating genetic variations which was strongly correlated with the differences within populations. The pathogen, infection was observed in all the cowpea varieties tested and cross infection on soya bean and lentils. The percentage disease severity ranged between 25.83% to 51.67%, which was significantly higher in cowpea varieties. The bio-efficacy of selected bio-agents showed varying levels of percentage inhibition against *X. axonopodis* pv. *vignicola,* depending on the method and the duration of exposure. *Bacillus subtilis* displayed the highest antibacterial activity between 68.33% and 87.79% by dual culture technique and between 38.33% to 71.33% in inverted plate method over seventy-two hours. *Bacillus amyloliquefaciens* showed a statistically significant antibacterial activity between 45.00% and 76.12% in dual culture technique and between 45.00% and 73.89% by inverted plate method. Cyprofloxacin the antibiotic used for positive check, ethanolic extracts of neem, garlic and ginger inhibited *X. axonopodis* pv. *vignicola* by 56.3 mm, 38.5 mm, 30.8 mm and 25.0 mm respectively, but no potency was noted for *Salvia nilotica*. Genetic variations of *Xanthomonas axonopodis* pv. *vignicola* was noted from different regions of Kenya. This study showed that *B. subtilis* and *B. amyloliquefaciens,* neem, garlic and ginger extracts are useful biocontrol options in management of *X. axonopodis* pv. v*ignicola* and therefore can be recommended for integration in the management of this pathogen in cowpea.

TABLE OF CONTENTS

DECLARATION ii

DEDICATION iii

ACKNOWLEDGEMENTS iv

ABSTRACT v

LIST OF TABLES xii

LIST OF FIGURES xiii

LIST OF PLATES xiv

LIST OF ABBREVIATIONS xv

LIST OF APPENDICES xvii

CHAPTER ONE 1

INTRODUCTION 1

1.1 Background information 1

1.2 Statement of the problem 3

1.3 Justification 4

1.4 Objectvies 5

1.4.1 Broad objective 5

1.4.2 Specific objectives 6

1.5 Research questions 6

CHAPTER TWO 8

LITERATURE REVIEW 8

2.1 The cowpea 8

2.1.1 Botanical classification of cowpea 8

2.1.2 Origin and geographic distribution of cowpeas 11

2.1.3 Cowpea cultivation and production 13

2.1.4 Importance of cowpea 16

2.1.5 Challenges and constraints in cowpea production 18

2.2 Diseases of cowpeas 18

2.2.1 Fungal diseases of cowpea 18

2.2.2 Viral diseases 20

2.2.3 Nematode diseases 21

2.2.4 Bacterial diseases 22

2.3 Bacterial blight disease of cowpeas 23

2.3.5 Symptoms and spread of Xanthomonas axonopodis pv. vignicola 24

2.3.6 Predisposing factors in the spread of *Xanthomonas axonopodis* pv. *vignicola*.. 25

2.3.1 Taxonomy of the genus Xanthomonas 26

2.3.2 Synopsis of *Xanthomonas axonopodis* 29

2.3.3 Morphological and biochemical features of *Xanthomonas axonopodis* 30

2.3.4 Pathological characteristics of *Xanthomonas axonopodis* pv. *vignicola* 30

2.3.5 Pathogenesis of *Xanthomonas axonopodis* pv. *vignicola* 31

2.4 Genetic variation of *Xanthomonas axonopodis* pv. *vignicol*a 32

2.4.1 Inter-simple sequence repeats (ISSR) for genetic diversity study of *Xanthomonas axonopodis* pv. *vignicola* 33

2.4. 2 Virulence determinants of *Xanthomonas axonopodis* pv. *vignicola* 35

2.4.3 Cross pathogenicity of *Xanthomonas axonopodis* 37

2.5 Management of *Xanthomonas axonopodis* 38

2.5.1 Cultural methods 38

2.5.2 Physical methods 38

2.5.3 Chemical methods 39

2.5.4 Use of resistant varieties 40

2.5.5 Biological methods 40

2.5.5.1 Microbial used in the management of *Xanthomonas axonopodis* 40

2.5.5.2. Plant botanical extracts in the management of *Xanthomonas axonopodis* 42

CHAPTER THREE 43

MATERIALS AND METHODS 43

3. 1 Determination of occurrence of bacterial blight of cowpea and collection of diseased samples 43

3. 1. 1 Data analysis 44

3. 2 Isolation and identification of the *Xanthomomonas axonopodis* pv. *vignicola* 44

3.2.1 Isolation of the *Xanthomonas axonopodis* pv. *vignicola* from diseased cowpea leaves 44

3. 2. 2 Morphological characterization of different isolates of *Xanthomonas axonopodis* pv. *vignicola* 45

3. 2. 2. 1 Gram staining reaction 45

3.2.3 Biochemical characterizations of different isolates of *Xanthomonas axonopodis* pv. *vignicola* 46

3.2.3.1 Starch hydrolysis 46

3.2.3.2 Catalase test 47

3.2.3.2 Liquification test (Gelatin liquefaction) 47

3.2.3.4 Lactose utilization 47

3.2.3.5 Acid production for sucrose and mannitol 48

3.2.3.6 Hydrogen sulphide (H_2S) production 48

3.2.3.7 Indole test, Methyl Red test, Voges Proskauer test and Citrate utilization test (IMVic reactions) 48

3.2.4 Pathological characterizations of different isolates of *Xanthomonas axonopodis* pv. *vignicola* 50

3.2.4.1 Planting of cowpea plants 50

3.2.4.2 Inoculum preparation 50

3.2.4.3 Inoculation 51

3.2.4.4 Data analysis 53

3.2.5 Molecular identification of the *Xanthomonas axonopodis* pv. *vignicola* isolates 53

3.2.5.1 DNA extraction and quantification from *Xanthomonas axonopodis* pv. *vignicola* 54

3.2.5.2 Amplification of 16S rDNA 55

3.2.5.3 Gel electrophoresis 56

3.2.5.4 DNA sequencing and interpretation 57

3.3 Determination of genetic diversity of *Xanthomonas axonopodis* pv. *vignicola* using Inter-Simple Sequence Repeats (ISSR) 57
3.3.1 DNA extraction and quantification of the isolated pathogens 57
3.3.2 Amplification of ISSR regions of the genome of the isolates 57
3.3.3 Gel Electrophoresis 59
3.3.4 Data Analysis 59
3.4. Cross pathogenicity of *Xanthomonas axonopodis* pv. *vignicola* 60
3.4.1 Inoculum preparation 60
3.4.2. Inoculation 61
3.4.3 Data analysis 62
3.6 Evaluation of the antibacterial potential of biological control agents 62
3.6.1 In vitro evaluation of the antibacterial potential of *Bacillus subtilis* and *Bacillus amyloliquefaciens* in controlling *Xanthomonas axonopodis* pv. *vignicola* 62
3.6.2 In vitro evaluation of the antibacterial potential of Salvia nilotica, neem, garlic and ginger against *Xanthomonas axonopodis* pv. *vignicola* 63
3.6.2.1 Preparation of plant parts for phytochemical extraction 63
3.6.2.2 Extraction of phytochemicals from plant samples 64
3.6.2.3 Evaluation of antibacterial activity of crude extracts of Salvia nilotica, garlic, neem and ginger 64
3.6.2.4 Data analysis 66
CHAPTER FOUR 68
RESULTS 68
4.1 Determination of the incidence and prevalence of bacterial blight on cowpea 68
4.2 Characterization of *Xanthomonas axonopodis* pv. *vignicola* 71
4.2.1 Cultural and morphological features of *Xanthomonas axonopodis* pv. *vignicola* isolates 71
4.2.2 Biochemical features of *Xanthomonas axonopodis* pv. *vignicola* isolates 74
4.2.3 Pathogenicity and virulence of *Xanthomonas axonopodis* pv. *vignicola* isolates on susceptible cowpea variety 76

4.2.4 Molecular characterization of the *Xanthomonas axonopodis* pv *vignicola* isolates 77

4.3 Analysis of genetic diversity of the *Xanthomonas axonopodis* pv. *vignicola* using inter-simple sequence repeat (ISSR) 78

4.3.2 Polymorphism information content 79

4.3.3 Polymorphic loci 80

4.3.4 Total band patterns for binary (haploid) data by populations 81

4.3.5 Analysis of molecular variance 83

4.3.6 Band frequencies, allele frequencies and estimated diversity by population for binary (haploid) data 84

4.3.7 Analysis of pairwise population matrix of Nei genetic distance of X*anthomonas axonopodis* pv. *vignicola* isolates 86

4.3.8 Pairwise population matrix of Nei genetic identity of the Xanthomonas axonopodis pv. vignicola populations 86

4.3.9 Principal coordinates analysis 87

4.3.9 Diversity phylogenetic tree of *Xanthomonas axonopodis* pv. *vignicola* isolates 89

4.4 Determination of the cross pathogenicity of *Xanthomonas axonopodis* pv. *vignicola* 92

4.5 Evaluation of the antibacterial potential of *Bacillus* sp. and selected botanicals against *Xanthomonas axonopodis* pv. *vignicola* 95

4.5.1 In vitro antibacterial activity of *Bacillus subtilis* against *Xanthomonas axonopodis* pv. *vignicola* through dual culture and inverted plate techniques 95

4.5.2 Antibacterial activity of *Bacillus amyloliquefaciens* against *Xanthomonas axonopodis* pv. *vignicola* through dual culture and inverted plate techniques 97

4.5.3 Comparison of antibacterial activities of *Bacillus subtilis* and *Bacillus amyloliquefaciens* 99

4.5.4 In vitro evaluation of *Salvia nilotica*, neem, garlic and ginger extracts against *Xanthomonas axonopodis* pv. *vignicola* 101

CHAPTER FIVE 103

DISCUSSION 103

5.1 The incidence and prevalence of bacterial blight on cowpea grown in different regions of Kenya 103
5.2.1 Morphological and biochemical features of *Xanthomonas axonopodis* pv. *vignicola* isolates 105
5.2.2 Pathogenicity and virulence of *Xanthomonas axonopodis* pv. *vignicola* isolates on susceptible cowpea variety 107
5.2.3 Molecular characterization of *Xanthomonas axonopodis* pv. *vignicola* using 16S rDNA 109
5.3 Genetic diversity of *Xanthomonas axonopodis* pv. *vignicola* using inter-simple sequence repeat 109
5.4 Cross pathogenicity of *Xanthomonas axonopodis* pv. *vignicola* on common beans, lentils, soybean, pigeon peas and green grams 114
5.5 The antibacterial potential of *Bacillus* sp. and selected botanicals against *Xanthomonas axonopodis* pv. *vignicola* 116
5.5.1 The antibacterial activities of *Bacillus subtilis* and *Bacillus amyloliquefaciens* against *Xanthomonas axonopodis* pv. *vignicola* 116
5.5.2 The antibacterial activity of crude extracts of *Salvia nilotica*, garlic, neem and ginger against *Xanthomonas axonopodis* pv. *vignicola* 118
CHAPTER SIX 121
CONCLUSIONS AND RECOMMENDATIONS 121
6.1 Conclusions 121
6.2 Recommendations 122
REFERENCES 123
APPENDICES 166

LIST OF TABLES

Table 1: Taxonomy and nomenclature of *Vigna unguiculata* species complex 10
Table 2: Productivity and production output of cowpeas in the world 14
Table 3: Cowpeas production and market performance in Kenya for the year 2017-2021 16
Table 4. The sequences of the Inter-Simple Sequence Repeats primers 58
Table 5. PCR reaction components for studying genetic diversity of *Xanthomonas axonopodis* pv. *vignicola* isolates 58
Table 6. PCR protocol for studying genetic diversity of *Xanthomonas axonopodis* pv. *vignicola* 59
Table 7: Incidence of bacterial blight of cowpea in Makueni, Isiolo, Kakamega, Uasin Gishu, Laikipia, and Meru counties 69
Table 8: Morphological features of *Xanthomonas axonopodis* pv. *vignicola* isolates 72
Table 9: Morphological features of *Xanthomonas axonopodis* pv. *vignicola* isolates collected from the six counties of Kenya 73
Table 10: Biochemical features of *Xanthomonas axonopodis* pv. *vignicola* isolates .. 75
Table 11: Virulence of *Xanthomonas axonopodis* pv. *vignicola* isolates on susceptible cowpea variety 77
Table 12: Polymorphism information content for the markers used in ISSR diversity studies of *Xanthomonas axonopodis* pv. *vignicola* isolates 80
Table 13: Percentage of polymorphic loci among the *Xanthomonas axonopodis* pv. *vignicola* isolates in different counties 81
Table 14: Total band patterns for binary (haploid) data by populations/counties 83
Table 15: Mean and standard error (SE) over loci for each *Xanthomonas axonopodis* pv. *vignicola* population 85
Table 16: Pairwise population matrix of Nei genetic distance for each *Xanthomonas axonopodis* pv. *vignicola* population 86
Table 17: Pairwise *Xanthomonas axonopodis* pv. *vignicola* population matrix of Nei genetic identity 87
Table 18: Cumulative variation spread across various main coordinates 88

LIST OF FIGURES

Figure 1: Prevalence of bacterial blight of cowpea from various counties in Kenya .. 70
Figure 2 Cowpea bacterial blight disease prevalence per county 70
Figure 3: Mean incidence of bacterial blight of cowpea in six counties surveyed in Kenya 71
Figure 4: Band patterns across populations from the different regions 82
Figure 6: PCoA via covariance matrix with data standardization *Xanthomonas axonopodis* pv. *vignicola* population from various counties 89
Figure 7: UPGMA Dendrogram generated from ISSR amplification sequences of 48 *Xanthomonas axonopodis* pv. *vignicola* isolates based on NEI 72 coefficient 91
Figure 8: Severity levels of cowpea bacterial blight disease for the different tested crops 95
Figure 9: Percent inhibition of *Xanthomonas axonopodis* pv. *vignicola* by *Bacillus subtilis* using inverted plate and dual culture techniques 96
Figure 10: Interaction of time of exposure and method of screening in the inhibition of *Xanthomonas axonopodis* pv. *vignicola* by *Bacillus subtilis* 97
Figure 11: Percent inhibition of *Xanthomonas axonopodis* pv. *vignicola* by *Bacillus amyloliquefaciens* using inverted plate and dual culture techniques 98
Figure 10: Interaction of time of exposure and method of screening in the inhibition of *Xanthomonas axonopodis* pv. *vignicola* by *Bacillus amyloliquefaciens* 99
Figure 11: Comparison of antibacterial activities of *Bacillus subtilis* and *Bacillus amyloliquefaciens* 100
Figure 12: Comparison of antibacterial activities of *Bacillus subtilis* and *Bacillus amyloliquefaciens* after 72 hours of exposure in both dual culture and inverted plate methods 101
Figure 13: Comparison of antibacterial activities of the tested bio-agents at the two levels of concentrations 102

LIST OF PLATES

Plate 1: Sample disease scores used during the severity studies 53

Plate 2: The axenic cultures of *Xanthomonas axonopodis* pv. *vignicola* (A-D). Typical yellow isolate (A and B); Creamy yellow isolate (C) and Light yellow pigmentation (D) 73

Plate 3: Agarose gel confirmation of DNA of 13 isolates of *Xanthomonas axonopodis* pv. *vignicola*..78

Plate 4: Agarose gel confirmation of PCR amplicons of 9 isolates of *Xanthomonas axonopodis* pv. *vignicola* 78

Plate 5: ISSR amplification patterns produced by primer D-3 and A-31 on *Xanthomonas axonopodis* pv. *vignicola* isolates 79

Plate 6: ISSR amplification patterns produced employing primer UBC8932800 and A-31 on *Xanthomonas axonopodis* pv. *vignicola* isolates 79

4.3.2 Polymorphism information content (PIC) 79

Plate 7: Fabaceae family members tested for cross-pathogenicity 93

Plate 8: Diseased and healthy leaves from tested plants...94

LIST OF ABBREVIATIONS

µl	Microliters
AEZ	Agro-ecological zones
AFA	Agriculture and Food Authority
AMOVA	Analysis of molecular variance
ANOVA	Analysis of Variance
BICMV	*Blackeye cowpea mosaic virus*
BLB	Bacterial leaf blight
CABMV	C*owpea aphid-borne mosaic potyvirus*
SBMV	S*outhern bean mosaic sobemovirus*,
cDNA	Complementary Deoxyribonucleic Acid
CFU	Colony forming units
CMV	*Cucumber mosaic cucumovirus*
CPCMV	C*owpea chlorotic mottle bromovirus*
CPMMV	C*owpea mild mottle Carlavirus*
CPMMV	C*owpea mild mottle virus*
CPMoV	C*owpea mosaic comovirus*
CPMoV	*Cowpea mottle virus*
CPSMV	C*owpea severe mosaic virus*
CYMV	C*owpea yellow mottle virus*
D.f	Degrees of freedom
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
EDTA	Ethylenediaminetetra acetic acid
FAO	Food and Agricultural Organisation
FAOSTAT	The Food and Agriculture Organization Corporate Statistical Database
G	Grams
IBPGR	International Board for Plant Genetic Resource
ISSR	Inter-simple sequence repeats
Mean Sq	Mean sum of squares
$MgCl_2$	Magnesium chloride

Ml	Millilitres
mRNA	Messenger Ribonucleic Acid
MYMIV	*Mungbean yellow mosaic India virus*
NA	Nutrient agar
PCoA	Principal Coordinate Analysis
PCR	Polymerase chain reaction
Psc	Pascal
qPCR	Quantitative Polymerase chain reaction
RAPD	Random amplified polymorphic DNA
RNA	Ribonucleic acid
Rpm	Revolutions per minute
rRNA	Ribosomal Ribonucleic acid
SBMV	S*outhern bean mosaic virus*
SDCDAR	State Department for Crops Development and Agricultural Research
SE	Standard error
SSR	Single sequence repeat markers
Sum Sq	Sum of squares
TBE	Tris-borate buffer
UoE	University of Eldoret
WHO	World Health Organization

LIST OF APPENDICES

Appendix I: Bacterial leaf blight (BLB) field survey questionnaire..........................166
Appendix II: ANOVA table for the cowpea bacterial blight incidence from the different sites 167
Appendix III. Tukey multiple comparison of means for disease incidence by sampled site with 95% family-wise confidence level 167
Appendix IV: ANOVA table for the cowpea bacterial blight disease incidence by county...171
Appendix V: Multiple comparison of mean disease incidence among the counties .171
Appendix VI: Per cent identity of *Xanthomonas axonopodis pv. vignicola* isolates.172
Appendix VII: Summary of analysis of molecular variance (AMOVA)..................173
Appendix VIII: The *Xanthomonas axonopodis* pv. *vignicola* diversity matrix data .173
Appendix IX: ANOVA table for the tested crops bacterial blight disease severity levels 176
Appendix X: Multiple comparison of mean disease severity levels of the tested legume crops 176
Appendix XI. ANOVA for *Bacillus subtilis* inhibition by the dual culture and inverted plate methods 177
Appendix XII: Tukey multiple comparison of means for *Bacillus subtilis* inhibition of *Xanthomonas axonopodis* pv. *vignicola* 177
Appendix XIII: ANOVA table for the interaction of time of exposure and method of screening in the inhibition of *Xanthomonas axonopodis* pv. *vignicola* by B. subtilis 177
Appendix XIV: ANOVA table for *Bacillus amyloliquefaciens* percent inhibition through both dual culture and inverted plate methods..178
Appendix XV: Tukey multiple comparison of means for *Bacillus amyloliquefaciens* percentage inhibition...178
Appendix XVI: ANOVA table for the interaction of time of exposure and method of screening in the inhibition of *Xanthomonas axonopodis* pv. *vignicola* by *Bacillus amyloliquefaciens* 178
Appendix XVII: ANOVA table for the inhibition of *Xanthomonas axonopodis* pv. *vignicola* by the different botanicals at concentration level of 12.5 mg/ml..............179

Appendix XVIII: Tukey multiple comparison of means for the action of the different botanical extracts against the growth of *Xanthomonas axonopodis* pv. *vignicola* at extract concentration of 12.5 mg/ml 179
Appendix XIX: ANOVA table for the inhibition of *Xanthomonas axonopodis* pv. *vignicola* by the different botanicals at concentration level of 25 mg/ml.................. 179
Appendix XX: Tukey multiple comparison of means for the action of the different botanical extracts against the growth of *Xanthomonas axonopodis* pv. *vignicola* at extract concentration of 25 mg/ml 180
Appendix XXI: ANOVA table for the comparison of the two levels of extract concentration for the inhibition of *Xanthomonas axonopodis* pv. *vignicola* 180
Appendix XXII: Similarity Report 181

CHAPTER ONE

INTRODUCTION

1.1 Background information

Cowpea (*Vigna unguiculata*) is a leguminous crop within the family Fabaceae that is globally cultivated by subsistence farmers especially in arid and semi-arid tropical regions (Lazaridi and Bebeli, 2023). It is an economically important crop cultivated in Asia, sub-Saharan Africa and the Americas (Affrifah et al., 2022). Its leaves and grains are utilized as food for humans and animals as well as the source of cash for the rural population (USDA, 2021). Cowpea is rich in plant protein providing amino acids such as isoleucine, lysine, phenylalanine, valine, methionine, histidine, and tryptophan (Loushigam and Shanmugam et al., 2023), crude fibre (Ogbonnaya et al., 2024), carbohydrates (Wanjiku et al., 2023), vitamins such as C, A, K, niacin and total choline (Affrifah et al., 2022) and iron, phosphorus, potassium, sodium, magnesium, zinc, and calcium minerals (Abebe et al., 2022). Cowpea provides health advantages including; anti-diabetic and anti-cancer (Owade et al., 2020), the leaves serve as antipyretic, diuretic, used in the control of ulcers, smallpox, burns, adenitis, and menstrual challenges (Abebe et al., 2022), and treatment of menopausal syndrome (Fithri et al., 2024).

Currently, Kenya is the largest producer of cowpea in East Africa with an estimated total land area under cultivation being more than 227, 800 hectares (Francis, 2017; Singh et al., 2023). The major improved cowpea varieties cultivated in Kenya include; M 66, KVU 27-1, KVU HB 48E 10, Nyekundu, MTW 610, KCP 022, MTW 63, ICV,

Katumani 80 (K80), and KVU 419, and other local land race varieties (Muindi et al., 2023; Odundo, 2023; Oyoo et al., 2017).

The production of cowpea is affected by various biotic factors among them are viral diseases which include cowpea mosaic virus, cucumber mosaic virus and southern bean mosaic virus (Ogunsola et al., 2023); Root-knot Nematodes (Joshua et al., 2023); Fusarium wilt, anthracnose and leaf spot are the most destructive fungal diseases (Dada et al., 2023; Obisesan et al., 2023), and bacterial diseases including the Bacterial spot and Bacterial blight are the major limiting diseases (Deshpande et al., 2023). Bacterial blight caused by *Xanthomonas axonopodis* pv. *vignicola* is among the destructive diseases hampering the production of cowpeas worldwide (Nantale et al., 2023a). The disease leads to a reduction in the quality and quantity of both the leaves and grains thus further reducing the market value of the leaf produce (Ogbuji and Isalar, 2021). The pathogen is mainly seed-borne (Obafemi et al., 2024). The pathogen has been known to differ in terms of pathological and physiological characteristics (Olatunde et al., 2024). The diversity of the pathogen studies has enabled the identification of the different pathogen characteristics which are crucial even in the development and identification of resistant germplasm. It has been established that several Xanthomonas intra-pathovar groups of strains interact with intra-species variants of the host (Manna et al., 2024). Xanthomonas patho-systems early detection is of paramount importance for the control and management of the diseases caused by this bacterium (Khan et al., 2022; Okoth, 2023). Among the diagnostic techniques, molecular diagnosis has proven as a cost-effective option for identifying pathogens with great genetic proximity (Clavijo et al., 2022). These molecular biology techniques have been extensively used for identifying various species of the genus Xanthomonas (Catara et al., 2021).

To date, disease management studies have not been adequately effective and harmless to satisfy the needs for the management of this disease. In the recent past, the use of chemical pesticides is highly discouraged due to effect on human health and environmental concerns as well as potentiality of re-infection in two or three years with susceptible cultivars and also build-up of resistant pathogen strains (Dugan et al., 2023; Khan et al., 2023). With the transformative agriculture scenario, the safest and most promising disease management technology is the use of biological control agents (Waghunde et al., 2023). However, the popularization of bio-control agents is very slow at only 2% available in contrast to chemicals (Rana et al., 2024). Due to this, there is need for continuous search for effective bio-agents because they are sustainable (Singh et al., 2023) in their workings in the suppression and management of the pathogen.

1.2 Statement of the problem

The yield of the cowpea, particularly among small-scale farmers, has been extremely low, averaging 0.5 t/ha, despite the many advantages of cowpea as food and an integral part of the cropping system (Owade et al., 2020). This is in contrast to the potential yield of 2.5 t/ha for certain cowpea varieties (Nderi, 2020). For a long time, bacterial blight caused by *X. axonopodis* pv. *vignicola* has threatened the global production and existence of cowpea. Bacterial blight has been documented to cause grain yield loss of about 64% both in quality and quantity (Owade et al., 2020). In Kenya the disease status in cowpea producing areas has however not been exhaustively defined, similar to the actual causal *Xanthomonas axonopodis* bacterial pathogen responsible for the disease. Cowpea because of its resiliency and versatility in the arid and semi-arid crop, has been recently promoted for production as a mitigation measure to climate change. The crop has further been introduced to none cowpea production regions in Kenya, despite this

there has been limited research on the disease status in cowpea grown in these regions. The disease has been mainly managed by chemical pesticides including copper based fungicides and antibiotics. Regular application of these pesticides has led to serious environmental hazards and harmful effects on both human health and other beneficial organisms. Due to the health hazards associated with the use of these chemicals, the application of other harmless biological techniques and other integrated methods in disease management is highly advocated. Several research findings have reported the potential of various bacteria and botanicals as promising options. in managing phytopathogenic bacteria (Mulatu et al., 2023). However, the potential activity of these biocontrol agents in the management of *Xanthomonas axonopodis* pv. *vignicola* is limited, as this is necessary to provide knowledge on their effect.

1.3 Justification

Cowpea provide a good source of vitamins, carbohydrates and mineral elements. It is one of the vegetables utilized in Kenya and other countries due to its nutritive value. Numerous challenges such as bacterial blight caused by *X. axonopodis* pv. *vignicola*, are mostly responsible for the severely poor yield of cowpea (Olal, 2015). The cowpea bacterial blight in cases of severe infection, result in a 92% decline in yield globally (Nantale et al., 2023a). The disease is highly prevalent in numerous agricultural regions in the tropical and sub-tropical states. The mortality caused by bacterial blight has been a great threat to the cultivation of cowpeas (Nantale et al., 2023b). Although the disease has been reported in the eastern part of Africa (Kenya, Tanzania and Uganda), information on bacterial blight is still scanty despite its presence in this region (Tollo et al., 2020). Further, the majority of cowpea farmers are small-scale, low-resource

farmers who are unable to fund the suggested management techniques, such as timely planting or routine spraying.

Since the prevalence of the disease was first documented in Kenya, no substantial advancement has been achieved in characterising the pathogen (*X. axonopodis* pv. *Vignicola)* diversity in Kenya. It is therefore significant to study the current status in terms of prevalence, incidence, genetic diversity, cross pathogenicity, and control of *X. axonopodis* pv. *vignicola* using bio-agents because of the destruction it causes on cowpeas. Saha et al. (2022) noted that the pathogen develops resistance to various pesticides after some period of application. Further, the use of synthetic pesticides has been implicated to hazardous health and environmental effects (Dugan et al., 2023). Consequently, this necessitates the continuous study of the pathogen diversity and further explore alternative bio-control options for the management. *Bacillus* sp. (*Bacillus amyloliquefaciens* and *Bacillus subtilis*) and plant botanicals can provide the complementary active natural bio-agents for disease management. Information on the disease incidences and the pathogen characteristics in different regions in Kenya is important in the realm of climate change, considering that cowpea has been considered a highly resilient crop and therefore promoted as one among other food crop in response to climate change.

1.4 Objectvies

1.4.1 Broad objective

To evaluate the genetic diversity, cross pathogenicity and control of *Xanthomonas axonopodis* pv. *vignicola* causing cowpea bacterial blight using *Bacillus* sp. and extracts from selected plants for effective control of this pathogen for increased cowpea productivity.

1.4.2 Specific objectives

i. To determine the incidence and prevalence of bacterial blight on cowpea in Makueni, Isiolo, Kakamega, Uasin Gishu, Laikipia and Meru counties in Kenya.

ii. To characterize *Xanthomomonas axonopodis* pv. *vignicola* responsible for bacterial blight of cowpea using morphological and biochemical characteristics, pathogenicity and 16S rDNA.

iii. To analyse the genetic diversity *Xanthomomonas axonopodis* pv. *vignicola* using inter-simple sequence repeat.

iv. To evaluate the cross pathogenicity of *Xanthomomonas axonopodis* pv. *vignicola* on common beans, lentils, soybean, pigeon peas and green grams.

v. To evaluate the antibacterial potential of *Bacillus* sp. and selected botanicals (*Salvia nilotica*, garlic, neem and ginger) against *X. axonopodis* pv. *vignicola*.

1.5 Research questions

i. What are the occurrence levels of bacterial blight on cowpea in Makueni, Isiolo, Kakamega, Uasin Gishu, Laikipia and Meru counties in Kenya?

ii. What are the morphological, biochemical, pathological and molecular characteristics of *Xanthomomonas axonopodis* pv. *vignicola* responsible for bacterial blight of cowpea?

iii. Are there genetic differentiation of *Xanthomomonas axonopodis* pv. *vignicola* infecting cowpea in different regions of Kenya?

iv. Do *Xanthomomonas axonopodis* pv. *vignicola* infect other crop members of the same family?

v. How effective are *Bacillus* sp. and selected botanicals in controlling *X. axonopodis* pv. *vignicola* causing bacterial blight in cowpea?

CHAPTER TWO

LITERATURE REVIEW

2.1 The cowpea

2.1.1 Botanical classification of cowpea

Linnaeus first identified the cowpea-cultivated form from the Antilles as *Dolichos unguiculatus* L. Walpers subsequently categorised it as *Vigna unguiculata* (L.) Walp in 1753 (Pasquet, 1998; Singh, 2020). Cowpea [*Vigna unguiculata* (L.) Walp] is a dicotyledonea with 2n = 22 chromosomes (Lonardi et al., 2019). Cowpea belongs to the order Fabales, family Fabaceae, subfamily Faboidea, tribe Phasaeolea, subtribe Phaseolinae, genus *Vigna,* section *Catiang*, Genus *Vigna*, Species *Vigna unguiculata* (L.) Walp, Subspecies (cultivated) *V. unguiculata* subsp. *unguiculata*, and Cultivar groups: *unguiculata*, *biflora*, s*eqsquipedalis*, *textilis* (Ebong, 1970; Singh, 2005; Singh, 2020). There has been a lot of debate and misunderstanding in the past regarding the classification and nomenclature of cowpeas due to their wide diversity in both cultivated and wild forms. However, it is now accepted that cowpeas belong to the botanical species *Vigna unguiculata* (L.) Walp., which formerly had more than twenty synonyms, including *V. cylindrical*, V. *sinensis, V. catjang, Dolichos sinensis,* and *Dolichos unguilculata* (Gbedevi et al., 2021; Lazaridi and Bebeli, 2023; Singh, 2020).

While different experts have identified different subgroups within the cultivated cowpea, they all agree that the cultivated cowpeas belong to the *Vigna unguiculata* subspecies *unguiculata*. It is now recognized that the four subspecies of cultivated cowpeas that were previously recognized as *unguiculata*, *biflora/cylindrical*, *sesquipedalis*, and *textilis* are actually four cultigroups within the subspecies

unguiculata (Choudhary et al., 2023; Pan et al., 2023; Pasquet, 1998). Cultigroup unguiculata is the widely grown, spreading and semi-erect cowpea that is cultivated worldwide. It is characterised by a variety of plant and leaf types, thick pods that are 10 to 30 cm long and have 5 to 12 mm long seeds, a variety of seed coat colours and textures and mostly pendant but occasionally erect hilums (Choudhary et al., 2023; Pan et al., 2023).

The Cultigroup biflora/cylindrical are varieties that are mostly farmed for vegetable pods, dry seeds that are used as pulses and fodder in India and other parts of Asia. Smaller and narrower pods (7.5–13 cm long) with self-coloured, light brown and cream seeds that are smooth and have a little hilum ring with the pods typically held erect. The Cultigroup textilis with small pods and small seeds with extremely long peduncles (50–80 cm) characteristic of this group were formerly farmed in West Africa along the banks of the Benue and Niger rivers, where fishermen would use the peduncles to fashion fishing lines. Nevertheless, the cultivation of these textilis is currently extremely restricted and rapidly dwindling. The textilis when fully grown, the dried peduncles are beaten to produce fibres that are used to make fishing ropes and lines. Lastly, the Cultigroup sequipedalis is one of the most widely grown vegetable crop in China, Korea, India, and Southeast Asia, is because of its extremely long, succulent pods that can reach a length of 90 cm. It is also known as the yard-long bean, snake bean, and asparagus bean. Compared to ordinary cowpeas, their kidney-shaped, longer (8–12 mm) seeds have smooth, brown, or cream seed coatings. Their growing tendency is to climb; thus, they require assistance from trellises (Boukar et al., 2023; Choudhary et al., 2023; Narayana and Angamuthu, 2021; Pan et al., 2023; Pasquet, 1998; Singh, 2020). Below is a synopsis of the four cultigroups that fall under the subspecies *unguiculata* (Table 1):

Table 1: Taxonomy and nomenclature of *Vigna unguiculata* species complex

Marechal et al., (1978)	Plenaar (1992)	Pasquet (1993)	Padulosi (1993)
V. unguiculata	*V. unguiculata*	*V. unguiculata*	*V. unguiculata*
	The cultivated forms of this are		
ssp, *unguiculata*	ssp, *unguiculata*	ssp, *unguiculata*	ssp, *unguiculata*
Cultigroups: unguiculata, textilis,sequipedalis and cylindrica/biflora			
	The wild types include		
ssp. *Dekindtiana* var. *dekindtiana*	ssp. *Dekindtiana* var. *huliensis* var. *dekindtiana*	ssp. *dekindtiana* var. *dekindtiana*	ssp. *dekindtiana* var. *huliensis* var. *grandiflora* var. *dekindtiana* var. *congolensis*
var. *mensensis*	ssp. *Mensensis*	ssp. *baoulensis* ssp. *Letouzeyi* ssp. *Burundlensis*	var. *ciliolata*
var. *protracta*	ssp. *mensensis*	ssp. *stenophylla*	ssp. *protracta* var. *rhomboidea* var. *protracta* var. *kgalagadiensis*
var. *pubescens*	ssp. *protracta*	ssp. *pubsescens*	ssp. *pubescens*
Ssp. *tenuis* Ssp. *stenophylla*	ssp. *Tenuis* ssp. *Stenophylla*	ssp. *Tenuis* ssp. *Stenophylla*	ssp. *tenuis* ssp. *stenophylla* var. *parviflora* var. *tenuis* var. *oblonga*

Verdcourt (1970) was the first to propose the classification and name of the immediate wild progenitors of cultivated cowpeas. Marechal et al. (1978), Pasquet (1993), Pedulosi (1993) and, Prinnar and Van Wyk (1992), refined this system. Ng and Padulosi (1991) and Padulosi, (1993) noted that IITA carried out a thorough characterization of over 400 wild *Vigna unguiculata* spp. Numerous species have been described, and some species' nomenclature has changed as a result of this study similar to the surveys of live materials in the field and specimens in significant herbaria collections in Europe and Africa, and cytological research (Ketema et al., 2020; Ng, 1995; Padulosi, 1993).

In the wild cowpea genotypes, there is a great deal of variation in plant morphology, including leaf type, growth habit, maturity, pod type, seed type, and colour (Panchta et al., 2021; Pasquet, 1993; Singh, 2005). The *pubescent* subspecies, p*ubescens* and protracta, have hairy stems, leaves, and pods. subsp. *pubescence* hairs, on the other hand, are smooth, straight, soft, and adhered to the stem and pod surfaces. Conversely, the hair types of subspecies *Protracta* are severely stiff, bristly, upright, and straight, whereas, the subspecies *burundiensis* mainly found in Burundi, Uganda, Kenya, and Zaire**.**

2.1.2 Origin and geographic distribution of cowpeas

Although there is a great deal of variability in cowpea in Asia and Africa, its exact origin has long been a source of conjecture and debate. Early findings revealed that cowpeas in Asia differed morphologically and were more diverse than those in Africa. Consequently, it was believed that cowpea originated independently in both Asia and Africa. Nonetheless, an Asian centre of origin has now been questioned in light of the lack of wild cowpeas in Asia as potential ancestors. While the exact location of the crop's domestication in Africa is unknown, all available evidence points to Southern Africa as the cowpea's original home (Lazaridi and Bebeli, 2023).

Numerous domestication centres have been proposed, such as Ethiopia (Sauer, 1952; Steele, 1976), Central Africa (Piper, 1913), West Africa (Faris, 1963; Lush and Evans, 1981), and South Africa (Zhukovashii, 1962). Utilising single-nucleotide polymorphism (SNP) markers Huynh et al. (2013) reported that cowpea originated from two distinct domestication processes. This was due to the identification of two gene pools, one located in West Africa and the other in East Africa. According to Guimarães et al. (2023), using SSR, SilicoDArT, and SNP for population structure analysis, two

distinct gene pools in Mozambique (Southern Africa) and Portugal (Southern Europe) were identified. Mozambican landraces and Portuguese landraces did not have a similar genetic heritage. Xiong et al. (2016), showed that the cowpea landraces from Southern Europe were less connected to those from Southern Africa, specifically Mozambique, and more related to those from West and Central Africa.

The team that met at the International Board for Plant Genetic Resource on *Vigna,* held in New Delhi (IBPGR, 1981), recommended as a priority the collection of both wild and cultivated forms of cowpea in Southern Africa, Transvaal, Natal, and Zimbabwe. This recommendation was based on the distribution of diverse wild cowpeas in Eastern Africa, which stretch from Ethiopia to Southern Africa. According to Baudoin and Marechal (1985) and Gbedevi et al. (2021), West and Central Africa are the secondary centres of diversity, while the Southern and East Africa are considered as the principal regions of diversity. Asia is suggested to be the third centre of diversity. The existence of diversity among the cowpea varieties was later confirmed by Gumede et al. (2022) through the genetic diversity studies they carried out on the cowpea grown in West, South, and East Africa, where a significant genetic diversity and population structure among the cowpea genotypes using single nucleotide polymorphism (SNP) markers was noted.

According to Herniter et al. (2020) and Ng and Marechal (1985), cowpeas are thought to have originated in Eastern Africa and travelled to India before 150 BC, West Asia and Europe before 300 BC, and the Americas around 1500 AD. Small-seeded and vegetable cowpeas were selected in South Asia and Southeast Asia, whereas less variability and selection happened in Europe and Western Asia since these regions lack the ideal climate for cowpeas. There is a large amount of variation and secondary wild

forms in East and West Africa due to the distribution of the very small seeds of wild cowpeas by birds long before the arrival of Christianity. Human selection in wild cowpeas for larger seeds and superior growth habits have been implicated to have produced a variety of cultigroups and their domestication in Asia and Africa (Herniter et al., 2020; Ng, 1995).

2.1.3 Cowpea cultivation and production

The indigenous cowpea (*V. unguiculata*) is a versatile crop that is mostly grown in sub-Saharan Africa's tropical regions (Owade et al., 2020; Sobda et al., 2018). According to FAOSTAT (2019), sub-Saharan Africa accounts for 95.6% of the land area under production of cowpea crop for leaves globally. The huge land mass under cowpea production have additionally been exacerbated by the drought-tolerant and short-term life span of cowpea (Mekonnen et al., 2022), which has made it easier to intercrop it with other major crops, including sorghum or maize (Mwenda et al., 2023). Cowpea is a leguminous plant hence helping in fixing nitrogen in the soil, thus improving soil fertility and thereby enhancing yields (Nunes et al., 2022; Owade et al., 2020).

In the world, the leading cowpea producing countries are; Nigeria, Mali, Niger, Senegal, Burkina Faso, Ghana, Togo, Cameroon, Benin and Chad in West and Central Africa; South Sudan, Sudan, Somalia, Malawi, Kenya, Uganda, Zambia, Tanzania, Botswana, Zimbabwe and Mozambique in East and Southern Africa (Table 2); Bangladesh, India, Nepal, Sri Lanka, Myanmar, China, Indonesia, and Philippines in Asia; Cuba, West Indies and Haiti, in Central America; USA in North America and Brazil in South America (FAOSTAT, 2020; Lazaridi and Bebeli, 2023; Munyao, 2023).

Table 2: Productivity and production output of cowpeas in the world

S/N	Country	Area harvested (Ha)	Yield per hectare	Production in tons	Inference on Production
1	Nigeria	2,853,097	9,137	2,606,912	1st
2	Niger	5,889,677	4,035	2,376,727	2st
3	Burkina Faso	1,307,336	4,826	630,965	3st
4	Ghana	11,898	19,862	215,350	4st
5	Tanzania	30,366	4,096	202,865	5st
6	Cameroon	258,898	4,043	185,832	6st
7	Kenya	11,154	4,367	179,399	7st
8	Mali	160,412	3,767	157,739	8st
9	Myanmar	119,398	11,425	136,411	9st
10	Sudan	333,638	2,678	104,667	10st
11	Mozambique	284,451	5,545	89,356	11st
12	Democratic Republic of Congo	95,803	4,432	72,726	12st
13	Senegal	260,408	6,889	60,422	13st
14	Malawi	159,345	13,515	42,456	14st
15	United States	169,279	4,296	23,632	15st
16	China	209,371	8,876	15,652	16st
17	Madagascar	14,596	8,907	13,000	17st
18	Uganda	208,059	9,750	12,439	18st
19	Sri Lanka	9,499	11,770	11,180	19st
20	South Africa	15,108	10,360	4,871	20st

Source: **FAOSTAT, 2020**

The key cowpea production regions in Kenya are Kisii, Kitui, Taita Taveta, Migori, Bungoma, Kakamega, Kwale, Makueni, Machakos, Tharaka Nithi, Laikipia, and Kilifi. Due to the need for crop diversification and climate resilience therefore, the crop has recently been introduced in other counties such as Meru, Isiolo, Uasin Gishu, Baringo, Trans Nzoia among others (Basweti and Achieng et al., 2022; Binacchi et al., 2022; Muindi et al., 2021; Mwenda et al., 2023; Owade et al., 2020). Depending on the cultivar, the crop can be produced anywhere from 0 to 2000 meters above sea level. Cowpeas may provide good yields with as little as 300–700 mm of rainfall per year since they are comparatively drought-resistant (Lazaridi and Bebeli, 2023; Verheyen et al., 2024). Yields are lowered by excessive rain or protracted dry spells (Mentari et al.,

2023). During flowering, excessive rainfall leads to floral abortion, whereas during harvesting, dry weather is crucial. Warm weather is ideal for cowpeas (Tolefack et al., 2023). For their growth, a temperature range of 20 to 35°C is ideal. Temperature extremes have an impact on crop development and growth (Thiombiano et al., 2023). Numerous types of soil can be used to raise cowpeas however, a better production is encouraged by fertile soils that drain well and have an ideal pH of 5.5 to 6.5 (Mugale et al., 2023; Singh et al., 2023). Grown varieties of cowpeas in Kenya include KVU 419, KCP 022, Kenkunde, Kunde 1, Katumani 80 (K80), KVU 27-1, 419, MTW 63, 610, Kitui black eye, Machakos 66 (M66), and other local land race varieties (Muindi et al., 2023; Munyao, 2023; Odundo, 2023; Wanjiku et al., 2023).

Kenya produces approximately 115,801 MT of cowpeas annually (Horticultural Crops Directorate, 2016), worldwide 8.9 million metric tonnes of cowpeas were produced in in 2019 (FAO, 2021) (Table 3). In Kenya there has been a fluctuating trend in cowpea production from 239,131 ha in 2020 to around 235,734 ha in 2021. Similarly, a drop in productivity has also been reported from 12.3 (90 kg) bags per hectare to 11.8 (90 kg) bags per hectare in 2021, leading to a corresponding production of 264,160 tonnes and 250,060 tonnes in 2020 and 2021 respectively (AFA, 2022).

Table 3: Cowpeas production and market performance in Kenya for the year 2017-2021

Year	**2017**	**2018**	**2019**	**2020**	**2021**
Area (Ha)	254,669	258,732	242,274.88	239,131	235,734
Production					
90 kg bag	1,627,300	1,990,078	2,471,045.20	2,935,111	1,655,788
Metric tonnes (MT)	146,457	179,107	222,394	264,160	250,060
Yield (90 kg/Ha)	6.4	7.7	10.2	12.3	11.8
Farm gate (KES/90 kg bag)	4,295	3,354	5,430	5,269	5,354
Wholesale (90 kg bag)	7,653	6,598	6,815	7,187	8,427
Imports- MT	238	0	0.3	1.1	136
Total Value (billion) KES	7	6.7	13.4	15	4

***Source*: State Department for Crops Development and Agricultural Research (SDCDAR)**

2.1.4 Importance of cowpea

Cowpeas play a significant role in nutrition, income generation, and food security, making them valuable for households and communities. It is essential to the support of all the different value chain participants, such as farmers, processors, merchants, and food vendors (Nwagboso et al., 2024). Cowpeas are produced as a fodder, cover crop, food (pods, leaves, and seeds), and green manure (Horn et al., 2022; Nwagboso et al., 2024). Bokelmann et al. (2022), Kirigia et al. (2018) and Owade et al. (2020), observed that cowpea is one of the African leafy vegetables that can help to increase food and nutrition security in sub-Saharan Africa because the crop is used for forage in addition to being used as food for humans. The majority of households in the main cowpea-growing regions of Kenya do consume boiled cowpea leaves, with a few consuming sundried or blanched types (Owade et al., 2020).

In Kenya and other parts of the world, the leaves are eaten raw, dried, or fermented by the native communities (Biama et al., 2020). By using the crop's leaves as food, one can obtain nutrients like protein, beta-carotene, iron, zinc, calcium, fibre, potassium,

non-digestible carbohydrates, sodium content, very low lipids, amino acids, polyphenols with antioxidant properties, and protein, which are highly valued among sub-Saharan Africa's vulnerable population (Affrifah et al., 2022; Kirigia et al., 2018; Oliveira et al., 2023). The USDA (2021) noted that cowpea is rich in essential amino acids including histidine, lysine, isoleucine, cysteine, leucine, tyrosine, threonine, valine, tryptophan, phenylalanine, globulin, albumins, alcohol-soluble prolamins, aspartic, and glutamic acids.

In addition to their nutritional value, cowpea leaves and green pods are employed in the management of several human ailments, such as smallpox, measles, adenitis, ulcers, and burns (Abebe et al., 2022). Similarly, cowpea plant seeds are useful for treating a variety of illnesses, including astringents, diuretics, and antipyretics. Decoction or soup is used for conditions related to the liver and spleen, intestinal cramp, leucorrhoea, irregular menstruation, and urine expulsions (Abebe et al., 2022; Ogbole et al., 2023; Pioltelli et al., 2023).

Cowpea haulms have been used as animal feed due to their highly nutritive value (Amole et al., 2022; Samireddypalle et al., 2017). Akplo et al. (2023) and Faye et al. (2024) explained the importance of cowpea among the dual-purpose crops that have been utilised for grain and fodder to improve the health status of both humans and domestic animals in semi-arid sub-Saharan Africa. It has also played a great role in improving food security, living standards, and malnutrition in sub-Saharan Africa and further being utilized as animal fodder (Nyaga et al., 2020; Owade et al., 2020). Additionally, cowpea encourages the building of soil organic matter as well as the fixation of carbon and nitrogen when included in crop rotation systems. Thus, soil fertility is increased, and physical properties like water infiltration and retention

capacity are improved (Beker et al., 2023; Faye et al., 2024; Ondieki et al., 2017). Muindi et al. (2021) noted that cowpea is a significant multifunctional legume crop. The cowpea also tends to improve biological soil fertility and crop productivity by interacting with a broad variety of highly ecologically valuable rhizobia bacteria in the soil (Wekesa et al., 2022).

2.1.5 Challenges and constraints in cowpea production

Cowpea (*Vigna unguiculata* L. Walp.) a significant leguminous crop mostly farmed by smallholder farmers for animal feed and food security mostly in sub-Saharan Africa (Lazaridi and Bebeli et al., 2023). Despite the wide usage and popularity of the cowpea in some communities in Kenya, because of its resiliency, a number of factors limit the production, productivity and utilisation of this vegetable in Kenya. The loss of cowpea yield potential is caused by a number of biotic and abiotic variables, including diseases (bacterial, viral, and fungal), insect pests, poor soil fertility, drought, and metal toxicity (Karikari et al., 2023; Nkomo et al., 2021; Omomowo et al., 2021). The contribution of in-availability of improved varieties that can tolerate these stressors, inadequate farming methods, and a lack of inputs required for higher profitability and productivity are other factors causing low yields. Microbial pathogens causing diseases have been found to be among the main factors limiting cowpea productivity in Kenya (Owade et al., 2020; Wanjiku et al., 2024).

2.2 Diseases of cowpeas

2.2.1 Fungal diseases of cowpea

The most damaging phytopathogens of cultivated crops worldwide are fungi (Akintunde et al., 2023; Fisher et al., 2012; Omomowo et al., 2021). In the field and

post-harvest phase, cowpea is destroyed by a large number of species belonging to several fungal genera. The cowpea production losses that occasionally reach 100% have been linked to fungal diseases that are transmitted through seeds and the soil. Da Silva et al., (2021) noted that fungal pathogens such as *Fusarium* sp., *Aspergillus* sp., *Macrophomina* sp., *Nigrospora* sp., and *Cladosporium* sp. carried in cowpea seeds lead to a serious reduction in the emergence of seeds. *Mucor* sp., *Aspergillus flavus,* and *Rhizopus* sp. infect cowpea seeds as post-harvest pathogens causing a drastic reduction in grain weight (Joy et al., 2020). According to Deepika et al. (2020) and, Mbeyagala et al. (2022) southern blight, also known as stem disease incited by *Sclerotium rolfsii*, damping-off incited by *Pythium* sp., seedling blight or collar caused by *Rhizoctonia solani,* and Fusarium wilt incited by *Fusarium oxysporum* f.sp. *tracheiphilum,* are among the major soil-borne fungal diseases limiting the cultivation and production of cowpeas globally.

Akitunde et al. (2023); Etaware et al., (2019) and Loeto et al., (2016) noted from their study that *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Rhizopus stolonifera*, *Aspergillus parasiticus*, *Rhizoctonia solani, Septoria vignae, Fusarium solani, Aspergillus temarii, Penicilllium chyrosogenum, Cladosporium cladosporioides, Colletotrichum gloeosporioides*, *Corynespora cassiicola*, and *Botryodiplodi theobromae* are some of the most prevalent fungal pathogens linked with cowpea seed deterioration and postharvest loss. Ndalira et al. (2020) and Kiprop et al. (1987) noted that cowpeas grown in Eastern and Western Kenya were infected with Septoria leaf spot disease and rust fungus, respectively. Similarly, Kankam et al. (2023) reported that rust disease infects cowpeas grown in Northern Ghana by significantly interfering with root development and mineral, water and nutrient uptake, resulting in poor crop growth. Sindushree et al., (2023) documented that cowpea rust incited by

Uromyces phaseoli var. *vignae* (Barcl.) Arth. leads to a significant reduction in grain development.

Cowpea seeds carry seed-borne pathogens such as *Alternaria* aff. *burnsii*, *Fusarium* sp., and *Epicoccum* sp. that cause serious crop infections at the seedling development stage (Pachoute et al., 2024). Bakhshi et al., (2021) and Edet et al., (2022) reported the infection of cowpea with Cercospora leaf spot disease caused by a complex of cercosporoid fungi complex (*Cercospora canescens*, *Cercospora* sp. G, C. *iranica, Cercospora* sp. T, *Cercospora* cf. *flagellaris*, and *Cercospora vignigena*) and two *Pseudocercospora* species (*Pseudocercospora* cf. *cruenta* and *Pseudocercospora griseola* f.sp. *griseola*). *Culvularia penniseti* and *Nigrospora sphaerica* fungi are responsible for the browning of the aerial parts of the cowpea crop, resulting in stunted growth of infected plants (Obisesan and Ojo, 2023).

2.2.2 Viral diseases

Viral infections can have a negative effect on cowpea production, in certain situations, they have been linked to yield losses of up to 90% in cowpeas or, to crop failure entirely. Globally, more than 20 viruses have been reported to infect cowpea (Horn and Shimelis, 2020; Nsa and Kareem, 2015). Incidence of cowpea mosaic virus has been found to decrease due to the application of diammonium phosphate fertiliser and farmyard manure (Kiptui et al., 2020; Mutebi and Moranga, 2022). Whitney and Gilmer (1974) associated the dissemination of the cowpea mosaic virus with thrips, chrysomelid beetles, and grasshoppers. Mangeni et al., (2020) demonstrated that the common bean mosaic virus transmitted by an aphid vector is capable of infecting cowpea plants, resulting in symptoms such as local lesions, curling, and stunting.

Eight viruses namely *Cowpea aphid-borne mosaic virus* (CABMV), *Bean common mosaic virus* (BMMV), *Clackeye cowpea mosaic strain* (BCMV), *Cowpea mild mottle virus* (CMMV), *Cucumber mosaic virus* (CMV), *Cowpea mottle virus* (CMV), *Southern bean mosaic virus,* and *Cowpea yellow mosaic virus* (CYMV)*,* were detected in diseased cowpeas in Southwest Nigeria, causing symptoms of stunted growth, puckering, mottling, systemic mosaic, leaf deformation, and vein banding (Ogunsola et al., 2023; Praneetha et al., 2022). Additionally, Abd El-Aziz (2024) and Adams et al. (2020) noted that CYMV, *Cowpea mosaic comovirus*, *Cowpea mottle virus* (CPMoV), *Cucumber mosaic cucumovirus* (CMV), *Southern bean mosaic sobemovirus*, CABMV, *Cowpea mild mottle virus* (CPMMV), *Cowpea mild mottle Carlavirus* (CPMMV), S*outhern bean mosaic virus* (SBMV), *Cowpea severe mosaic virus* (CPSMV), and *Cowpea chlorotic mottle bromovirus* (CPCMV) are among the economically significant cowpea pathogenic viruses capable of causing devastating infections in sub-Saharan Africa.

Mungbean yellow mosaic India virus (MYMIV) is the most devastating pathogenic virus in India, inciting yellow mosaic disease in cowpeas during the rainy season, especially late-sown crops where grain yield losses of 58.38% have been recorded (Kumar et al., 2022). *Cowpea mottle virus* (CPMoV) and *Blackeye cowpea mosaic virus* (BICMV) have the ability to infect cowpea with the production of severe symptoms that cause losses in yield (Ahmed et al., 2022).

2.2.3 Nematode diseases

Nematodes are one of the factors preventing cowpea productivity from improving and are also the reason for significant losses in cowpea yield (Dareus et al., 2021; Joshua, 2023; Omomowo et al., 2021). Divya et al., (2021); Krishna and Nisha (2023) and

Ribeiro et al., (2022) reported the infection of cowpea by the *Meloidogyne incognita* nematode, causing root-knot disease. Similar findings were reported by Kusakabe et al. (2023) and Simion and Palle (2022), who noted that *M. incognita* in cowpeas is responsible for the devastating destruction of cowpea plants. Severe infections by the root knot *M. incognita* nematode leads to premature senescence of the cowpea plant (Thakur et al., 2023). *Aphelenchoides besseyi* nematode was reported to be responsible for foliar dark spots on cowpea (Favoreto et al., 2022 and Noronha et al., 2023). Reniform *(Rotylenchulus reniformis)* soil nematode, infects the roots of cowpea crops, causing root destruction (Lira et al., 2020; Ria et al., 2023; Zaki et al., 2024).

2.2.4 Bacterial diseases

Bacterial infections are some of the major factors affecting cowpea yields, causing massive crop losses of up to 90% in the form of reduced pod sizes, seed grain, leaf, and fodder (Agbicodo et al., 2010; Praneetha et al., 2022). A number of these detrimental pathogens spread through seeds and others spread through the soil (Constantin et al., 2016; Timilsina et al., 2020). The detrimental symptoms of bacterial pathogen infection in cowpeas are brownish leaf spots, observable stem splits, necrotizing and yellow halo leaf forms, blotches, and water-filled pods (de Lima-Primo et al., 2019; Saha et al., 2022). Mahesha et al., (2022) further noted that bacterial blight is the main biotic stress affecting cowpea. Under severe infections, cowpea bacterial blight can result in up to 92% yield loss (Nantale et al., 2023a).

Bacterial wilt and tan spot disease of cowpea, caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens,* has been documented as among the most devastating bacterial infections of cowpea. It causes disease symptoms such as systemic wilt and interveinal chlorosis on leaflets, resulting in necrotic zones, seed discoloration

to orange, yellow, or pink (Osdaghi et al., 2020). El-Nagdi et al., (2021) noted that *Pseudomonas fluorescens* is a plant pathogen capable of infecting cowpea. The Xanthomonas genus of bacteria is associated with some of the most harmful cowpea diseases, especially the bacterial blight (Durojaye et al., 2019; Nantale et al., 2023b; Saha et al., 2022).

2.3 Bacterial blight disease of cowpeas

Cowpea bacterial blight, also known as canker, is common in cowpea production regions where it is severe during the windy and rainy seasons, due to the contribution of these factors in speeding up the bacterial spread and growth (Durojaye et al., 2019; Mahesha et al., 2022). The occurrence of bacterial blight disease in Kenya have been documented. The improved cowpea varieties in Kenya were reported to be more resistant to bacterial blight compared to local varieties (Njonjo et al., 2018). The bacterial pathogen, *Xanthomonas axonopodis* pv. *vignicola*, is the main causative agent of the destructive seed-borne cowpea bacterial blight disease (Agbicodo et al., 2010; Saha et al., 2022). The pathogen has been observed to overwinter in plant detritus and can endure for up to fourteen years in seeds with the disease being mainly transmitted through seed infection (Karavina et al., 2011).

Nantale et al., (2023a) reported the occurrence of bacterial blight with devastating effects of crop yield loss of up to 92% in severe conditions in Uganda with the improved varieties showing appreciable resistance to bacterial blight disease. Comparable conclusions had been reported by Adipala et al., (1995), who noted that cowpea blight in Uganda caused by *X. axonopodis* pv. *vignicola* was commonly damaging with the most promising mitigation measure being the use of resistant genotypes. Similar findings were reported by Saha et al., (2022) in Bangladesh explaining that cowpea

bacterial blight hinders production, and the most promising intervention is the development of resistant crop varieties. The bacterial blight causal Xanthomonas genus have undergone significant evolutionary changes, including the horizontal transfer of genes coding for traits responsible for pathogenicity and adaptation (Chen et al., 2021).

Several reports by Agbicodo et al. (2010); Durojaye et al. (2019); Ganiyu et al. (2017) and Oguntade et al. (2021), have documented the occurrence of cowpea bacterial blight disease in various cowpea-growing agro-ecological regions in Nigeria causing significant losses in grain yield, harvested leaves and fodder. This has been noted to endanger food security in sub-Saharan Africa. Ogbuji and Isalar (2021) blamed bacterial blight disease for the serious losses in both harvested leaves and grain yield in the sub-Saharan Africa. Shi et al., (2016) noted cowpea bacterial blight disease caused by *Xanthomonas axonopodis* pv. *vignicola* as among the most economically devastating bacterial infection of cowpea, due to its prevalence in major cowpea production areas globally, and that the deployment of resistant cultivars serves as the critical technique to controlling the disease.

2.3.5 Symptoms and spread of *Xanthomonas axonopodis* pv. *vignicola*

The initial symptom of cowpea bacterial blight appears as wrinkled and reddish cotyledons of seedling originating from contaminated seeds (Ganiyu et al., 2017; Kumar et al., 2018). At first, the leaves develop as necrotic lesion which eventually extends to infect the stem. Once the infectious agent enters the vascular bundles, the disease spreads throughout the plant body, infecting the growing tip and ultimately destroying the plant (Agbicodo et al., 2010; Praneetha et al., 2022). The primary effect of *X. axonopodis* pv. *vignicola* infection is on the leaves, and it might result in total defoliation based on the genotype's susceptibility (Claudius-Cole et al., 2016; Ibrahim,

2023). The secondary symptoms on the leaf are manifested as light yellow round spots dispersed across the lamina, with a diameter ranging from 4 to 10 mm. These spots have a brown, necrotic centre with crimson veins. Pods develop streaks of deep green colour or wetness. The infected pods shrink and turn yellow; smaller, wrinkled, and contaminated seeds are produced by the sick pods (Agbicodo et al., 2010; Durojaye et al., 2019; Omomowo et al., 2021). The major source of the primary inoculum is the contaminated seeds, while wind, rain, and insects disseminate the pathogen to cause the secondary infection (Durojaye et al., 2019).

2.3.6 Predisposing factors in the spread of *Xanthomonas axonopodis* pv. *vignicola*

Inadequate field preparation, little or no fertiliser application, low altitude, a substantial weed infestation, and a single cropping system are linked to the incidence and severity of bacterial blight, which have been known to play a major role in the disease's epidemics (Mengesha and Yetayew., 2018). Girma et al., (2022), showed a strong linkage between the bacterial blight disease intensity and the independent factors, namely altitude, weed management practices, sowing date, cropping system, crop growth stage, crop cultivar, seed sources and fertiliser application. Mengesha and Yetayew et al., (2018) found that planting early or late lowers, the average severity of the bacterial blight infection as compared to the optimal planting time.

The utilisation of infected seeds majorly accounts for the persistence and dissemination mechanisms of cowpea bacterial blight. When *X. axonopodis* pv. *vignicola* infects flower buds and early pods, it can spread to the seed through the vascular system and harm the eventual seedlings upon germination (Dell'Olmo et al., 2023; Mahesha et al., 2022). Warm temperatures and heavy rains throughout the growing season promote leaf moisture and infection on leaves and pods (Marcuzzo and Füchter., 2021). Weeds

encircling cropping fields and volunteer plants inside the crop area can harbour insect pests and plant pathogens, or they might establish a microclimate that is conductive to disease development and therefore, could increase the occurrence of bacterial blight. Weeds in infested fields retain the pathogen that causes cowpea bacterial blight for longer period (Zang et al., 2021). Decreased crop resilience as a result of weeds' fierce competition for resources predisposes host crops to both aerial and soil-borne diseases including bacterial blight (Degu et al., 2023; Yimer et al., 2018). Interestingly the high-altitude regions are less prone to bacterial blight disease development (Girma et al., 2022). The variability of plant populations, has been known to be influenced by intercropping, because it impacts the disease cycles by modifying wind speed, host density, microclimate (relative humidity, temperature, and leaf wetness) and vector transmission (Degu et al., 2023; Girma et al., 2022).

2.3.1 Taxonomy of the genus Xanthomonas

Majority of the bacteria in the genus *Xanthomonas* are plant-pathogenic and occur all over the world, causing diseases in a variety of plants. Numerous taxonomic and determinative investigations have been conducted on this genus because of its economic significance (Vauterin et al., 1995; Olatunde et al., 2024). DNA-rRNA hybridizations have demonstrated that the genus belongs to a distinct rRNA branch within the Proteobacteria's gamma taxon (Stackebrandt et al., 1988). The taxonomy in the Bergey's Manual of Systematic Bacteriology, indicated that the genus is split into the following five species: *Xanthomonas axonopodis*, *Xanthomonas fragarie*, *Xanthomonas ampelina*, *Xanthomonas albilineans,* and *Xanthomonas campestris* (Bradbury et al., 1984). Vauterin et al. (1990) noted a number of these suggestions were developed to enhance the classification of the genus *Xanthomonas* and included the; i)

Ride and Ride (1978) who named "*Xanthomonas populi*"; (ii) *Pseudomonas maltophilia* was transferred to the genus *Xanthomonas* and named as *Xanthomonas maltophilia* by Swings et al., (1983); (iii) Van der Mooter et al. (1987a) suggested that the pathovars *Xanthomonas campestris* pv. *oryzae* and *Xanthomonas campestris* pv. *graminis* as phenotypically distinct from the other *Xanthomas campestris* pathovars and, hence renamed as distinct species *Xanthomonas oryzae* and *Xanthomonas graminis*. Further, Willems et al. (1987) suggested that *Xanthomonas ampelina* should be renamed as *Xylop hilus ampelinus* and that it is not a member of the *Xanthomonas* genus.

Van der Mooter et al. (1987b) suggested eight species of the Xanthomonas genus namely: *Xanthomonas*: *Xanthomonas albilineans*, *X. campestris*, *X. axonopodis*, X. *graminis*, *X. fragarie*, *X. maltophilia*, *X. populi,* and *X. oryzae*. Among these species, *Xanthomonas campestris* is by far the most complicated due to its division into over 123 pathovars, as detailed by Dye et al. (1980). Vauterin et al. (1995) conducted a thorough DNA-DNA hybridization on 183 strains of the genus *Xanthomonas* and noted that the genus was composed of twenty genomic homology associations. They noted that the following groupings matched the species that had previously been described and could not be affected by reclassification: *Xanthomonas fragariae*, *Xanthomonas albilineans*, *Xanthomonas populi,* and *Xanthomonas oryzae*. They noted that *Xanthomonas campestris*, the previously described species, was diverse, with sixteen DNA homology groups with a significant degree of DNA homology between one of these groups and *Xanthomonas axonopodis*.

According to amendment of Vauterine et al. (1995) the type species of the genera *Xanthomonas campestris* (Dowson, 1939; Pammel, 1985) comprised only the

pathovars isolated from crucifers *X. campestris* pv. *armoraciae, X. campestris* pv. *aberrans, X. campestris* pv. *barbareae, X. campestris* pv. *incanae, X. campestris* pv. *campestris,* and *X. campestris* pv. *raphanin. Xanthomonas axonopodis,* as classified by Starr and Garces (1950), was amended to encompass 34 previous pathovars of *Xanthomonas campestris*. They suggested the following species names *Xanthomonas arboricola* sp. *nov*., encompassing *X. arboricola* pv. *juglandis*, *X. arboricola* pv. *corylina, X. arboricola* pv. *poinsettiicola*, *X. arboricola* pv. *pruni*, *X. arboricola* pv. *populi* and *Xanthomonas bromi* sp. *nov.*; *Xanthomonas cassavae* sp. nov., nom. rev. (Wiehe and Dowson 1953); *X. codiaei* sp. *nov*., comprising isolates of type B of *X. campestris* pv. *poinsettiicola*, the extant taxon; *Xanthomonas hortorum* sp. nov., comprising *X. hortorum* pv. *hederae*, *X. hortorum* pv. *pelargonii,* and *X. hortorum* pv. *vitians; Xanthomonas cucurbitae* (Bryan, 1926) sp. nov., nom. rev.; *Xanthomonas melonis* sp. nov.; *Xanthomonas hyacinthi* (Wakker, 1883) sp. nov., nom. rev.; *Xanthomonas sacchari* sp. nov. for strains isolated from infected sugarcane in Guadeloupe; *Xanthomonas theicola* sp. nov.; *Xanthomonas pisi* (Goto and Okabe 1958) sp. nov., nom. rev.; *Xanthomonas translucens* (Jones et al., 1917) sp. nov., nom. rev., comprising *X. translucens* pv. *arrhenatheri, X. translucens* pv. *graminis, X. translucens* pv. *cerealis, X. translucens* pv. *hordei, X. translucens* pv. *phleipratensis, X. translucens* pv. *phlei, X. translucens* pv. *poae, X. translucens* pv. *undulosa, X. translucens* pv. *secalis; Xanthomonas vasicola* sp. *nov.,* comprising *X. vasicola* pv. *vasculorum and X. vasicola* pv. *holcicola* (type B strains of the earlier taxon *X. campestris* pv. *vasculorum*); and the type B strains of the extinct taxon *Xanthomonas campestris* pv. *vesicatoria,* which are included in *Xanthomonas vesicatoria* (Doidge, 1920) sp. nov., nom. rev.

2.3.2 Synopsis of *Xanthomonas axonopodis*

The *Xanthomonas axonopodis* is described in the same way as the genus. The phytopathogenic specificity on a broad range of host plants distinguishes the following pathovars: *X. axonopodis* pv. *alfalfae*, *X. axonopodis* pv. *axonopodis*, *X. axonopodis* pv. *bauhiniae*, *X. axonopodis* pv. *cajani*, *X. axonopodis* pv. *begoniae*, *X. axonopodis* pv. *cassavae*, *X. axonopodis* pv. *citri* (previously *Xanthomonas campestris* pv. *citri* group A strain) and *X. axonopodis* pv. *cassia* (Hartung and Civerolo, 1987), Gabriel et al., (1989) reclassified *Xanthomonas axonopodis* pv. *citrumelo* as *Xanthomonas campestris* pv. *citrumelo*; *Xanthomonas axonopodis* pv. *aurantifolii* as *Xanthomonas campestris* pv. *aurantifolii*. Gabriel et al., (1989), Palleroni et al., (1993) and Vauterin et al., (1995) named the following as 38 members of *Xanthomonas axonopodis*; *X. axonopodis* pv. *clitoriae*, *X. axonopodis* pv. *cyamopsidis*, *X. axonopodis* pv. *coracanae*, *X. axonopodis* pv. *desmodii*, *X. axonopodis* pv. *desmodiilaxiflori*, *X. axonopodis* pv. *desmodiigangetici*, *X. axonopodis* pv. *dieffenbachiae*, *X. axonopodis* pv. *desmodiirotundifolii*, *X. axonopodis* pv. *erythrinae*, *X. axonopodis* pv. *lespedezae*, *X. axonopodis* pv. *glycines*, *X. axonopodis* pv. *malvacearum*, *X. axonopodis* pv. *patelii, X. axonopodis* pv. *manihotis*, *X. axonopodis* pv. *phaseoli*, *X. axonopodis* pv. *phyllanthi, X. axonopodis* pv. *phaseoli-fuscans*, *X. axonopodis* pv. *poinsettiicola*, *X. axonopodis* pv. *ricini*, *X. axonopodis* pv. *rhynchosiae*, *X. axonopodis* pv. *sesbaniae*, *X. axonopodis* pv. *vasculorum, X. axonopodis* pv. *tamarindi*, *X. axonopodis* pv. *vesicatoria*, *X. axonopodis* pv. *vignicola*, *X. axonopodis* pv. *vignaeradiatae*, *X. axonopodis* pv. *vitians*, *X. axonopodis* pv. *betlicola*, *X. axonopodis* pv. *fascicularis*, *X. axonopodis* pv. *biophyti*, *X. axonopodis* pv. *khayae*, *X. axonopodis* pv. *martyniicola*, *X. axonopodis* pv. *maculifoliigardeniae*, *X. axonopodis* pv. *melhusii*, *X. axonopodis* pv. *pedalii*, *X.*

axonopodis pv. *nakataecorchori*, *X. axonopodis* pv. *punicae*, and *X. axonopodis* pv. *physalidicola*.

2.3.3 Morphological and biochemical features of *Xanthomonas axonopodis*

The members of *Xanthomonas axonopodis* have common morphological and biochemical features despite the differences in host specificity. They are gram-negative rods that measure 1.0 to 2.9 by 0.4 to 0.6 μm and typically appear either singly or in pairs, or in chains (Anmod et al., 2022; Chormale et al., 2021; Rothe et al., 2022). Typically, the cells move by using a single polar flagellum; have yellow mucoid, smooth, circular, slightly or highly elevated, and convex colonies (which may be light or brownish yellow colonies) in nutrient agar media (Van den Mooter and Swings, 1990; Nauman et al., 2023). Andrews et al. (1976) and Suke et al. (2022) noted that the yellow pigment of Xanthomonas is mono- or dibromoarylpolyenes referred to as Xanthomonadins, which are the key feature of the genus. Further, they noted that the viscous or mucoid nature of the cultures is due to the xanthan exopolysaccharide.

Xanthomonas axonopodis generally are strict aerobes, catalase positive, esculin, starch, gelatin negative, and milk proteolysis; they lack the ability to reduce nitrate to nitrite, are oxidase negative or weakly reactive; Indole, acetoin, and urease are not generated (Anmod et al., 2022; Khan et al., 2024; Oguntade et al., 2021). When cultured in mediums that contain carbohydrates like arabinose, glucose, mannose, cellabiose, and trehalose, it produces very little acid (Chaudhari et al., 2022; Kabade et al., 2020).

2.3.4 Pathological characteristics of *Xanthomonas axonopodis* pv. *vignicola*

Xanthomonas axonopodis pv. *vignicola* is one of the pathovar members of the *X. axonopodis* with pathogenicity specificity to cowpea (*Vigna unguiculata*). The cowpea

bacterial blight disease severity significantly differs among the different cowpea varieties, with disease severity ranging from 0.0 to 2.0 on severity scale of 0-5 (Durojaye et al., 2019; Nantale et al., 2023a). Agbicodo et al., (2010) and Oguntade et al., (2021) noted that sometimes the virulence of *X. axonopodis* pv. *vignicola* isolates on the susceptible cowpea host variety is only obtained after a combination and co-inoculation with two or more isolates, which increases in the improved synergistic potential of the pathogens to initiate a disease in combination rather than singly. Individual isolates functioning in tandem share a common pathovar (Oguntade et al., 2021). Kumar et al., (2022) noted that 30 to 40-day-old plants were more susceptible to *Xanthomonas axonopodis* pv. *vignicola,* with older leaves being less susceptible when compared to the newly emerged leaves.

2.3.5 Pathogenesis of *Xanthomonas axonopodis* pv. *vignicola*

The disease symptoms manifest in the form of a small, chlorotic, brownish, water-soaked lesion that eventually grows and merges to produce vast necrotic patches on the leaves, causing stem cankers, leaf fall, pod and flower abortion, and seedling death (Durojaye et al., 2019; Olatunde et al., 2024). Lin et al. (2020) noted that the pathogen produces irregular brown necrotic areas with yellow margins on the leaves of infected plants, with visualization of bacterial streaming on a light microscope upon cutting the lesion. Joshua (2023), Praneetha et al. (2022) and Chen et al. (2021) noted that tiny water-soaked patches that eventually turn into necrotic lesions are the first signs of the disease; these are then followed by yellow haloes on the leaves that are then located in accordance with the bacterial infection course; brown crack stripes on the stems, dark green water-soaked lesions and pustules on the pods, a swollen canker, and, at the end, discoloration in the seeds. Kumar et al. (2022) reported similar disease symptoms on

the infected plant leaves and that the initial symptoms were observed within 5 to 10 days of infection. Saha et al. (2022) observed further, that the bacterial blight symptoms include small brown patches that eventually increase to bigger necrotic areas on leaves, which are highly noticeable with an increase in the number of days.

On the seedlings, the symptoms noted are: blight, discoloration of leaf veins, necrotic spots, wilting, yellowing of the leaves, drying of the leaves, and sparse fibrous roots (Isalar and Ogbuji, 2021). When a seed is infected, it manifests as small, creamy yellow patches that eventually turn brown (Chen et al., 2021; Mahesha et al., 2022). These spots can be found on the hilum region in cases of vascular dissemination, at the micropyle in cases of floral infection, or all over the seed coat in cases of infection by touch (Chen et al., 2021; Kumar et al., 2022). Strong infections can cause seeds to shrink, which has a negative impact on the vigour and rate of germination (Darrasse et al., 2018). The entire plant may die in warm weather conditions and in cases of severe infestation. Plants and seedlings can be infected but may not even show symptoms (Joshua et al., 2023). These diseased plants experience early leaf fall and tiny, translucent, wet patches that are easier to see on the abaxial side of the leaf (Omoigui et al., 2020). Other symptoms noted when a pod becomes infected include, patches of dark green water on the sites where the bacteria enter the seeds causing shrivelling and discoloration.

2.4 Genetic variation of *Xanthomonas axonopodis* pv. *vignicola*

Genetic diversity of *X. axonopodis* pv. *vignicola* has been mainly evaluated using the molecular techniques. Several researchers have employed varied techniques in quest of understanding genetic diversity that exist among the different *X. axonopodis* pv. *vignicola* isolates. Several molecular markers have been employed to study the genetic

variations among *X. axonopodis* pv. *vignicola* isolates. These markers which have been previously used analyse the genetic variation of *X. axonopodis* pv. *vignicola* include the single sequence repeat markers (SSR) (Duche et al., 2015; Sharma et al., 2022), random amplified polymorphic DNA (RAPD) markers (Fatima et al., 2012; Madavi, 2022; Olatunde et al., 2024; Oliveira et al., 2023; Ramdas and Patil 2020) and inter-simple sequence repeat (ISSR) (Fatima et al., 2012).

2.4.1 Inter-simple sequence repeats (ISSR) for genetic diversity study of *Xanthomonas axonopodis* pv. *vignicola*

Genetic variations among *X. axonopodis* pv. *vignicola* isolates have been previously illustrated through single sequence repeat (SSR) (Duche et al., 2015), random amplified polymorphic DNA (RAPD) (Fatima et al., 2012; Olatunde et al., 2024) and inter-simple sequence repeat (ISSR) (Fatima et al., 2012) in other regions of the world. Examining the genetic diversity of the various *X. axonopodis* pv. *vignicola* isolates genome is critical into determining the genetic linkages and genotypes associated with virulence geographic regions among various isolates. The finding is crucial for the development of resistant cowpea genotypes with high degrees of resistance to *X. axonopodis* pv. *vignicola* pathogens (Timilsina et al., 2020).

Inter-simple sequence repeats (ISSR) are semi-arbitrary dominant identifiers that replicate target nucleotide sequences scattered throughout the genome (El-Fatah et al., 2023). As long as the annealing temperature is high, this approach produces multilocus and substantially polymorphic results. ISSR markers are used to study the genetic variation of bacteria isolates as it is a powerful technique for detecting pathotypes (Ebrahimi et al., 2021). ISSR-PCR has proven to be rapid, accurate, and effective for establishing genetic connections between Xanthomonas isolates (Igwe et al., 2022;

Samanta and Mandal, 2014). The presence of genetically diverse *Xanthomonas axonopodis* isolates in different geographical regions could possibly be linked to the pathogen's horizontal spread, which is facilitated by genetically homogeneous clones of elite crop cultivars' and further also by the planting material that appears healthy but is actually latently diseased (Kumar et al., 2020).

Great genetic diversity does exist among the *X. axonopodis* pv. *vignicola* isolates, and it is significant in identifying the genotypes and genetic links between various isolates as well as their virulence genotypes (Olatunde et al., 2024; Sharma et al., 2022). Duche et al. (2015) and Verdier et al. (1998) noted the existence of genetic variations among the *X. axonopodis* pv. *vignicola* isolates isolated from different cowpea-growing regions in Nigeria. They noted that the diversity analysis has been used in the identification of polymorphic traits associated with virulence characters that aids in the analysis of plant pathogen interactions at the genetic level. Further, they noted that understanding plant pathogen genetic variation dynamics is important in the identification for the development of resistance genotypes, as it is significant in preventing the selection of new virulence that responds effectively against the genetic sources of resistance genes that are now available. Khatri-Chhetri et al. (2003) further, noted that *X. axonopodis* pv. *vignicola* metabolic variations were not associated with its virulence diversity.

The genotype factors among *X. axonopodis* pv. *vignicola* might likely account for their modifications to various cowpea hosts and varied pathogenic traits (Olatunde et al., 2024). Fatima et al., (2012) expalined that when genetic diversity studies for *X. axonopodis* isolates were carried out via the ISSR technique, high levels of polymorphisms were observed among the isolates with intraspecific diversity at the

amplification (prominent bands) in the range of 200 to 1000 bp (base pairs). Kumar et al., (2018) and Madavi et al., (2023) reported similar results regarding the banding patterns, ranging from 100 to 1000 bp, produced by ISSR primers when they analysed the genetic diversity of *Xanthomonas axonopodis* isolates from the different geographical regions in India, they further observed that isolates from the same geographical region were clustered together despite the difference in virulence or race. According to Samanta and Mandal (2014), a band size of 275 to 3050 bp in genetic variability studies among the *Xanthomonas axonopodis* isolates from four agro-ecological zones of India was reported. The diversity was mainly associated with the geographic zone of the isolates. According to Kumar et al., (2018) the geographical zone of *Xanthomonas axonopodis* isolates played an important role in the genetic composition and virulence of the isolates. Pathogen diversity enhances the host specificity, the expression of susceptibility genes, the emergence of new strains, and mechanisms of host avoidance which are important in the management of the disease (Timilsina et al., 2020).

2.4. 2 Virulence determinants of *Xanthomonas axonopodis* pv. *vignicola*

Toxins, cell wall-degrading enzymes, siderophore biosynthesis, and flagellum are the basal pathogenicity determinants in *X. axonopodis* which are involved in suppressing pathogen-triggered immunity and effector-triggered immunity (Arrieta-Ortiz et al., 2013; Timilsina et al., 2020). Apart from the above, Ariute et al., (2022) noted that several genes responsible for the virulence of the Xanthomonas genus are found in the bacterial DNA, including *htp*B, *che*Y, *fli*N, *pil*G, *vip*A, *ugd*, *clp*V1, *vipB,* and *pil*T. The Cytolysin *cyl*A and 16SrRNA are the dominant virulence factors (genes) responsible

for the pathogenicity of *X. axonopodis* pv. *vignicola,* which act by causing suppression and/or altering of the host responses (Olatunde et al., 2024).

Other *Xanthomonas axonopodis* virulence factors noted, include Type I, II, III, IV, and VI protein secretion systems (Dey & Raghuwanshi, 2024). The type III effector proteins included *xopN, xopZ, xopV, avrBs2, xopQ, xopX, xopE4, xopR,* and *xopA01* (Medina et al., 2018). The *Xanthomonas* secretion systems are significant in translocating protein effectors to the extracellular surroundings or straight into eukaryotic cells (Shah et al., 2023). Different secretion systems serve various functions: adhesion to the host cell (type I secretion system), degradation of the host cell wall (type II secretion system), phytopathogenesis and manipulation of the host cell (type III secretion system), pathogen conjugation and competition (type IV secretion system), autoagregation and adhesion to the host cell (type V secretion system), and microbial competition (type VI secretion system) (Alvarez-Martinez et al., 2021; Pfeilmeier et al., 2024; Carezzano et al., 2023).

Further, pathogenic *Xanthomonas* bacteria possess a xyloglucan degradation mechanism associated with pathogenesis that is responsible for breaking down the xyloglucan which is a highly modified and resistant polysaccharides present in the vascular plants as the principal cell walls and it's a barrier deployed by the host against pathogens (Vieira et al., 2021). The pathogen xyloglucan degradation machinery is composed of specialised membrane transporters, a modular xyloglucan acetylesterase, and glycoside hydrolases (Giuseppe et al., 2023; Vieira et al., 2021).

The xyloglucan utilisation loci are remarkably conserved throughout the Xanthomonas genera, despite the wide diversity of hosts and tissue specificity. A small number of Xanthomonas lack the anticipated GH74 xyloglucanase; however, endoglucanases

express downstream the xyloglucan utilisation locus (Brennan et al., 2019; Giuseppe et al., 2023; Petrova et al., 2022). According to Bonfim et al. (2023) and Terrapon et al., (2018) the genomic investigation showed that the xyloglucan utilisation locus is conserved in the majority of *Xanthomonas* species.

2.4.3 Cross pathogenicity of *Xanthomonas axonopodis*

Xanthomonas axonopodis has a wide host range, infecting crop members of the same family or even from a different family (Arcila and Trujillo, 1990). The pathogen causes severe losses in production to different crops, with losses amounting to as high as 70% (Zafar et al., 2024). *Xanthomonas axonopodis* pv. *vignicola* infects several cultivars of cowpea plants, both domesticated and wild types, causing bacterial blight (Oguntade et al., 2021; Olatunde et al., 2024). Saha et al. (2022) noted that the infectiveness of *X. axonopodis* pv. *vignicola* varied depending on the crop cultivar and the production season. *Xanthomonas axonopodis* has active infective mechanisms against several cultivars of citrus fruits, serving as the main agent leading to low citrus fruit production (Khan et al., 2024). Patil et al. (2022) noted that *X. axonopodis* produces an exopolysaccharide that plays an important role in crop infection. The main host of *X. axonopodis* pv. *vignicola* is the cowpea, but it has been reported to cause infections in *Phaseolus acutifolius, Pisum sativum, Macroptilium lathyroides, Lablab purpureus, Vigna aconitifolia, Vigna mungo, Vigna angularis, Phaseolus vulgaris*, *and Phaseolus acutifolius* (Chen et al., 2021). *Xanthomonas axonopodis* pv. *eucalyptorum* has the infective ability on castor bean, tomato, common bean and eucalypt plants, causing bacterial blight (Ferraz et al., 2024). Sena-Velez et al. (2022) noted that members of the *Xanthomonas axonopodis* use various chemotaxis responses to carbon sources from

various plants, enabling the sensing of host-specific signals important in plant entry and subsequent infection.

2.5 Management of *Xanthomonas axonopodis*

The cultivation of cowpea has been faced by a myriad of challenges including pests and diseases for which farmers have employed various management strategies including cultural, physical, legislative, chemical, use of resistance breeding and biological techniques.

2.5.1 Cultural methods

The cowpea bacterial blight can best be managed by practising crop rotation, proper selection of farming land which is free of the pathogen, field hygiene by deep burial or burning crop residues and the use of pathogen free seeds (da Silva Júnior et al., 2022 and Karavina et al., 2011). When cowpeas were cultivated as an intercrop with maize over short-term rains and as a relay crop following maize during the long rains, a decrease in disease dissemination inside and between plants were noted (Ouko et al., 1989). The cultivation of cowpeas with sorghum or maize intercropping leads to decrease in disease incidence because these plants seem to act as a mechanical barrier to prevent the pathogen from moving between the cowpea plants (da Silva Júnior et al., 2022).

2.5.2 Physical methods

Zafar et al., (2024) observed that X-rays and UV-rays greatly reduce the cowpea bacterial blight disease incidence and severity levels. The effectiveness and efficacy depended on the crop cultivar and the duration of exposure with longer exposure time producing better results. Hot water treatment of the cowpea seeds was found to have

significantly reduced the severity of cowpea bacterial blight disease (Nandini and Shripad, 2015). Kiran et al., (2021) and Thakur et al., (2024) reported the effectiveness of thermotherapy of legume seeds through hot water and air treatment, vapour heat treatment and microwave treatment as safe and cheap methods of managing and controlling *X. axonopodis* pathogens. Further, Thakur et al. (2024) noted that thermophysical treatments of seeds promotes both biological and physiological activity of legume seeds leading to higher seed germination as well as promoting environmental health.

2.5.3 Chemical methods

Nandini and Shripad (2015) indicated that seed treatment with *Pseudomonas fluorescens* with foliar spray of *Pseudomonas fluorescens* at 25 days and Copper oxychloride + Streptocycline (0.3% + 0.05) spray at 45 days, reduced the severity of cowpea bacterial blight significantly and improved both the germination per cent and yield. Chemicals like potassium methyl-dithiocarbamate, copper hydroxide and copper sulphate are effective in controlling foliage infection (Karavina et al., 2011). Further, Gul et al. (2024) reported that nanoparticles are highly effective in the control of *X. axonopodis* pathogens. Copper oxychloride has been established to have the potential to effectively inhibit the growth of *X. axonopodis* (Godara et al., 2025). Kumar et al., (2023) noted that a combination of copper oxychloride and antibiotics (streptocycline, validamycin and kasugamycin) were effective in inhibiting the growth of *X. axonopodis*. Jat et al. (2022) opined that anti-biotics including neomycin and streptocycline were effective in the management of bacterial blight disease. The agrochemical 1,3,4-oxadiazole was effective in the management of *X. axonopodis* resulting to improved crop and yield increase (de Faria et al., 2024). Jat et al., (2022)

noted that copper hydroxide and copper oxychloride were effective in the management of bacterial blight caused by *X. axonopodis*.

2.5.4 Use of resistant varieties

One of the best strategies to prevent significant crop losses is to use legume cultivars resistant to various diseases (Adila et al., 2021). Saha et al. (2022) evaluated several improved cowpea varieties for their resistance to *X. axonopodis* pv. *vignicola* and noted that a number of high-yielding cultivars were resistant to bacterial blight. Similarly, Nantale et al., (2023a) and Simons et al. (2021) tested several cowpea varieties resistance to bacterial blight disease and opined that a number of genotypes could be included in breeding for high-yielding resistant cultivars. The breeding for resistant cowpea cultivars is an effective way in the management and control of cowpea bacterial blight (Mahesha et al., 2022). Development of disease resistant cowpea genotypes is the cheapest, resilient and, effective way of ensuring constant growth and supply of cowpea in the tropics (Manu et al., 2024).

2.5.5 Biological methods

2.5.5.1 Microbial used in the management of *Xanthomonas axonopodis*

Sunyar et al. (2024) reported that *Bacillus substilis, B. pumilus* and *B. amyloliquefaciens* were effective in the control of common bacterial blight disease of legumes. Shivaji (2020) opined that *Bacillus substilis* and *Pseudomonas fluorescens* can also be used to manage *Xanthomonas axonopodis* as it highly inhibits its growth using its secondary metabolites. Kanthaiah and Velu (2019) noted that *Xanthomonas axonopodis* are sensitive to octadecanoic acid 2-oxo methyl ester metabolite produced by biocontrol bacteria. Dimkić et al. (2022) and Fira et al. (2018) further noted that the

key inhibitory metabolites in *Bacillus* spp. are lipopeptides, iturin, surfactin, bacillomycin and fengycin. In addition, volatile compounds including aldehydes, alcohols, ketones, sulfides and aromatics produced by these bacteria are capable of inducing systemic resistance in plants. The findings were confirmed by Sampathkumar et al. (2023) who noted the efficacy of the secondary metabolites as well as their promising positive impact on crop growth promotion and high yields. *Bacillus* spp. plays a significant role in plant nutrient acquisition, production of important plant hormones and protection against pathogens (Saxena et al., 2020). Etesami et al. (2023) and Zhang et al. (2023) noted that *Bacillus* spp. play an important role in plant disease suppression through competition for nutrients, production of secondary metabolites and stimulation of plant systemic resistance.

Akinsemolu et al. (2024); Ercole et al. (2025) and Karačić et al. (2024) noted that *Bacillus* spp. play an important role in defence of plants against diseases through production of peptides, bacteriocins, siderophores, extracellular enzymes, and polyketides as well as production of phytohormones including absciscic acid, ethylene, gibberellins and cytokinins that play a significant role in plant growth. Similar findings were reported by Riseh et al. (2024). Godara et al. (2025) noted that streptocycline antibiotic had the potential to inhibit the growth of *X. axonopodis* under *in vitro* conditions. Omar et al. (2022) and Yanti et al., (2024) observed high efficacy levels of *Micromonospora shersina* and *Streptomyces* spp. against *X. axonopodis* causing bacterial blight disease of shallot crops. The purified secondary metabolites from *B. substilis* displayed high efficacy levels against *X. axonopodis* (Nargund et al., 2022). Jiang et al. (2023) noted that an environment friendly metabolite, *p*-Aminobenzoic acid, from *Lysobacter antibioticus* was effective in inhibiting the growth of *X. axonopodis* pv. *glycine*.

2.5.5.2. Plant botanical extracts in the management of *Xanthomonas axonopodis*

Ganiyu et al. (2017) and Godara et al. (2025) established that extracts from neem, garlic, *Azadirachta indica*, pawpaw and *Acalypha wilkisiana* were effective in reducing the incidences and severity of bacterial blight disease in cowpea. Garlic extracts displayed high efficacy levels against *X. axonopodis* as reported by Godara et al. (2025). Ganiyu et al. (2022) evaluated the antibacterial activity of pepper extracts and noted that it was able to inhibit the growth of *X. axonopodis* up to inhibition zones of 23.50 mm under *in vitro* conditions. Iftikhar et al. (2025) and Praneetha et al. (2022) evaluated the antibacterial potential of clove, neem and cinnamon oils as well as garlic extracts against *X. axonopodis* and reported that the botanicals were able to inhibit the bacterium but at varying levels with neem and cinnamon showing the highest and lowest inhibitory effect respectively. Adamu et al. (2021) opined that ginger oils were able to disrupt the normal growth of bacterial cells as well as limit the production of biofilms. Further, the ginger nanoparticles showed high levels of antibacterial potential against the bacterial leaf blight disease caused by *Xanthomonas*. The findings were affirmed by Liang et al., (2025) who showed that apart from ginger extracts inhibiting biofilm production, it also inhibits the polysaccharide formation and some virulence factors. Abd El-Hameid et al. (2025) reported that ginger and garlic extracts as well as *B. amyloliquefaciens* greatly improved the health of pear plants against fire blight disease caused by *Erwinia amylovora*. Furthermore, the effectiveness of *Aloe vera* extracts against *X. axonopodis* pv. *citri* have been reported by Kankamol et al. (2021).

CHAPTER THREE

MATERIALS AND METHODS

3. 1 Determination of occurrence of bacterial blight of cowpea and collection of diseased samples

Field surveys were conducted during the dry and wet seasons in the selected regions of the counties of Meru, Kakamega, Uasin Gishu, Isiolo, Laikipia and Makueni, which represented different agro-ecological zones (AEZ) growing cowpeas in Kenya. In each of the agro-ecological zone surveyed, farmers' fields were sampled at about five kilometres apart having cowpeas at various stages of development. A structured cowpea bacterial blight survey questionnaire was used during the survey (Appendix I).

The bacterial blight incidence on individual farms surveyed was noted as a percentage of symptomatic samples per the total number of plants sampled. In each farm, four $1m^2$ quadrants were drawn at random. In each quadrat all cowpea plants were counted and also those showing disease symptoms were counted separately and the individual farm incidence was computed as illustrated by Sampathkumar et al. (2023) and Wang et al. (2021).

$$\text{Percentage disease incidence (\%)} = \frac{\text{The number of infected cowpea plants}}{\text{Total cowpea plants in the quadrant}} \times 100\%$$

The disease prevalence was determined as illustrated by Sampathkumar et al. (2023).

$$\text{Disease prevalence (\%)} = \frac{\text{Total number of the diseased farms per County}}{\text{Total sampled cowpea farms per County}} \times 100\%$$

From the diseased cowpea plants, five leaves (three leaves with intermediate disease appearances and two at the start of disease development) were collected and brought to the Laboratory. The collected samples in the field were maintained in a keep cool box

having ice (4°C) and taken to the laboratory for the isolation of the pathogen and further studies.

3. 1. 1 Data analysis

The bacterial blight disease incidence per farm was first calculated into percentage per quadrant and the mean of the three quadrants taken as the disease incidence of the farm. The obtained percentage incidence levels for bacterial blight disease were then entered into excel and analysed by ANOVA procedures using the R-program computer software package, R version 4.1.2 (2021-11-01). Means were separated using Tukey multiple comparison of means at $p \leq 0.05$.

3. 2 Isolation and identification of the *Xanthomomonas axonopodis* pv. *vignicola*

3.2.1 Isolation of the *Xanthomonas axonopodis* pv. *vignicola* from diseased cowpea leaves.

The bacterium was isolated from the diseased cowpea leaves gathered from the different Agro-ecological zones (AEZ) of Kenya as described by Omar et al. (2024); Otieno et al. (2023).

The collected leaves for isolating the bacterium was first washed in running tap water to remove soil debris. Approximately 0.5 cm parts of the diseased leaf sections with early stages of infection were cut out and thereafter disinfected in 2.5% sodium hypochlorite in a sterile petri-dish and rinsed in 3 changes of sterile distilled water. The cut leaf parts were then placed in a sterile petri dish containing 10 ml of sterile distilled water and the tissues teased apart using a sterile scalpel until the lesions were very small and allowed to stand for 45 minutes. Eight serial dilutions were made from the above suspension, and from the 4th to 8th dilution, 1 ml suspension was pipetted aseptically

into petri-dishes containing nutrient agar (NA) and carefully spread evenly with a sterile glass rod. The inoculated petri dishes were inversely incubated at 30±2 °C for 24 to 48 hours.

Pure cultures of the suspected pathogen were obtained by picking selected colonies and streaking following the techniques elaborated by Kones (2024); Wekesa et al. (2023).

The most prevalent colony with typical morphological characteristics of the target pathogen (*X. axonopodis* pv. *vignicola*) was chosen from the diverse bacterial colony populations for transfer from a mixture of colony populations and was re-streaked out aseptically using a wire loop into a freshly prepared nutrient agar media with 5% glucose then incubated at 30±2 °C in an inverted position for 24 hours.

3. 2. 2 Morphological characterization of different isolates of *Xanthomonas axonopodis* pv. *vignicola*

The morphological features of *X. axonopodis* pv. *vignicola* isolates such as Gram staining, growth pattern, shape, colony colour, and size were assessed for identification of the isolates as described by Kabade et al. (2020) and Lin et al. (2020).

3. 2. 2. 1 Gram staining reaction

A small amount of the pure bacterial culture was picked using a sterile wire loop and a thin smear made on a glass slide, then the underside was slightly flamed to fix the bacterial smear on the side. The smear was then flooded with crystal violet stain and left for about 30 seconds before pouring off the stain and washed in tap water for 15 seconds. Two drops of iodine solution were then placed to cover the smear for 30 seconds. The excess iodine solution was then poured off, and the slide washed with running tap water. Excess water was then drained off and the smear decolourised by

flooding with 95% alcohol until the solvent was not coloured. The smear was then washed with tap water for about 5 seconds and counterstained with safranin for about 40 seconds before briefly washing in tap water and gently blotting dry with filter paper. The microscopic examination was then done under x40 objective to examine the smear distribution, and then under oil immersion (×100 objective) and the description of shape and colour was done, and whether it was Gram-negative or Gram-positive.

The colony morphology of the bacterium under study was compared with descriptions in the literature for identification (Kabade et al., 2020; Lin et al., 2020).

3.2.3 Biochemical characterizations of different isolates of *Xanthomonas axonopodis* pv. *vignicola*

Biochemical tests done to identify *X. axonopodis* pv. *vignicola* were starch hydrolysis, mannitol test, sucrose test, Catalase activity, Gelatin liquefaction, lactose utilization, hydrogen sulphide production, indole test, methyl red test, Voges Proskauer test and citrate utilization as described by Kabade et al. (2020) and Ogundate et al. (2021).

3.2.3.1 Starch hydrolysis

Starch broth medium containing peptone (100 g), starch soluble (20 g), beef extract (15 g), agar (2 g), sterile distilled water (1000 ml) and pH (7). The starch broth was then sterilized by autoclaving at 121°C and 15 psc for 15 minutes and aseptically poured onto a sterilized petri dish then allowed to cool before a spot of the bacterial culture was aseptically made on three plates. The plates were inoculated at 30±2 ºC and starch hydrolysis tested, one plate at a time, after 2, 4, and 6 days by flooding the agar surface with Lugol’s iodine and allowing to stand for about 5 minutes for the development of a colourless zone surrounding the bacterial growth to indicate starch hydrolysis.

3.2.3.2 Catalase test

A loopful of bacterial culture of 36 hours was smeared on a glass side and covered with two drops of 20% hydrogen peroxide. The production of gas bubbles then indicated the reaction was positive.

3.2.3.2 Liquification test (Gelatin liquefaction)

The nutrient gelatin medium consisting of peptone (10 g), Gelatin (20 g) beef extract (5 g), and distilled water (1000 ml) with a pH of 7.0 was prepared. All the ingredients were then mixed, and heated over a water bath until the gelatin was fully dissolved and autoclaving performed at 121 °C and 15 psc for 15 minutes. The media was left to cool to approximately 45 °C and poured onto the sterile petri dishes, allowed to solidify and then a spot was inoculated with a 24-hour culture of the bacterial isolate. The plates were then inversely incubated at 20 ±1°C for 24 hours, then the surface of the plate flooded with a 0.2% mercuric chloride solution. The development of white precipitate was taken as a positive test.

3.2.3.4 Lactose utilization

An axenic culture of *X. axonopodis* pv. *vignicola* isolate was transferred aseptically to sterile tubes containing sterile 5 ml phenol red lactose broth. The inoculated tubes were then incubated at 30±1°C for 24 hours and the results were determined; when a test is positive, the colour changes from red to yellow, signifying an acidic pH shift and vice versa when it was negative.

3.2.3.5 Acid production for sucrose and mannitol

Xanthomomonas axonopodis pv. *vignicola* acid production ability was tested using medium C dye. Ten millilitres (10 ml) of medium C Dye were dispensed in each test tube and sterilized in an autoclave at 121 °C and 15 psc for 15 minutes. Filtered and sterile sucrose, mannitol, dextrose and maltose carbohydrates were put into the tubes at 0.14 % concentration then the tubes inoculated with 0.1 ml of the 24 hours old *X. axonopodis* pv. *vignicola* culture and incubated at 30±1°C for three days. The change in the colour of the medium confirmed the acid production.

3.2.3.6 Hydrogen sulphide (H_2S) production

Peptone water medium consisting of Peptone (10 g), sodium chloride (NaCl) 5 g, sterile distilled water (1000 ml), and pH (7.0) were dispensed in 5 ml volumes in test tubes and sterilized by autoclaving. To determine H_2S production, lead acetate test strips were prepared as follows; Whatman N0. 1 filter paper was cut into 50 × 5 mm strips which were then soaked in a warm saturated solution of lead acetate. The strips were then dried at 60 °C, autoclaved and again dried at 60 °C. The peptone water medium in each test tube was then inoculated with a loopful 48-hour slant growth of the test bacteria. After inoculation, the test strip was inserted in between the inner wall of the tube and the plug (to hang just above the broth). The test tubes were then incubated at 30±1°C and the observations noted at regular intervals for 6-12 days. The blackening of the test strip indicated the production of H_2S.

3.2.3.7 Indole test, Methyl Red test, Voges Proskauer test and Citrate utilization test (IMVic reactions)

The four reactions tested were;

1. Indole test (Tests the potential of the bacteria to generate indole from tryptophan (amino acid) using the enzyme tryptophanase.

The test bacterium was inoculated in a sterile peptone water (amino acid-tryptophan) and incubated overnight at 35±1 °C. Further, one set of the organism contained in a test tube was incubated and maintained as a negative control. After incubation, approximately 5 drops of Kovac's reagent were added and shaken gently then allowed to stand for 2 minutes. The observation of red or pink coloured ring at the top was taken as positive for indole generation.

2. Methyl red test

The test bacterium was inoculated into 5 ml glucose phosphate broth (glucose and phosphate buffer) and incubated at 35±1 °C for 48 hours. Thereafter, the pH of the media was tested by adding 4 drops of methyl red reagent. The development of the red colour was taken as positive while the yellow colour was taken as negative.

3. Voges Proskauer test

The suspected test bacterium was inoculated into 5 ml glucose phosphate broth (glucose and phosphate buffer) and incubated at 35±1°C for 48 hours and then 0.5 ml of alpha-naphthol was added to the medium and gently shaken. Thereafter, 0.2 ml of 40 % KOH was added to the broth and shaken, and the test tube allowed to stand for 30 minutes. The development of red colour was taken as positive.

4. Citrate utilization test

The suspected test bacterium was picked up from a pure isolate with a straight wire loop and inoculated into the slope of sterile Simmon's citrate agar and incubated for 24

hours at 37±1°C. The ability of an organism to utilize citrate as the sole source of carbon and energy changes the medium colour from green to blue, therefore the colour change indicated the citrate utilization.

3.2.4 Pathological characterizations of different isolates of *Xanthomonas axonopodis* pv. *vignicola*

The pathogenicity test to confirm Koch's postulates was carried out on a three-week-old healthy, susceptible cowpea variety (Kunde Mboga) as noted by Chormale et al., (2021).

3.2.4.1 Planting of cowpea plants

Approximately 250 kilograms of loam soil was sterilized in the autoclave at 121 ºC and 15 Pascal's (psc) for 45 minutes, and allowed to cool for six days then transferred to the nursery at the greenhouse. *Xanthomonas axonopodis* pv. *vignicola* susceptible cowpea variety (Kunde Mboga) seeds purchased from Kenya Seed Company Limited, Nairobi Kenya. One seed was grown on the plastic polythenes measuring ten centimetres in diameter by 15 centimetres, containing 1.5 kilograms of sterile loam soil. Regular watering (once a day) and monitoring of the seedlings growth and development was done daily until they were two weeks old.

3.2.4.2 Inoculum preparation

The inoculum was prepared from each isolate by flooding 24 hours' *X. axonopodis* pv. *vignicola* cultures growing on nutrient agar with 10 ml of sterile distilled water, gently rubbed with a sterile glass rod then put in sterile universal bottles and shaken for 10 minutes in a shaker at 125 revolutions per minute (rpm) to dislodge the bacterial cells. The bacterial suspension was then aseptically filtered through two layers of sterile

cheesecloth. Serial dilutions of 10^{-1} up to 10^{-10} were made by pipetting 1ml of the mother suspension (stock solution) into the first test tube containing 9 ml of sterile distilled water and shaking gently to mix (dilution 10^{1}). One millilitre of the dilution was then pipetted into the second test tube containing 9 ml of sterile distilled water and shaken gently to mix (dilution 10^{2}). The same procedure was followed for dilution 10^{3}, 10^{4} up to 10^{10}.

The inoculum concentration was estimated using the haemocytometer; A Pasteur pipette was used to place one drop of the filtrate suspension from each of the dilutions onto the ruled surface of the haemocytometer and then the suspension covered with a coverslip and, examined under a light microscope at ×40 and ×100. The bacterial cell number per millilitre of the suspension for each of the dilution was calculated as follows;

Area of each square = 0.2 mm (length) × 0.2 mm (width) = 0.04 mm^2.

Volume of each square = 0.04 mm^2 × 0.1 ml = 0.004 mm^3;

Then if 1 ml = 1 cc = 1000 mm^3 and Y cells are in 0.004 mm^3

Therefore, cells in 1 ml = $Y \times 1000/0.004 > Y \times 0.25 \times 10^6 = Y \times 25 \times 10^4$

Where Y= Average number of bacterial cells from 4 squares.

3.2.4.3 Inoculation

Three-weeks-old cowpea plants (2-4 leaves) were inoculated with adjusted (1×10^8 CFU/ml) bacterial inoculant by injecting bacteria into the mesophyll spaces of leaves with a 1 ml plastic syringe. The control cowpea plants were inoculated with sterile

distilled water instead of bacterial inoculum suspension. The complete randomised experimental design with three replicates was adopted.

The cowpea plants were kept in a greenhouse at approximately 30°C and humidity of approximately 80%, and then tracked for typical symptom development. Initial disease symptoms were observed after five days of inoculation and final observations was recorded after 28 days.

The severity was computed as illustrated by Wang et al. (2021).

$$\text{Percentage disease severity (PDS)} = \frac{\Sigma \text{ of numerical ratings}}{(\text{Total number of leaves rated}) \text{ x } (\text{maximum scale})} \times 100\%$$

Disease severity was evaluated on a scale of 0 to 4 as illustrated by Ndalira et al. (2020) and Agbicodo *et al.,* (2010), where 0 = no notable symptoms, 1 = leaf spots affecting 1-25% of the leaf surface, 2 = blight covering 26–50% of the leaf surface, 3 = 50-75% leaf surface infected, and 4 = 75-100% of the leaf surface infected.

The *X. axonopodis* pv. *vignicola* isolates were further grouped into less virulent (<15% in severity), moderately virulent (15-30 % in severity) and highly virulent (>30% in severity) depending on the disease severity score and the period it took for the symptoms to appear after inoculation. The disease scale of 0 to 4 used during the cowpea bacterial blight severity studies with the scores based on the approximate percentage of the leaf surface affected is illustrated in Plate 3.1.

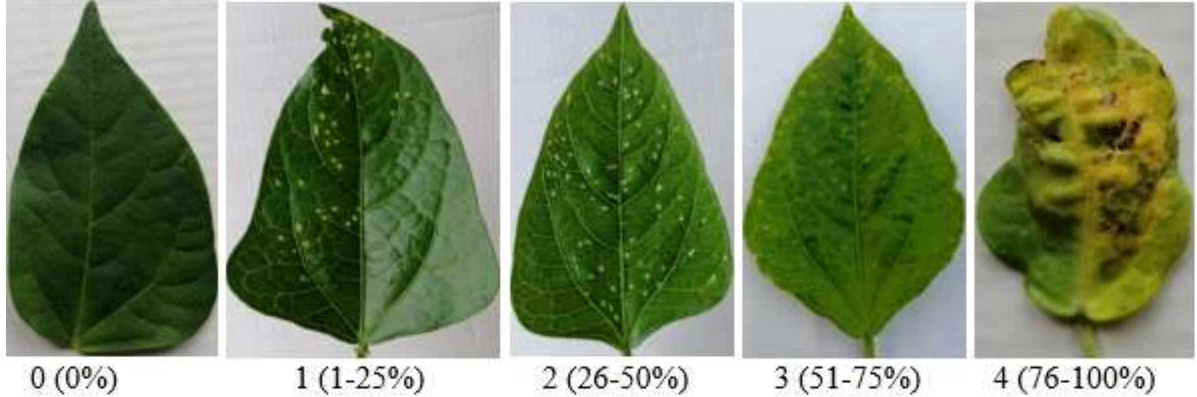

Plate 1: Sample disease scores used during the severity studies

3.2.4.4 Data analysis

The bacterial blight disease severity was first calculated into percentage per crop and the mean of the three replicates taken as the disease severity of the crop. The obtained percentage severity levels data for bacterial blight disease of the tested *X. axonopodis* isolates was entered into excel and analysed by ANOVA procedures using the R program computer software package, R version 4.1.2 (2021-11-01). Means were separated using Tukey multiple comparison of means at $p \leq 0.05$.

3.2.5 Molecular identification of the *Xanthomonas axonopodis* pv. *vignicola* isolates

The protocol for DNA extraction of *X. axonopodis* pv. *vignicola* isolates was performed based on the description of Hebert et al. (2021) and Singh et al. (2023) using Quick-DNA ™ Fungal/bacterial Miniprep extraction kit (Zymo Research Corp., USA).

3.2.5.1 DNA extraction and quantification from *Xanthomonas axonopodis* pv. *vignicola*

Prior to the start of bacterial DNA extraction, beta-mercaptoethanol was added to the Genomic Lysis Buffer to a final dilution of 0.5 % (v/v) i.e., 500 µl per 100 ml for optimal performance following the manufacturer's instruction.

A single colony of the axenic isolates was selected from nutrient broth culture and aseptically inoculated into sterile nutrient agar plates, then incubated at 30±2°C for 36 hours. A 100 mg of the bacterial cells (equivalent to approximately 10^9 bacterial cells) was gently scrapped from the nutrient agar plates and suspended in 200 µl of sterile molecular grade water and vortexed for 15 seconds. The mixture was then aseptically pipetted to the ZR bashingBead ™ Lysis Tube (0.1 mm and 0.5 mm) and 750 µl of BashingBead ™ Buffer added to the tube. The contents in the tube were then disrupted in a bead beater (A49 Mini Bead beater ™ Biospec) at the speed of 2500 revolutions per minute (rpm) for 3 minutes. The contents in the ZR bashingBead ™ Lysis Tube were centrifuged in a micro-centrifuge (5418: Eppendorf AG 22331 Hamburg, Germany) at 10,000 x g for 1 minute and then 400 µl of the supernatant transferred to a Zymo-Spin™ III-F Filter in a clean sterile collection tube and centrifuged at 8,000 x g for 1 minute. 1, 200 µl of Genomic Lysis Buffer was aseptically pipetted to the filtrate in the collection tube above and then 800 µl of the mixture transferred to Zymo-Spin™ IICR Column[3] in a new sterile collection tube and centrifuged at 10,000 x g for 1 minute. The flow in the collection tube was discarded and the preceding procedure repeated. The Zymo-Spin™ IICR Column[3] was aseptically placed in a clean fresh sterile collection tube and 200 µl of the DNA pre-wash buffer aseptically added and centrifuged at 10,000 x g for 1 minute then 500 µl g-DNA wash buffer was added to

the Zymo-Spin™ IICR Column[3] and centrifuged at 10,000 x g for 1 minute. The Zymo-Spin™ IICR Column[3] was transferred aseptically to a clean sterile 1.5 ml microcentrifuge tube and 100 µl DNA elution buffer added directly to the column matrix and centrifuged at 10,000 x g for 30 seconds to elute the DNA. The extracted DNA was quantified with Nano drop (Nanodrop 2000 Spectrophptometre- Thermo Scientific) at A_{260}/A_{280}. The DNA microcentrifuge tube containing the ultra-pure bacterial DNA was aseptically closed, labelled and stored at -20 °C as it awaited utilization in the downstream processes. The entire DNA extraction process was carried out under ice.

3.2.5.2 Amplification of 16S rDNA

The polymerase chain reaction was carried out at the Biotechnology laboratory at the University of Eldoret, Kenya.

The DNA amplification procedure adopted was by Hebert *et al.,* (2021) and Singh et al., (2023) with slight modification. The amplification of DNA was done using the *Xanthomonas axonopodis* 16S rDNA species specific primers; 16F27-5' AGAGTTTGATCCTGGCTCAG3' and 1492R -5'-GGT TACCTTGTTACGACTT-3', forward and backward primers respectively.

The polymerase chain reaction (PCR) components of total volume of 25 µl were prepared aseptically following the manufacturer's (New England Biolabs Inc.) guidelines as follows: 12.5 µl of One *Taq* Quick-Load 2X Master Mix with standard buffer that contained: dNTPs, $MgCl_2$, buffer components and stabilizers, was aseptically pipetted into a 1.5 ml PCR microtube, and then 1 µl of the forward and reverse primers and 2 µl of template genomic DNA were pipetted aseptically into the microtube respectively. 8.5 µl of nuclease-free water was aseptically added to the

reaction components, closed, and thoroughly mixed. All the reaction components were aseptically assembled on ice.

The PCR reaction components were transferred quickly to a preheated thermocycler (Eppendorf AG 22331 Hamburg: No. 5345-028097. Germany) employing the following program: initial denaturation step at 94 °C for 5 minutes; denaturation at 94 °C for 30 seconds; annealing at 56 °C for 30 seconds; extension for 60 seconds at 68 °C for 40 cycles and last extension at 72 °C for 10 minutes. The polymerase chain products were removed immediately the run time was completed and then kept at 4 °C awaiting electrophoresis.

3.2.5.3 Gel electrophoresis

A 0.8 % agarose gel was prepared by dissolving 2.4 grams of agarose in 300 ml of 1X Tris-borate ethylenediaminetetra acetic acid (EDTA)TBE buffer, mixed well and boiled in the microwave (LG MOD: MS1947C. S/N 804TAEJ00460, China) until it had fully dissolved. The molten agarose gel was left to cool up to about 60 °C and 30 µl of the nucleic acid gel stain- 10,000X (SafeView classic) was added and mixed gently. The agarose gel was gently poured into a sealed gel casting tank with combs already inserted in the required orientation and then left for about 15 minutes to settle. Once the gel had solidified, the seal was carefully removed from gel casting plate. A 1X TBE electrophoresis buffer was carefully poured in to the gel tank (Kirkhouse Trust- Model 81:2325 Class II. Calileo Bioscience, Belgium) up to the indicated level and combs were carefully removed to prevent the formation of air bubbles inside the wells as this could interfere with the DNA movement. A 8 µl of the PCR products was carefully loaded into the individual wells (12 mm). The gel pilot 1kb molecular marker ladder prepared in the ratio of 1:1:4 [DNA ladder, Gel Loading Dye, Purple (6X) and, 1X TE

buffer (made up of 10Mm Tris-HCl containing 1Mm $EDTA.Na_2$)] respectively and loaded into the first wells at both ends and at the middle for band size evaluation of the PCR amplicons. Electrophoresis was carried out at 100 volts for 90 minutes through electrophoresis power supply adapter (Consort EV215. S/N 80406).

After the 90 minutes, the gel was photographed with a gel-documentation system (2UV Transilluminator- BioDoc-it^{TM} Imaging System) and the dimensions of the amplified fragments resolved by comparing the gel pilot 1kb molecular marker ladder.

3.2.5.4 DNA sequencing and interpretation

The amplified PCR products were sent to Inqaba Biotec East Africa Ltd. for sequencing and the resulting sequences exposed to BLAST search both at NCBI and at 16S bacterial cultures Blast server for the naming of Prokaryotes.

3.3 Determination of genetic diversity of *Xanthomonas axonopodis* pv. *vignicola* using Inter-Simple Sequence Repeats (ISSR)

The genetic diversity of the *X. axonopodis* pv. *vignicola* isolates was determined by inter-simple sequence repeats (ISSR) makers as described by Kharde et al., (2018) with slight modifications.

3.3.1 DNA extraction and quantification of the isolated pathogens

The isolated DNA (section 3.2.5.1) above was used for genetic diversity studies using ISSR markers.

3.3.2 Amplification of ISSR regions of the genome of the isolates

The inter-simple sequence repeats (ISSR) regions of the genome of the bacteria was amplified by polymerase chain reaction (PCR) for studying the pathogen diversity. First

a set of 9 primers were initially pre-tested for polymorphism. The DNA was amplified using the six *X. axonopodis* ISSR polymorphic primers indicated in Table 4 below as noted by Fatima et al., (2012); Kharde et al. (2018) and Kumar et al. (2018).

Table 4. The sequences of the inter-simple sequence repeats primers

Marker name	Primer sequence (5'-3')
ISSR820	GTGTGTGTGTGTGTGTC
ISSR816	CACACACACACACACAT
UBC8932800	AGCAGCAGCAGCGT
D-3	GACAGACAGACAGACA
A-31	AGCAGCAGCAGC
A-16	CACACACACACAR

A reaction volume of the 25 µl polymerase chain reaction mixture was used. The polymerase chain reaction (PCR) components of 25 µl were prepared aseptically following the manufacturer's guidelines as shown in Table 5:

Table 5. PCR reaction components for studying genetic diversity of *Xanthomonas axonopodis* pv. *vignicola* isolates

Reaction components	Reaction volumes
One *Taq* Quick-Load 2X Master Mix with Standard Buffer that contained: dNTPs, $MgCl_2$, buffer components and stabilizers, (New England Biolabs Inc.)	12.5 µl
ISSR primers	2 µl
Template genomic DNA	2 µl
Nuclease-free water	8.5 µl
Total volume	25 µl

All the reaction components were aseptically assembled on ice. The PCR reaction components were transferred quickly to a preheated thermocycler (Eppendorf AG 22331 Hamburg, Germany) employing the following program as shown in Table 6.

Table 6. PCR protocol for studying genetic diversity of *Xanthomonas axonopodis* pv. *vignicola*

Cycle step	Temperature	Time	Cycles
Initial Denaturation	94°C	5 minutes	1
Denaturation Annealing Extension	94°C 55°C 68°C	30 seconds 30 seconds 1 minute	40
Final extension	72°C 30	10 minutes	1
Hold	4°C	∞	1

The polymerase chain products were removed immediately the run time was completed and then kept at 4 °C awaiting electrophoresis.

3.3.3 Gel Electrophoresis

The gel electrophoresis was performed as described in section 3.2.5.3 above. During the entire process the PCR products were kept on ice. The entire process (amplification, gel electrophoresis and gel documentation) was repeated for each *X. axonopodis* pv. *vignicola* isolates until all the ISSR primers had been analysed.

3.3.4 Data Analysis

The ISSR bands were scored (1) for presence and (0) for absence, the data was recorded from each line and, entered into Microsoft Excel and used for downstream analysis. A diversity matrix was produced from the binary data using Jaccard's diversity coefficient in the SIMQUAL software of the NTSYS-pc 2.10e, (C) 2000, Applied Biostatistics Inc. program.

Cluster analysis was done with the unweighted pair group arithmetic mean method (UPGMA) in the SAHN software of the NTSYS-pc program. The graphic dendrogram

of the genetic distance among the 48 isolates was generated by means of UPGMA cluster computation of averaged diversity index.

The polymorphism information content (PIC) for each primer was calculated using the Microsoft Excel. The Genetic Distance (GC) was done by Pairwise Population Matrix of Nei Genetic Distance in the Distance Based program of the GenAIEx 6.503 software. Principal Coordinate Analysis (PCoA) and Analysis of Molecular Variance (AMOVA) was performed using the Distance Based program of the GenAIEx 6.503 software.

3.4. Cross pathogenicity of *Xanthomonas axonopodis* pv. *vignicola*

The most aggressive and virulent isolate of *X. axonopodis* pv. *vignicola* as determined in section 3.2.3 during pathogenicity test was used to evaluate the cross pathogenicity potential on common beans, soya, lentils, pigeon pea and green grams established in pots as noted by Sampathkumar et al. (2023) and severity measured as per the method reported by Ndalira et al. (2020).

Approximately 120 kilograms of loam soil was sterilized in the autoclave at 121 ^{o}C and 15 Pascal’s (psc) for 45 minutes, and allowed to cool for six days then transferred to the nursery at the greenhouse. Cowpea varieties, beans, soya, lentils, pigeon pea and green grams’ seeds were obtained from Kenya Seed Company Ltd, Nairobi; Kenya. On the plastic pots (15 cm diameter) containing 2 kilograms of sterile loam soil, one seed was sown per pot. Regular watering (once a day) and monitoring of the seedlings was done daily until they were two weeks old.

3.4.1 Inoculum preparation

The inoculum was prepared from the most virulent and aggressive isolate (selected from section 3.2.3 during pathogenicity) by flooding nutrient agar having *X. axonopodis* pv. *vignicola* cultures which had grown for 36 hours with 10 ml of sterile

distilled water. The agar surface was then gently rubbed with a sterile glass rod then transferred to sterile universal bottles and shaken for 10 minutes in a shaker at 125 revolutions per minute (rpm) to dislodge the bacterial cells. The bacterial suspension was then filtered through two layers of sterile cheesecloth. Serial dilutions of 10^{-1} up to 10^{-8} was made by pipetting 1ml of the mother suspension (stock solution) into the first test tube containing 9 ml of sterile distilled water and shaking gently to mix (dilution 10^{-1}). One millilitre of the dilution was then pipetted into the second test tube containing 9 ml of sterile distilled water and shaken gently to mix (dilution 10^{-2}). The same procedure was then followed for dilution 10^{-3} 10^{-4} up to 10^{-8}.

The inoculum concentration was estimated using the haemocytometer as described in section 3.2.4.2 above.

3.4.2. Inoculation

Two-weeks-old cowpea, common beans, soya, lentils, pigeon pea and green grams' plants (2-4 leaves) were inoculated with adjusted (1×10^8 CFU/ml) bacterial inoculant by injecting bacteria into the mesophyll spaces of leaves with a 1 ml plastic syringe. The control plants were inoculated with sterile distilled water instead of bacterial inoculum suspension. Complete randomised experimental design with three replicates was adopted.

The plants were kept in a greenhouse at about 30°C and monitored for typical symptom development. Initial disease symptoms were noted from the time of inoculation and final observations were recorded after 28 days.

The disease severity was computed as illustrated by Bdliya and Alkali (2010) and Wang et al., (2021).

$$\text{Percentage disease severity (PDS)} = \frac{\Sigma \text{ of numerical ratings}}{\text{(Total number of leaves rated) x (maximum scale)}} \times 100\%$$

Disease severity was evaluated on a scale of 0 to 4 as illustrated by Ndalira et al. (2020) and Agbicodo et al. (2010), where 0 = no notable symptoms, 1 = leaf spots affecting 1-25% of the leaf surface, 2 = blight covering 26-50% of the leaf surface, 3 = 50-75% leaf surface infected, and 4 = 75-100% of the leaf surface infected as illustrated in section 3. 2. 4. 3 above. The bacterial blight disease severity per crop species was first calculated into percentage per crop and the mean of the three crops taken as the disease severity of the crop.

3.4.3 Data analysis

The obtained percentage severity levels data for bacterial blight disease of the tested crops was entered into excel and analysed by ANOVA procedures using the R program computer software package, R version 4.1.2 (2021-11-01). Means were separated using Tukey multiple comparison of means at $p \leq 0.05$.

3.6 Evaluation of the antibacterial potential of biological control agents

3.6.1 *In vitro* evaluation of the antibacterial potential of *Bacillus subtilis* and *Bacillus amyloliquefaciens* in controlling *Xanthomonas axonopodis* pv. *vignicola*

The antibacterial potential of *Bacillus subtilis and Bacillus amyloliquefaciens* against the *Xanthomonas axonopodis* pv. *vignicola* was tested by the dual culture and inverted plate methods as described by Palacios-Rodriguez et al., (2024) and Soliman et al. (2023).

A fresh bacterial colony (24 hours old) of the test *X. axonopodis* pv. *vignicola* was aseptically streaked at one end of a 90 mm petri dish containing nutrient agar medium for the dual culture technique and on the middle of nutrient agar plate for the inverted

plate method. The biocontrol agents of *Bacillus subtilis* and *Bacillus amyloliquefaciens* (obtained from Moi University, Biological Science Laboratory) were then aseptically streaked individually on the opposite end and on the middle of the nutrient agar petri plate respectively. The inverted plates were wrapped and sealed with a sterile parafilm and incubated at 30±1°C. Observation was made until bacterial cell growth of the test pathogen had fully covered the entire petri plate in the control plate which had only a streak of the test pathogen made in the middle. When the control had covered the entire plate, the inhibition region was measured by measuring the gap between the two edges of the tested microbes (the pathogen and tested bio-agent) in the petri dish in millimetres.

The efficacy test for the *B. subtilis* and *B. amyloliquefaciens* was carried out in a completely randomized design. The screening was done separately for each bio-agent and in aseptic conditions in the safety cabinet (Aura 2000 M.A.C; TUV Product; Saveen Werner Life Science).

3.6.2 *In vitro* evaluation of the antibacterial potential of *Salvia nilotica,* neem, garlic and ginger against *Xanthomonas axonopodis* pv. *vignicola*

3.6.2.1 Preparation of plant parts for phytochemical extraction

Salvia nilotica and neem (*Azadirachta indica*) twigs, garlic (*Allium sativum*) (bulbs and ginger (*Zingiber officinale*) rhizomes were washed through flowing tap water to eliminate dirt and any other undesired foreign material. They were then dried under the shade until the moisture content was approximately below 12%. The dried plant materials were then ground into powder using an electric grinder. The resulting ground plant parts were then carefully stored in wrapped and marked glass bottles until later use.

3.6.2.2 Extraction of phytochemicals from plant samples

The phytochemicals from the dried selected plants were extracted by the maceration technique as illustrated by Bitwell et al. (2023) and Mungwari et al. (2025). The sample-to-solvent ratio of 1:10 (v/v) was adopted. Two hundred grams of the dried and ground respective plant materials were each separately homogenised in 2000 ml of absolute ethanol in a conical flask with constant agitation on a shaker (VWR ADV 500 Shaker ECN: 444-2916) at 50 rpm for 72 hours. The homogenates were then filtered through two-ply cheese-fabric netting and centrifuged at 10,000 rpm for 15 minutes in a centrifuge (6000 series centrifuge, Centurion Scientific Ltd, Switzerland) to take off suspended small fragments of utilised plant material. The filtrates were further filtered through a 0.45 µm membrane filter to remove fine plant materials and then evaporated to dryness under vacuum at 45-50°C in a rotary evaporator (Buchi Rotavapor R-3000, Switzerland). The extract from the rotary evaporator was removed and subjected to further drying in the water bath at 45°C for 10 hours to fully remove any remaining solvent. The resultant extract was then stored in sterile, well-wrapped and marked vials at 4°C until utilisation.

3.6.2.3 Evaluation of antibacterial activity of crude extracts of *Salvia nilotica*, garlic, neem and ginger

Inoculum preparation

The pathogen inoculum was prepared as described by Hudzicki (2009) and Ibn and Ahmed (2025). Six isolated colonies of *X. axonopodis* pv. *vignicola* from a 24-hour-old culture were picked using a sterile inoculating loop and suspended in 4 ml of sterile saline in screw-capped universal bottles. The inoculated saline universal bottles were vortexed to create a homogenised suspension. The turbidity of the suspension was

adjusted to 0.5 McFarland standard by adding more bacteria since the suspension was slightly light. The suspension was utilised within 20 minutes of preparation.

Extract dilutions preparation

Salvia nilotica, garlic, neem and ginger extracts were removed from the fridge and two dilutions of 12.5 mg/ml and 25 mg/ml of each extract were prepared on 28 ml sterile universal screw-capped bottles as illustrated by Ibn and Ahmed (2025). The dilutions were prepared by aseptically weighing 62.5 grams and 125.0 grams of each of the plant extracts and then dissolving them in 200 µl of dimethyl sulphoxide (DMSO) in well-labelled sterile universal bottles. Once the extract had fully dissolved in DMSO, 5 ml of sterile distilled water were aseptically pipetted into each bottle and vortexed until it had uniformly mixed, resulting in extract concentrations of 12.5 mg/ml and 25 mg/ml depending on the respective weighted plant extract.

Agar plates preparation and inoculation

The disc diffusion technique as described by Hudzicki (2009) and Ibn and Ahmed (2025) was used. Nutrient agar (NA) media was prepared following the manufacturers guidelines. After autoclaving the media, it was left to cool up to 45°C, then approximately 15 ml of the media was aseptically poured onto sterile 90 mm glass plates in a safety cabinet. The poured media was left for one hour to solidify well.

After media solidification, a sterile swab was dipped into the *X. axonopodis* pv. *vignicola* inoculum, and the swab was rotated against the side wall of the bottle slightly above the fluid mark with application of pressure to remove excess fluid. The dried surface of the nutrient was then seeded by streaking the swab four times over the entire NA surface with rotation of the plate at about 60° each time for even distribution of the bacterial inoculum. The same procedure was followed until all the plates had been

seeded. The plates were then left to sit at 28°C for 10 minutes with their lids slightly ajar for the agar surface to dry before moving forward with downstream steps.

Sterile paper discs (10 mm in diameter) were aseptically dipped into respective plant extracts and left for 2 minutes to absorb the extract. The lid of the petri dish was partially removed, and an appropriate antibacterial-loaded disc (with plant extracts) was aseptically placed on the middle of the agar surface using sterile forceps. The disc was gently pressed with handling forceps to ensure firm contact with the agar surface. The same process was repeated until all the discs had been placed in the respective plates. Filter paper discs loaded with 5 µg of ciprofloxacin antibiotic were used as a positive control. All the analysis was done in replicates of three. When all the plates had been inoculated the plates were inverted and incubated randomly at 30±1°C for 24 hours.

Measuring zone of inhibition and interpretation of the findings

After the incubation, the inhibition zones were measured to the nearest millimetre using the Vernier calliper, including the diameter of the paper disc in the reading. The measurements were visually made without any aid, with the viewing of the plate being on the vertical line of sight to avoid any parallax error in the reading. The sensitivity of the bacteria to the botanical extracts was interpreted as either potent or non-potent based on the ability to inhibit the growth of *X. axonopodis* pv. *vignicola*. The plant extracts with antibacterial potential were interpreted as potent while those while those without any antibacterial effect were noted as non-potent.

3.6.2.4 Data analysis

The obtained inhibition levels were entered into Excel, and the treatment efficacy was analysed by one-way ANOVA procedures using the R program computer software

package. Means of the zones of inhibition across various bio-agents were assessed using Tukey multiple comparison of means at $p \leq 0.05$.

CHAPTER FOUR

RESULTS

4.1 Determination of the incidence and prevalence of bacterial blight on cowpea

In the individual farms, the cowpea bacterial blight disease incidence varied from 26.87% to 64.67%, with a mean of 44.89% throughout all the sites surveyed. However, the highest disease incidence was noted in farm KK09 (64.67%) in Kakamega county and the least disease incidence at UGO8 (26.87%) in Uasin Gishu county (Table 7). The disease incidence levels were statistically significant, $P \leq 0.001$, (Appendix II). Means comparison by Tukey test at 95% family-wise confidence level (Appendix III), showed the significantly different sites on the recorded mean disease incidence.

Cowpea bacterial blight disease was prevalent in all the six counties of Makueni, Isiolo, Kakamega, Uasin Gishu, Laikipia and Meru surveyed. Out of eighty (80) cowpea farms sampled, 60% tested positive for the cowpea bacterial blight disease in 48 farms. The highest disease prevalence was recorded in Kakamega and Makueni counties both at 93.33% and the lowest disease prevalence recorded in Meru county (20%) (Figure 1). There was statistically significant difference ($P \leq 0.001$) in disease incidence among the counties (Appendix IV). The multiple comparison of mean disease incidence by Tukey post-hoc test at 95.0% family-wise confidence level (Appendix V), showed the significant difference in regions on the observed mean disease incidence.

Table 7: Incidence of bacterial blight of cowpea in Makueni, Isiolo, Kakamega, Uasin Gishu, Laikipia, and Meru counties

Site	% Mean incidence
IS01	35.77
IS02	40.60
IS04	42.93
IS06	53.13
IS06-2	45.30
IS08	38.90
IS09	47.67
ISO4	39.73
KK01	49.13
KK02	41.20
KK03	42.20
KK04	46.80
KK05	45.50
KK06	62.43
KK07	51.60
KK08	50.50
KK09	64.67
KK10	57.10
KK12	48.10
KK13	46.47
KK14	50.13
KK15	51.07
LK01	41.10
LK02	31.90
LK04	39.50
LK05	33.90
MK01	61.30
MK02	52.13
MK03	61.37
MK04	48.30
MK05	54.93
MK06	60.70
MK07	52.87
MK08	52.17
MK09	43.77
MK10	42.47
MK12	37.57
MK13	29.07
MK14	37.83
MK15	52.63
MU01	37.93
MU02	30.80
UG02	41.80
UG04	30.80
UG05	36.27
UG07	31.13
UG08	26.87
UG09	34.53

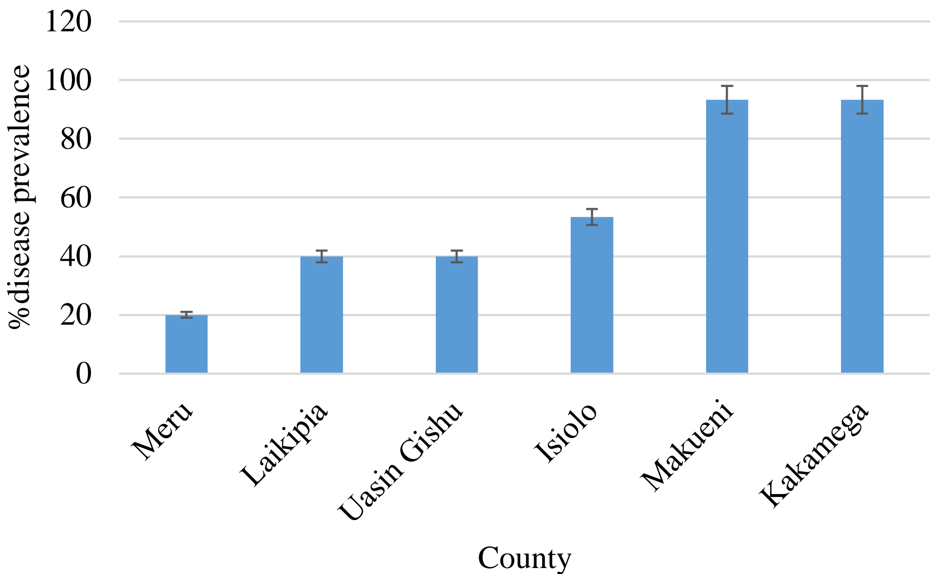

Figure 1: Prevalence of bacterial blight of cowpea from various counties in Kenya

From the analysis, Isiolo, Makueni and Kakamega, displayed a comparatively elevated median prevalence rates, while Uasin Gishu and Meru had lower median levels (Figure 2). Kakamega showed the lowest difference in disease incidence, with two notable outliers while Makueni showed the highest difference in disease incidence among the farms where sampling was done.

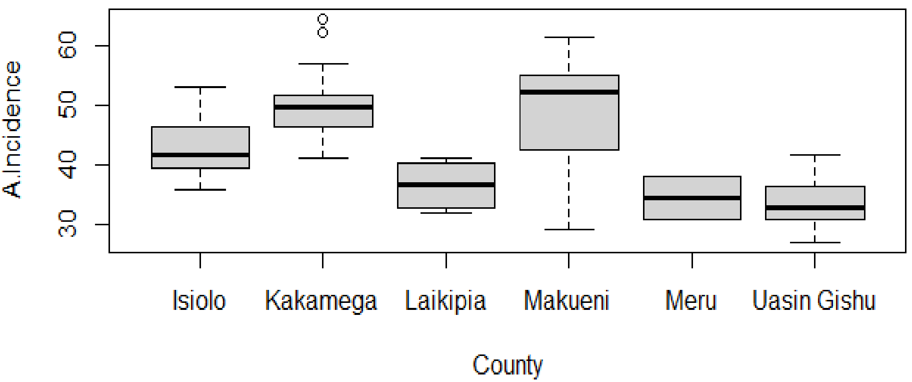

Figure 2 Cowpea bacterial blight disease prevalence per county

The cowpea bacterial blight disease incidence was recorded in all the six counties surveyed. The highest mean disease incidence was recorded in Kakamega county (50.49%) and the least disease incidence being recorded in Uasin Gishu county (33.57%) (Figure 3).

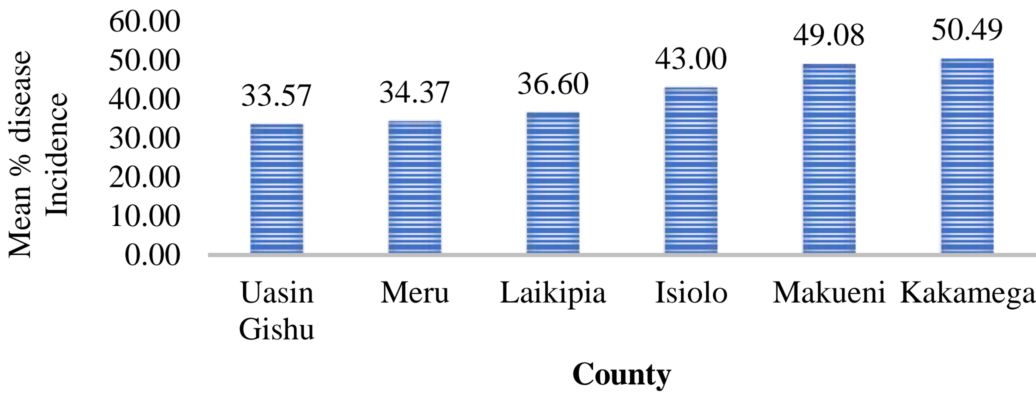

Figure 3: Mean incidence of bacterial blight of cowpea in six counties surveyed in Kenya

4.2 Characterization of *Xanthomonas axonopodis* pv. *vignicola*

4.2.1 Cultural and morphological features of *Xanthomonas axonopodis* pv. *vignicola* isolates

The *X. axonopodis* pv. *vignicola* isolates displayed slight variance in morphological and cultural characteristics on nutrient agar (NA) (Table 8). The dominant pigmentation was typical yellow which was noted in 79.17% of the isolates, 14.58% had creamy yellow pigmentation and 6.25% had light yellow colour (Table 9; Plate 2). All the Xanthomonas isolates produced Xanthomonadin and had rod arrangement; entire margin; mucoid surface and convex elevation (Table 8). In terms of form, 89.58% of the isolates were circular while 10.42% were irregular. Further, 87.5% of the isolates were opaque while 12.5% were translucent.

Table 8: Morphological features of *Xanthomonas axonopodis* pv. *vignicola* isolates

		Morphological character						
Isolate	X-p	Pigmentation	Arrangement	Margin	Surface	Elevation	Form	Opacity
IS01	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
IS02	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
IS04	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
IS05	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
IS06	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
IS06-2	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
IS09	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
IS08	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
KK01	+	Light yellow	Rod	Entire	Mucoid	Convex	Irregular	Translucent
KK02	+	Cream yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
KK03	+	Typical yellow	Rod	Entire	Mucoid	Convex	Irregular	Opaque
KK04	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
KK05	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
KK06	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
KK07	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
KK08	+	Creamy yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
KK09	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
KK10	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
KK12	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
KK13	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
KK14	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
KK15	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Translucent
LK01	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
LK02	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
LK04	+	Typical yellow	Rod	Entire	Mucoid	Convex	Irregular	Opaque
LK05	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
MU01	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
MU02	+	Light yellow	Rod	Entire	Mucoid	Convex	Circular	Translucent
MK01	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
MK02	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
MK03	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
MK04	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
MK05	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
MK06	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
MK07	+	Creamy yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
MK08	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circulr	Opaque
MK09	+	Creamy yellow	Rod	Entire	Mucoid	Convex	Irregular	Translucent
MK10	+	Creamy yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
MK12	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
MK13	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
MK14	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
MK15	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
UG02	+	Light yellow	Rod	Entire	Mucoid	Convex	Circular	Translucent
UG04	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
UG05	+	Typical yellow	Rod	Entire	Mucoid	Convex	Irregular	Opaque
UG07	+	Creamy yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque
UG08	+	Creamy yellow	Rod	Entire	Mucoid	Convex	Circular	Translucent
UG09	+	Typical yellow	Rod	Entire	Mucoid	Convex	Circular	Opaque

NB: + =Positive, X-P= Xanthomonadin production

Table 9: Morphological features of *Xanthomonas axonopodis* pv. *vignicola* isolates collected from the six counties of Kenya

Feature		Percentage of Xanthomonas isolates displaying the characters
Xanthomonadin production		100.00
Pigmentation		
	Typical Yellow	79.17
	Creamy yellow	14.58
	Light yellow	6.25
Arrangement	Rod	100.00
Margin	Entire	100.00
Surface	Mucoid	100.00
Elevation	Convex	100.00
Form		
	Circular	89.58
	Irregular	10.42
Opacity		
	Opaque	87.50
	Translucent	12.5.0

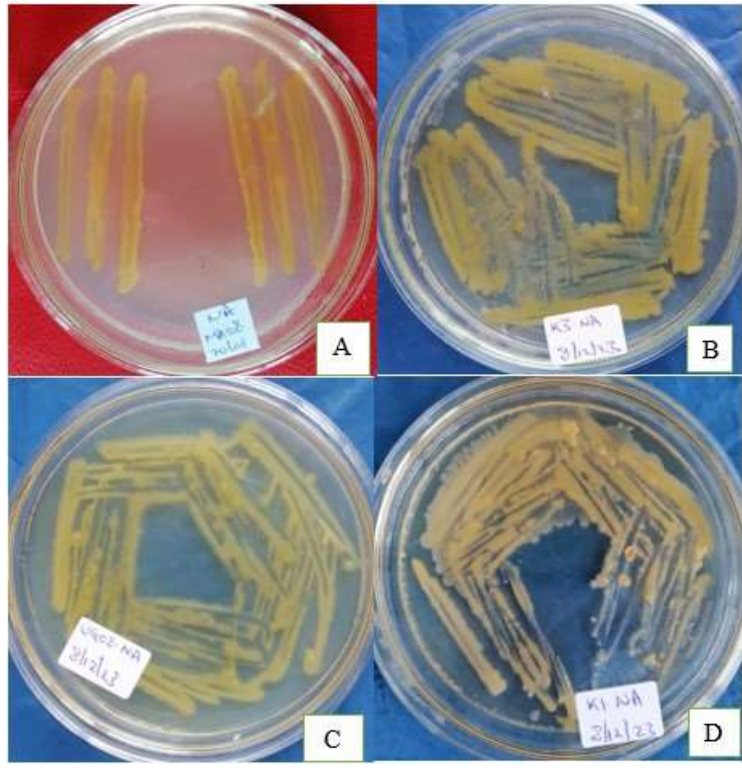

Plate 2: The axenic cultures of *Xanthomonas axonopodis* pv. *vignicola* (A-D). Typical yellow isolate (A and B); Creamy yellow isolate (C) and Light yellow pigmentation (D)

4.2.2 Biochemical features of *Xanthomonas axonopodis* pv. *vignicola* isolates

It was noted that all the studied bacterial cells were Gram negative rods. All the 48 *X. axonopodis* pv. *vignicola* isolates had the ability to hydrolyse starch, forming a colourless zone surrounding the bacterial colony (Table 10). In mannitol reaction, all the isolates were negative due to their inability to develop a colour change in a mixture of Medium C dye and mannitol. The 48 isolates developed a colour change in a mixture of Medium C dye and sucrose indicating a positive reaction. All the 48 Xanthomonas isolates produced gas bubbles upon addition of two drops of 20% hydrogen peroxide which confirmed a positive reaction. In addition, the 48 Xanthomonas isolates developed a white precipitate on the surface of the medium plate upon flooding with 0.2% mercury chloride solution indicating a positive response. Similarly, all the isolates were lactose positive as a result of colour change from red to yellow as well as hydrogen sulphide production as a result of the blackening of the test strip. The isolates failed to synthesis indole from utilization of tryptophan as shown by the failure to form a red or yellow ring nor generate adequate acid during the fermentation of glucose in methyl red test. In regard to the ability to breakdown citrate as a sole source of energy and carbon, a positive test was recorded as shown by change in medium's colour from green to yellow. Further, the glucose fermentation to produce 2,3-butanediol as a by-product leading to red colour development upon addition of potassium hydroxide was observed in all the isolates as shown in Table 10.

Table 10: Biochemical features of *Xanthomonas axonopodis* pv. *vignicola* isolates

	Biochemical Test											
Isolate	G-R	S-H	M-T	S-T	C-A	G-L	L-U	H_2S	I-T	M-R	V-P	C-U
IS01	-	+	-	+	+	+	+	+	-	-	+	+
IS02	-	+	-	+	+	+	+	+	-	-	+	+
IS04	-	+	-	+	+	+	+	+	-	-	+	+
IS05	-	+	-	+	+	+	+	+	-	-	+	+
IS06	-	+	-	+	+	+	+	+	-	-	+	+
IS06-2	-	+	-	+	+	+	+	+	-	-	+	+
IS09	-	+	-	+	+	+	+	+	-	-	+	+
IS08	-	+	-	+	+	+	+	+	-	-	+	+
KK01	-	+	-	+	+	+	+	+	-	-	+	+
KK02	-	+	-	+	+	+	+	+	-	-	+	+
KK03	-	+	-	+	+	+	+	+	-	-	+	+
KK04	-	+	-	+	+	+	+	+	-	-	+	+
KK05	-	+	-	+	+	+	+	+	-	-	+	+
KK06	-	+	-	+	+	+	+	+	-	-	+	+
KK07	-	+	-	+	+	+	+	+	-	-	+	+
KK08	-	+	-	+	+	+	+	+	-	-	+	+
KK09	-	+	-	+	+	+	+	+	-	-	+	+
KK10	-	+	-	+	+	+	+	+	-	-	+	+
KK12	-	+	-	+	+	+	+	+	-	-	+	+
KK13	-	+	-	+	+	+	+	+	-	-	+	+
KK14	-	+	-	+	+	+	+	+	-	-	+	+
KK15	-	+	-	+	+	+	+	+	-	-	+	+
LK01	-	+	-	+	+	+	+	+	-	-	+	+
LK02	-	+	-	+	+	+	+	+	-	-	+	+
LK04	-	+	-	+	+	+	+	+	-	-	+	+
LK05	-	+	-	+	+	+	+	+	-	-	+	+
MU01	-	+	-	+	+	+	+	+	-	-	+	+
MU02	-	+	-	+	+	+	+	+	-	-	+	+
MK01	-	+	-	+	+	+	+	+	-	-	+	+
MK02	-	+	-	+	+	+	+	+	-	-	+	+
MK03	-	+	-	+	+	+	+	+	-	-	+	+
MK04	-	+	-	+	+	+	+	+	-	-	+	+
MK05	-	+	-	+	+	+	+	+	-	-	+	+
MK06	-	+	-	+	+	+	+	+	-	-	+	+
MK07	-	+	-	+	+	+	+	+	-	-	+	+
MK08	-	+	-	+	+	+	+	+	-	-	+	+
MK09	-	+	-	+	+	+	+	+	-	-	+	+
MK10	-	+	-	+	+	+	+	+	-	-	+	+
MK12	-	+	-	+	+	+	+	+	-	-	+	+
MK13	-	+	-	+	+	+	+	+	-	-	+	+
MK14	-	+	-	+	+	+	+	+	-	-	+	+
MK15	-	+	-	+	+	+	+	+	-	-	+	+
UG02	-	+	-	+	+	+	+	+	-	-	+	+
UG04	-	+	-	+	+	+	+	+	-	-	+	+
UG05	-	+	-	+	+	+	+	+	-	-	+	+
UG07	-	+	-	+	+	+	+	+	-	-	+	+
UG08	-	+	-	+	+	+	+	+	-	-	+	+
UG09	-	+	-	+	+	+	+	+	-	-	+	+

Key: + =positive reaction, - =negative reaction, G-R= Gram's reaction, S-H= starch hydrolysis, M-T= mannitol test, S-T= sucrose test, C-A= Catalase activity, G-L= Gelatin liquefaction, L-U= lactose utilization, H_2S= Hydrogen sulphite production, I-T= indole test, M-R= methyl red test, V-P= Voges Proskauer test, and C-U= citrate utilization**.**

4.2.3 Pathogenicity and virulence of *Xanthomonas axonopodis* pv. *vignicola* isolates on susceptible cowpea variety

Forty-one (85.42%) of the forty-eight (48) *X. axonopodis* pv. *vignicola* isolates were virulent to Ken Kunde cowpea variety, while seven isolates showed no clear disease symptoms. The bacterial blight symptoms on the susceptible cowpea were observed from the ninth day of inoculation. The symptoms observed started as small brownish water-soaked lesions that eventually grew and merged to produce larger chlorotic patches on the leaves. Yellowing and falling of the lower leaves was also observed in the later stage of the disease. There was a difference in the virulence of the isolates and they were classified into three main groups based on the time it took to produce characteristic disease symptoms on the susceptible cowpea plants; less virulent, moderately virulent and highly virulent. Nineteen (39.58%) of the isolates were very virulent isolates that caused the cowpea bacterial blight disease within 9 to 12 days after inoculation (Table 11). The moderately virulent *X. axonopodis* pv. *vignicola* isolates caused the disease between 13 to 16 days after inoculation, made up of 27.08% (13) of the isolates, and 18.75% (9) were grouped as less virulent, able to cause a disease between 17 to 21 days after inoculation. Kakamega and Makueni showed a higher percentage of the extremely aggressive *X. axonopodis* pv. *vignicola* isolates due to the short period it took to produce noticeable disease symptoms on the cowpea plants. Further, it was noted that the isolates obtained from the same county showed difference in virulence with isolates showing varying degrees of aggressiveness.

Table 11: Virulence of *Xanthomonas axonopodis* pv. *vignicola* isolates on susceptible cowpea variety

Aggressiveness degree of the Xanthomonas isolates	Percentages and number	Isolates
Avirulent isolates	14.58 (7)	IS04, IS06-2, KK04, LK01, MK04, MK09, and UG07.
Less virulent isolates	18.75 (9)	KK06, KK08, LK02, IS02, ISO8, MU01, MK03, UG09 and UG04.
Moderately virulent isolates	27.08 (13)	KK02, KK10, KK12, LK04, UG02, UG05, MK02, MK05, MK10, MK14, IS06, IS09, and MU02.
Highly virulent isolates	39.58 (19)	KK01, KK03, KK05, KK07, KK09, KK10, KK13, KK14, KK15, MK01, MK06, MK07, MK08, MK12, MK15, IS01, IS05, LK05, and UG08.

4.2.4 Molecular characterization of the *Xanthomonas axonopodis* pv *vignicola* isolates

The DNA extracted from the 48 isolates of *X. axonopodis* pv. *vignicola* (Plate 3) and run on 0.8% agarose gel showed amplicons base pair band of approximately 1500 bp (Plate 4). When the unpurified PCR output was sequenced and analysed through the BLAST program by comparing the available sequence in the National Centre for Biotechnology Information (NCBI) database with the aligned sequences, the results showed that all the isolates were 95 to 99 per cent identical to previously identified *X. axonopodis* pv *vignicola* in the NCBI database (Appendix VI).

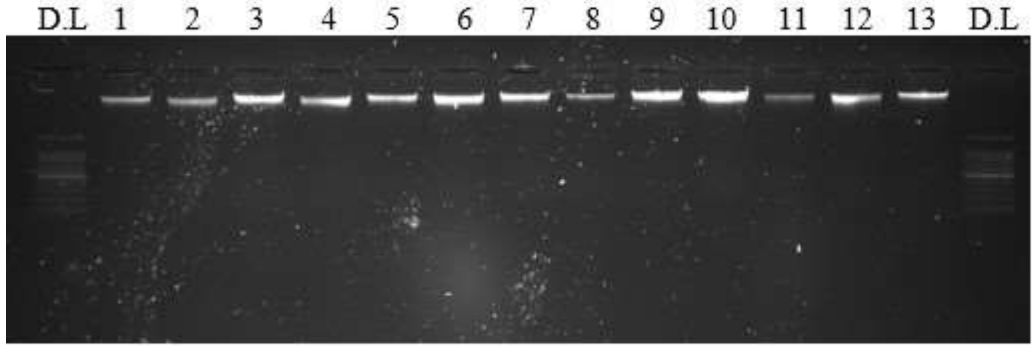

Plate 3: Agarose gel confirmation of DNA of 13 isolates of *Xanthomonas axonopodis* pv. *vignicola*

Legend: D.L: DNA ladder, 1: IS01, 2: IS06, 3: IS08, 4: KK02, 5: KK05, 6: KK09, 7: KK15, 8: LK01, 9: LK04, 10: MU01, 11: MK03, 12: MK12 and 13: UGO7.

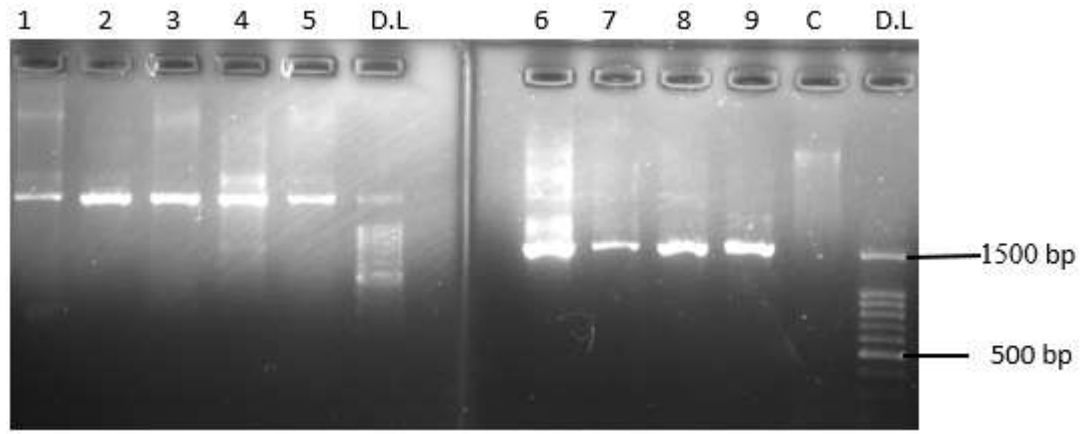

Plate 4: Agarose gel confirmation of PCR amplicons of 9 isolates of *Xanthomonas axonopodis* pv. *vignicola*

Legend: D.L: DNA ladder, C: negative control, 1: IS01, 2: IS06, 3: IS08, 4: KK02, 5: KK05, 6: KK09, 7: KK15, 8: LK01, 9: LK04.

4.3 Analysis of genetic diversity of the *Xanthomonas axonopodis* pv. *vignicola* using inter-simple sequence repeat (ISSR)

A total of ten ISSR markers were tested for polymorphism and six markers (ISSR820, ISSR816, UBC8932800, D-3, A-31, and A-16) which showed polymorphism were

adopted for diversity studies. The amplification patterns generated using primers D-3 and A-31 on the same isolates (1-12) are shown in Plate 5. Similarly, Plate 6 shows the banding patterns for primers UBC8932800 and A-31. The banding patterns clearly demonstrated great genetic variability among the different *X. axonopodis* pv. *vignicola* isolates.

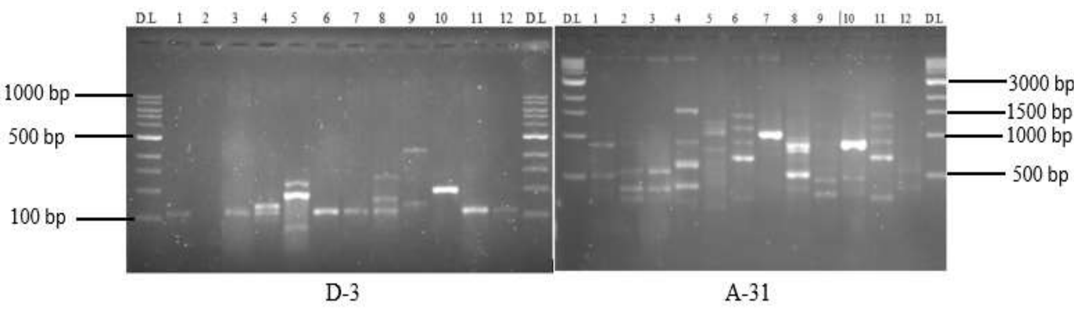

Plate 5: ISSR amplification patterns produced by primer D-3 and A-31 on *Xanthomonas axonopodis* pv. *vignicola* isolates

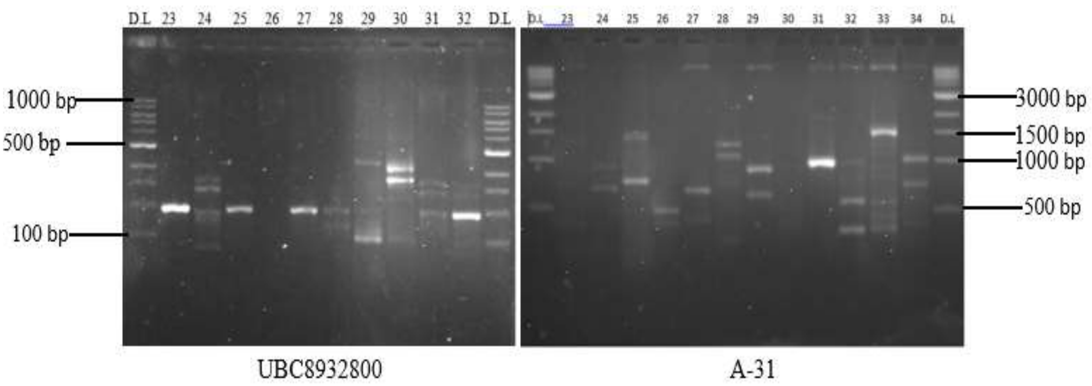

Plate 6: ISSR amplification patterns produced employing primer UBC8932800 and A-31 on *Xanthomonas axonopodis* pv. *vignicola* isolates

4.3.2 Polymorphism information content (PIC)

The genetic informativeness of each molecular marker used in diversity studies when assessed by calculating the polymorphism information content of each marker. The PIC values ranged from 0.2384 to 0.4486. The highest value of the polymorphism

information content was of the A-31 primer (0.4486), followed by the UBC8932800 marker (0.4256), while the lowest PIC value was shown by the A-16 maker (0.2384) (Table 12).

Table 12: Polymorphism information content for the markers used in ISSR diversity studies of *Xanthomonas axonopodis* pv. *vignicola* isolates

Marker	PIC
ISSR820	0.3717
ISSR816	0.3806
UBC8932800	0.4256
D-3	0.2578
A-31	0.4486
A-16	0.2384

4.3.3 Polymorphic loci

Xanthomonas axonopodis pv. *vignicola* isolates from various counties showed varying percentages of polymorphic loci. The highest percentage of polymorphic loci was observed in Makeuni County (44.62%), followed by Kakamega County (43.33%), and the least percentage of polymorphic loci was noted in the Meru County isolates (27.95%). The mean percentage of polymorphic loci was 37.78% (Table 13).

Table 13: Percentage of polymorphic loci among the *Xanthomonas axonopodis* pv. *vignicola* isolates in different counties

Population (Counties)	% Polymorphism
Isiolo	42.05%
Kakamega	43.33%
Laikipia	39.49%
Meru	27.95%
Makueni	44.62%
Uasin Gishu	29.23%
Mean	37.78%
Standard Error	3.99%

4.3.4 Total band patterns for binary (haploid) data by populations (counties)

The six molecular makers amplified a total of 140 scored bands ranging from 100 bp to 3.5 kb in size of which 124 (88.57%) bands were polymorphic and sixteen (11.43%) were unique bands (Figure 4; Table 14). *Xanthomonas axonopodis* pv. *vignicola* isolates from Isiolo County had a total of twenty-seven different bands, with all 27 bands having a frequency of >= 5%. Five of the 27 bands were unique to a single population, while three bands were locally common bands (<=50%). In Kakamega, there were a total of twenty-six different bands, with all the bands having a frequency of >= 5%. Three of the 26 bands were unique to a single population, while an equal number were locally common bands (<=50%). Further, in Laikipia, there were a cumulative of twenty-five different bands, with all the bands having a frequency of >= 5%. Three of the 25 bands were unique to a single population, while two were locally common bands (<=50%). In Meru, there were a total of nineteen different bands, with all the bands having a frequency of >= 5%. One of the 19 bands was unique to a single population, while two were locally common bands (<=50%). In Makueni, there were a total of twenty-seven different bands, with all the bands having a frequency of >= 5%. Four of the 27 bands were unique to a single population, while an equal number were

locally common bands (<=50%). In Uasin Gishu County, there were a total of sixteen bands, all being different bands and all the bands had a frequency of >= 5%. There were no private or locally common bands in Uasin Gishu County *X. axonopodis* pv. *vignicola* isolates (Figure 4).

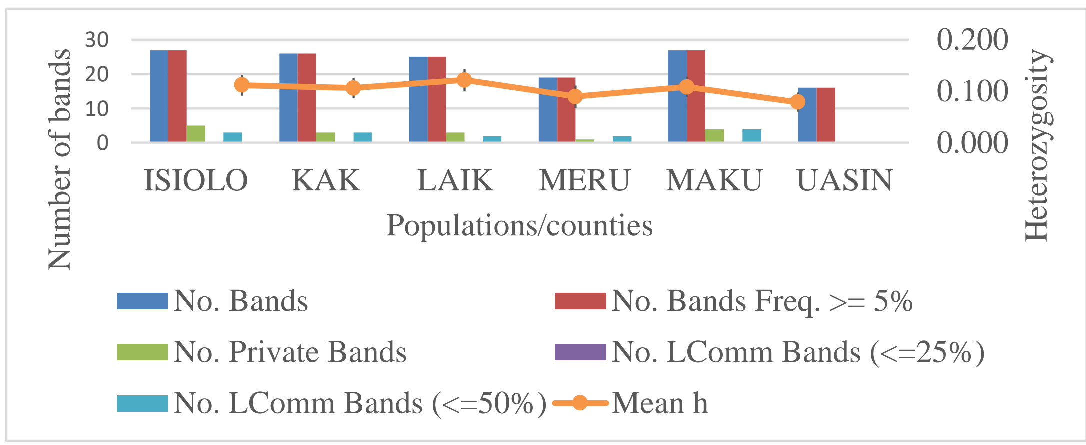

Figure 4: Band patterns across populations from the different regions

Legend: KAK- Kakamega; LAIK-Laikipia; MAKU- Makueni and UASIN- Uasin Gishu.

Table 14 below provides a detailed comparison of genetic diversity metrics of the *X. axonopodis* pv. *vignicola* isolates across the six populations/regions (Isiolo, Kakamega, Laikipia, Meru, Makueni, and Uasin Gishu) based on the presence and frequency of various genetic bands. Isiolo had the highest number of unique bands (5), suggesting a distinct genetic profile. No population had bands that are locally common in 25% or fewer Xanthomonas populations, indicating some degree of commonality in genetic bands across these populations. The number of locally common bands in 50% or fewer populations varied, with Isiolo and Makueni having the highest (4) and Laikipia and Meru having the lowest (2). On the measure of genetic diversity (mean h) within each Xanthomonas population, Isiolo isolates had the highest mean diversity of 0.112, while Uasin Gishu had the lowest of 0.079. The standard error of the mean diversity (SE of

Mean h) indicated that there was a great variation in the precision of the diversity estimate. For example, Isiolo had a standard error of 0.020, suggesting a higher precision compared to Laikipia, which had 0.022. The genetic diversity (h) varied across the populations, with Laikipia showing the highest mean diversity (0.122) and Uasin Gishu the lowest (0.079). The unbiased diversity (uh) followed a similar trend, with Laikipia having the highest (0.162) and Uasin Gishu having the lowest (0.095). The standard errors for both mean h and mean uh were relatively small, indicating precise estimates of genetic diversity.

Table 14: Total band patterns for binary (haploid) data by populations/counties

Population	Isiolo	Kakamega	Laikipia	Meru	Makueni	Uasin Gishu
Number of different bands	27	26	25	19	27	16
Number of different bands with a frequency >= 5%	27	26	25	19	27	16
Number of bands unique to a single population	5	3	3	1	4	0
Number of locally common bands (freq. >= 5%) found in 25% or fewer populations	0	0	0	0	0	0
Number of locally common bands (Freq. >= 5%) found in 50% or fewer populations	3	3	2	2	4	0
Mean h	0.112	0.106	0.122	0.090	0.109	0.079
SE of Mean h	0.020	0.019	0.022	0.022	0.019	0.019
Mean uh	0.128	0.114	0.162	0.179	0.117	0.095
SE of Mean uh	0.023	0.021	0.029	0.044	0.020	0.023

Where for haploid binary data, p = band frequency and q = 1 – p and h = diversity = 1 - (p^2 + q^2), and uh = unbiased diversity = (N / (N-1)) * h.

4.3.5 Analysis of molecular variance

The analysis of molecular variance (AMOVA) performed on *X. axonopodis* pv.

vignicola isolates from the six different regions and populations of Kenya, showed that among *X. axonopodis* pv. *vignicola* populations, only 5% of the total genetic variance existed between the six regions. This indicated that the genetic differences among the populations of *X. axonopodis* pv. *vignicola* isolates are relatively small. However, within the populations, a significant 95% of the total genetic variance can be attributed to differences within the populations. This suggests that individual populations, rather than different populations, contain the majority of the genetic diversity in the *X. axonopodis* pv. *vignicola* isolates (Figure 5). The AMOVA results indicate that the majority of genetic variation in *X. axonopodis* pv. *vignicola* isolates from the six regions in Kenya exists within the populations rather than among them. This suggests a high level of genetic diversity within the *X. axonopodis* pv. *vignicola* population in each region (Appendix VII).

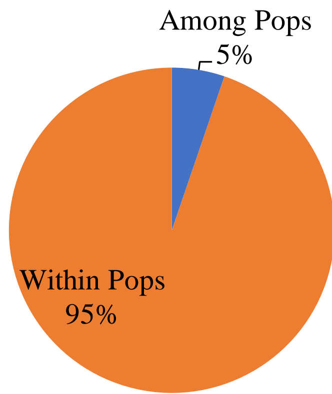

Figure 5: Percentages of molecular variance among the *Xanthomonas axonopodis* pv. *vignicola* isolates

4.3.6 Band frequencies, allele frequencies and estimated diversity by population for binary (haploid) data

The number of different alleles (Na) varied among *X. axonopodis* pv. *vignicola* populations, indicating differences in genetic richness. Makueni had the highest

average number of alleles per locus (0.692), while Uasin Gishu had the lowest (0.397) (Table 15). The number of effective alleles (Ne) varied among the *X. axonopodis* pv. *vignicola* populations, with Laikipia having the highest effective number of alleles (1.213), suggesting a more balanced allele frequency distribution compared to Uasin Gishu (1.140). There existed greater genetic diversity among the *X. axonopodis* pv. *vignicola* populations, as indicated by Shannon's Information Index (I), with higher values suggesting greater diversity. For example, Laikipia showed the highest diversity (0.178), while Uasin Gishu showed the least (0.115). On the diversity (h) and unbiased diversity (uh), there existed genetic variability within *X. axonopodis* pv. *vignicola* populations, with Laikipia having the highest diversity (h = 0.122) and unbiased diversity (uh = 0.162), indicating significant genetic variability. Uasin Gishu had the lowest (h = 0.079, uh = 0.095), suggesting less genetic diversity as regards to the standard errors for each measurement given, which gave an indication of the precision of these estimates. For example, the diversity estimates (h) for Makueni and Uasin Gishu had similar precision (SE = 0.019). Generally, the lower the standard error (SE) values indicates more precise estimates.

Table 15: Mean and standard error (SE) over loci for each *Xanthomonas axonopodis* pv. *vignicola* population

Population		N	Na	Ne	I	h	uh
Isiolo	Mean	8.000	0.667	1.189	0.168	0.112	0.128
	SE	0.000	0.106	0.037	0.029	0.020	0.023
Kakamega	Mean	14.000	0.667	1.174	0.163	0.106	0.114
	SE	0.000	0.107	0.034	0.028	0.019	0.021
Laikipia	Mean	4.000	0.615	1.213	0.178	0.122	0.162
	SE	0.000	0.104	0.039	0.032	0.022	0.029
Meru	Mean	2.000	0.423	1.179	0.124	0.090	0.179
	SE	0.000	0.088	0.044	0.030	0.022	0.044
Makueni	Mean	14.000	0.692	1.175	0.167	0.109	0.117
	SE	0.000	0.108	0.033	0.028	0.019	0.020
Uasin gishu	Mean	6.000	0.397	1.140	0.115	0.079	0.095
	SE	0.000	0.090	0.035	0.027	0.019	0.023

Key; N=sample size, Na= number of different alleles, Ne= number of effective alleles,

I=Shannon's Information Index, h=Diversity, uh=Unbiased Diversity.

4.3.7 Analysis of pairwise population matrix of Nei genetic distance of *Xanthomonas axonopodis* pv. *vignicola* isolates

The pairwise population matrix of Nei genetic distance provided a quantitative measure of genetic divergence between populations. Kakamega and Uasin Gishu showed the closest genetic similarity (0.009), suggesting minimal genetic divergence, similar with Makueni, Kakamega (0.010) and Uasin Gishu (0.012). Meru showed the most genetically divergent population from the other populations, especially from Uasin Gishu (0.054) and Makueni (0.053). Some populations like Laikipia and Isiolo had moderate genetic distances from all other populations, indicating it neither falls into the closest nor the most distant category significantly (Table 16).

Table 16: Pairwise population matrix of Nei genetic distance for each *Xanthomonas axonopodis* pv. *vignicola* population

Isiolo	Kakamega	Laikipia	Meru	Makueni	Uasin Gishu	
0.000						Isiolo
0.020	0.000					Kakamega
0.040	0.027	0.000				Laikipia
0.048	0.043	0.029	0.000			Meru
0.032	0.010	0.035	0.053	0.000		Makueni
0.036	0.009	0.041	0.054	0.012	0.000	Uasin Gishu

4.3.8 Pairwise population matrix of Nei genetic identity of the *Xanthomonas axonopodis* pv. *vignicola* populations

The pairwise population matrix of Nei genetic identity provided a measure of genetic similarity between different populations. Kakamega and Uasin Gishu *X. axonopodis* pv. *vignicola* isolates shared the highest genetic identity (0.991), indicating very high genetic similarity, while Makueni also had a high genetic identity with Kakamega

(0.990) and Uasin Gishu (0.988), suggesting strong genetic relatedness. Meru generally showed lower genetic identity with other populations, especially with the Makueni and Uasin Gishu (both 0.948), indicating it is somewhat genetically distinct. Isiolo had moderately high genetic identities with the other populations, indicating it is neither the most nor the least similar to other populations (Table 17).

Table 17: Pairwise *Xanthomonas axonopodis* pv. *vignicola* population matrix of Nei genetic identity

Isiolo	Kakamega	Laikipia	Meru	Makueni	Uasin Gishu	
1.000						Isiolo
0.980	1.000					Kakamega
0.961	0.973	1.000				Laikipia
0.954	0.958	0.971	1.000			Meru
0.968	0.990	0.966	0.948	1.000		Makueni
0.965	0.991	0.960	0.948	0.988	1.000	Uasin Gishu

4.3.9 Principal coordinates analysis (PCoA) of *Xanthomonas axonopodis* pv. *vignicola* populations

To demonstrate the genetic relatedness between the 48 isolates, all the data from ISSR polymorphism experimental scores were combined and subjected to principal coordinates analysis (PCoA). Principal Coordinate Analysis (PCoA) was used to visualise the similarities or dissimilarities of data. In this case, the PCoA plot represents the genetic relationships between populations of *X. axonopodis* pv. *vignicola* isolates from the six different regions in Kenya. Coord. 1 and Coord. 2 are the first two principal coordinates, representing the major axes of genetic variation in the data. Each point on the plot corresponds to a population, positioned based on its genetic similarity to other populations. A larger spread indicates more genetic diversity. The first two axes capture the most significant proportion of the genetic variation (Table 18; Figure 6). The first three axes cumulative account for 32.74% of the total variation in the dataset. Each

population is represented by a specific marker type and colour, corresponding to the county it originates from (Figure 6).

With regard to genetic relationships between counties, populations from Kakamega and Uasin Gishu are positioned close to each other, reflecting high genetic similarity, which aligns with their high Nei genetic identity values. Populations from Meru and Laikipia are more spread out, indicating greater genetic diversity within the populations from these counties. The Isiolo and Makueni populations are relatively close to the centre, suggesting moderate genetic similarity with other populations (Figure 6). Population (pop 4) from Meru is positioned further from the centre, indicating they may have unique genetic characteristics compared to other populations (outliers).

Table 18: Cumulative variation spread across various main coordinates

Axis number	Eigen value	% variation	% cumulative variability
1	4.26	13.14	13.14
2	3.37	10.16	23.30
3	3.13	9.04	32.74

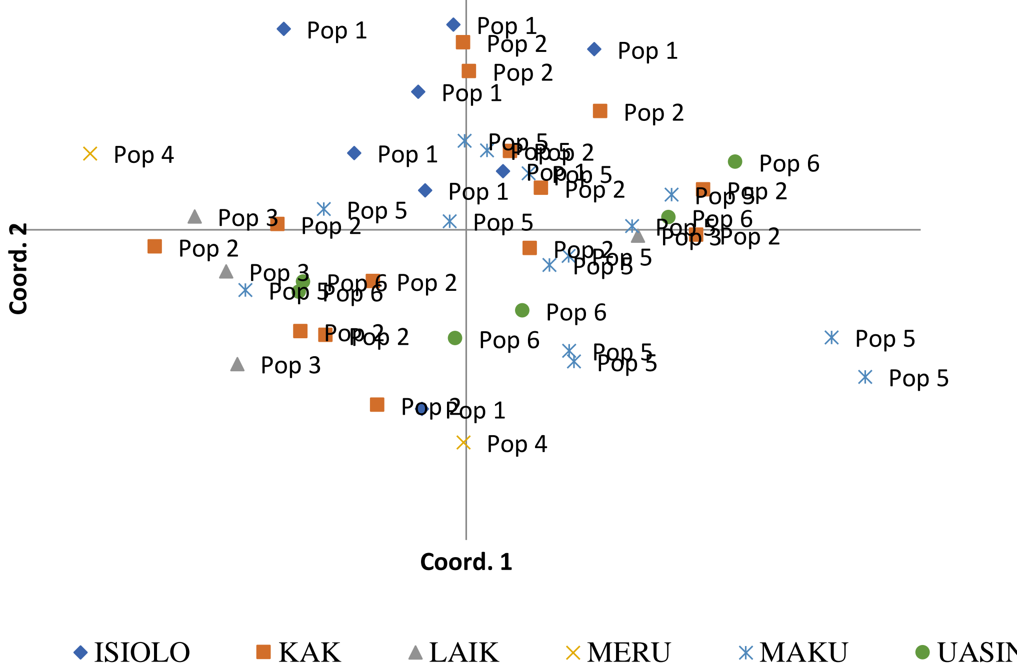

Figure 6: PCoA via covariance matrix with data standardization *Xanthomonas axonopodis* pv. *vignicola* population from various counties

Key: ISIOLO- Isiolo (blue diamonds), KAK- Kakamega (orange squares), LAIK- Laikipia (grey triangles), MAKU- Makueni (light blue crosses), UASIN- Uasin Gishu (green circles), and Meru (yellow x's).

4.3.9 Diversity phylogenetic tree of *Xanthomonas axonopodis* pv. *vignicola* isolates

The genetic relatedness between the 48 isolates from all the data from ISSR polymorphism scores were combined and subjected to cluster analysis by unweighted pair group arithmetic mean method (UPGMA) - based dendrogram displayed a dissimilarity coefficient which ranged from 0.056 to 1.14. The dendrogram below (Figure 7) provides a hierarchical clustering representation of the genetic distances among the *X. axonopodis* pv. *vignicola* isolates from the six different regions in Kenya. This dendrogram represents the genetic diversity between *X. axonopodis* pv. *vignicola* isolates, with shorter branches indicating greater genetic similarity. Based on the dissimilarity index, all the isolates were grouped into two main clusters (Figure 7:

Appendix VIII). Cluster A comprised the highest (47) *X. axonopodis* pv. *vignicola* isolates, which were from different areas. While the cluster B had only one isolate diverging (MK05) from Makueni. Cluster A_1 comprised two *X. axonopodis* pv. *vignicola* isolates from two regions (Isiolo and Kakamega; IS01 and KK03, respectively). In cluster A_2 it contained 45 *X. axonopodis* pv. *vignicola* isolates from all the regions sampled. The *X. axonopodis* pv. *vignicola* isolates from Isiolo (IS01, IS02, and IS03) were grouped in a distinct subgroup. These isolates were genetically similar, as indicated by their close grouping.

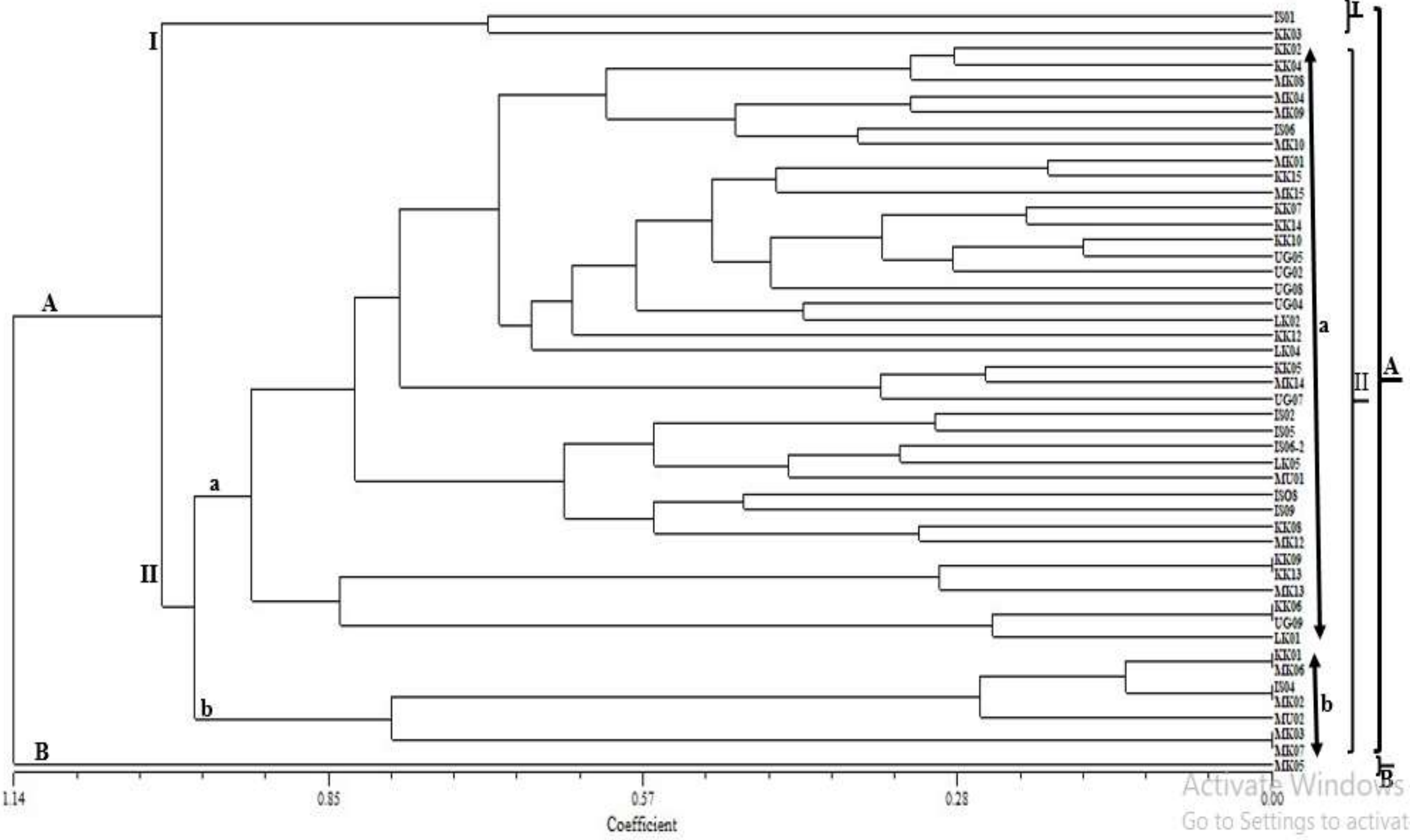

Figure 7: UPGMA Dendrogram generated from ISSR amplification sequences of 48 *Xanthomonas axonopodis* pv. *vignicola* isolates based on NEI 72 coefficient. **Key:** IS-Isiolo, KK- Kakamega (KK), LK- Laikipia, MU- Meru, MK- Makueni, and Uasin Gishu (UG).

4.4 Determination of the cross pathogenicity of *Xanthomonas axonopodis* pv. *vignicola*

The *X. axonopodis* pv. *vignicola* isolate MK08 (the highly virulent isolate) when inoculated to the cowpea (*V. unguiculata*) varieties (Bumala brown, Machakos 66 (M66), Cowpea brown, Ken Kunde, and local landrace cowpea): These include red chief, a variety of brown lentils (*Lens culinaris* ssp. *culinaris*), the KS20 variety of green grammes (*Vigna radiata*), the Nyala variety of soybean (*Glycine max*), and several common bean varieties (*Phaseolus vulgaris* L.), specifically Mwitemania GLP92, Kazuri bean, Chelelang, and yellow kidney beans, all sourced from Kenya Seed Co. Ltd., Nairobi (Plate 7).

Xanthomonas axonopodis pv. *vignicola* (MK08 isolate) caused an infection in all the cowpea varieties, soybeans, and lentils tested. However, it was observed that the green grams and all the common bean varieties were not susceptible to *X. axonopodis* pv. *vignicola.* The susceptible crops showed symptoms of bacterial blight (Plate 8) ten days after inoculation. The initial disease symptoms noted on the 10th day after inoculation were for the Bumala Brown, M66, and local landrace cowpea varieties, while for the Ken Kunde and Cowpea Brown varieties, they were observed on the 11th and 13th days, respectively. For the soybean and lentils, the initial disease symptoms were recorded on the 15th day after inoculating the pathogen onto the plant leaves.

Plate 7: Fabaceae family members tested for cross-pathogenicity:

Key: A, D, E, and H-cowpea (Bumala brown, Cowpea brown, Ken Kunde, and M66 varieties, respectively); B, C, and G-phaseolus *vulgaris* (Mwitemania GLP92, Kazuri bean-chelelang, and yellow kidney beans-local varieties, respectively); F-*vigna radiata* (KS20 variety); I-glycine *max* (Nyala variety); and J-lentils (Red chief variety).

The notable initial disease symptoms were necrotic lesions on the plant leaves, which progressed to form light yellow, round, or irregular spots spread across the leaf lamina. As the disease progressed, the pathogen developed the typical light brown spots and necrotic centre with crimson veins. The re-isolated pathogen from inoculated crops showed the disease symptoms, and their cultural characteristics were similar to those of the pathogen used for inoculation. Cross-inoculation research findings demonstrate that *X. axonopodis* pv. *vignicola* can cause bacterial disease in some of the crops in the same family. The diseased leaves (with the letter 'D' in Plate 8) exhibited typical bacterial symptoms consistent with *X. axonopodis* pv. *vignicola* infection, while healthy leaves (letter 'H') showed no visible symptoms of the disease and appeared green and normal.

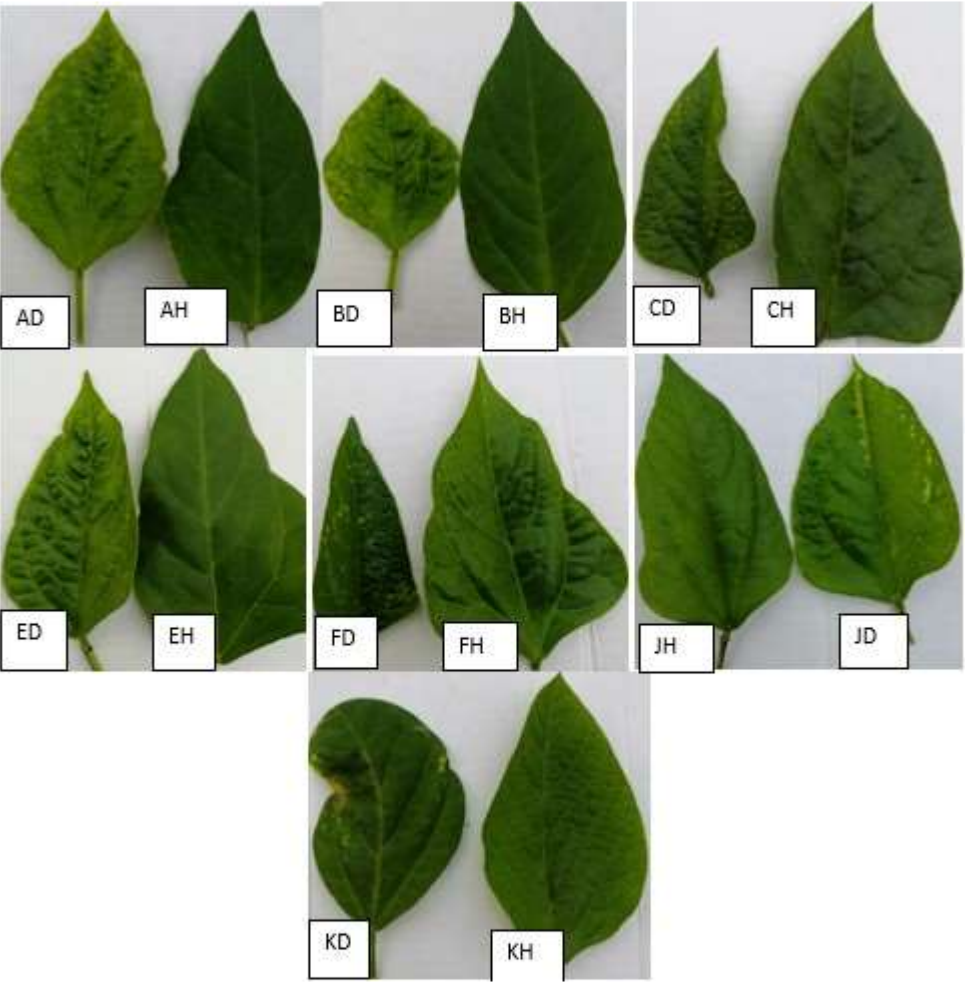

Plate 8: Diseased and healthy leaves from tested plants

Key; - A- Bumala brown cowpea; B- M66 cowpea; C- Cowpea brown; F- Local cowpea; J- Ken Kunde, and soyabean (K).

The percentage of disease severity ranged from 25.83% to 51.67%, with higher disease severity levels being observed in cowpea varieties. The disease severity in cowpea varieties ranged from 45.83% in Ken Kunde to 51.67% in the local landrace variety. Soya and lentils showed lower disease severity levels of 26.67% and 25.83%, respectively (Figure 8) below. The disease severity levels in the different crops differed significantly ($p \leq 0.001$) (Appendix IX) when they were compared using the Tukey test at a 95% family-wise confidence level (Appendix X).

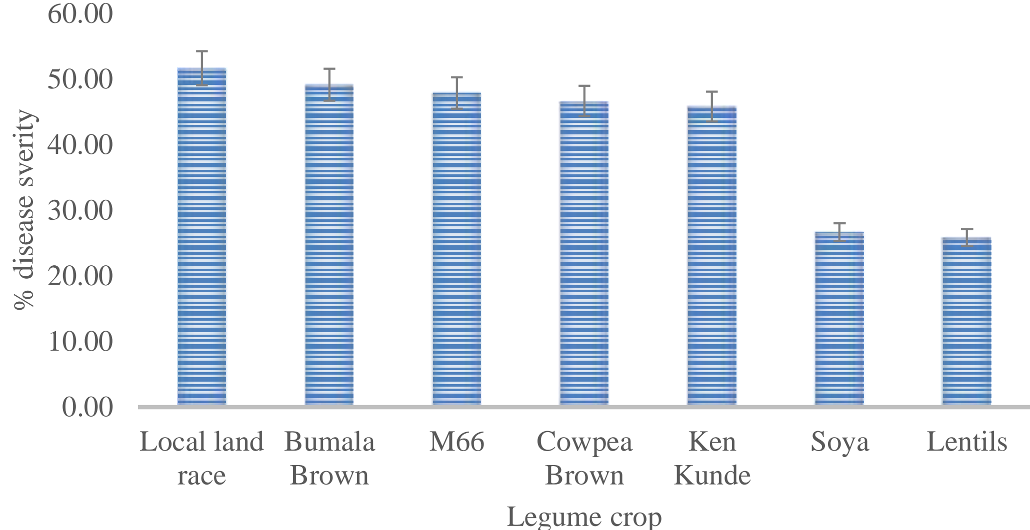

Figure 8: Severity levels of cowpea bacterial blight disease for the different tested crops

4.5 Evaluation of the antibacterial potential of *Bacillus* sp. and selected botanicals against *Xanthomonas axonopodis* pv. *vignicola*

4.5.1 *In vitro* antibacterial activity of *Bacillus subtilis* against *Xanthomonas axonopodis* pv. *vignicola* through dual culture and inverted plate techniques

Bacillus subtilis showed varying levels of inhibition against *X. axonopodis* pv. *vignicola,* which differed with the method of exposure and the duration of exposure (Figure 9). *Bacillus subtilis* displayed the highest antibacterial activity of between 68.33% and 87.79% in the dual culture technique, while inhibition of between 38.33% and 71.33% was recorded in the inverted plate method over an exposure period of seventy-two hours. Both techniques displayed an increasing trend in inhibition over time. The percentage inhibition increased from 24 hours to 72 hours for both the dual culture and inverted plate methods. As time progressed, the variation in inhibition levels between the two methods decreased. By 72 hours, the inhibition levels were almost similar, indicating that both techniques might have similar results on the continuous

exposure (Figure 9). There was a statistically significant difference (P≤0.001) in the inhibition of *X. axonopodis* pv. *vignicola* by *B. subtilis* through both the dual culture and inverted plate techniques through the 24, 48, and 72 hours of exposure (Appendix XI).

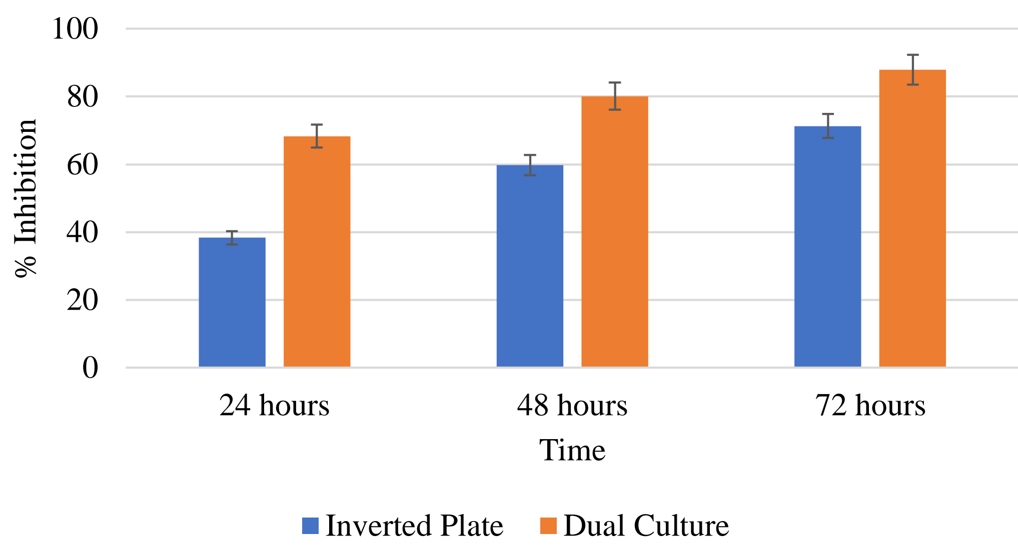

Figure 9: Percent inhibition of *Xanthomonas axonopodis* pv. *vignicola* by *Bacillus subtilis* using inverted plate and dual culture techniques

When Tukey multiple comparison of means was done at 95% family-wise confidence level for *B. subtilis* percent inhibition by the two methods (dual culture and inverted plate methods). It was noted that the period of exposure and the method of screening had a statistically significant effect on *Bacillus subtilis* at P≤0.05 (Appendix XII).

The interaction plot (Figure 10: Appendix XIII) clearly illustrated the significant factors in the *B. subtilis* inhibition activity over varied time ranges (24 hours, 48 hours, and 72 hours) using the two methods. The dual culture method showed a higher initial inhibition percentage at 24 hours compared to the inverted plate method. From 24 to 48 hours, both methods displayed a significant rise in inhibition, with the inverted plate method increasing by approximately 15% and the dual culture method increasing by

approximately 10%. From 48 to 72 hours, the rise was more gradual for both methods, at a rate of about 10%. The non-parallel nature of the lines implies an interaction effect between the methods and the duration of exposure. The difference in inhibition levels between the two methods changes with time. Generally, the dual culture method consistently exhibited higher inhibition levels across all times of exposure compared to the inverted plate method. The interaction between time and method was statistically not significant (Appendix XIII).

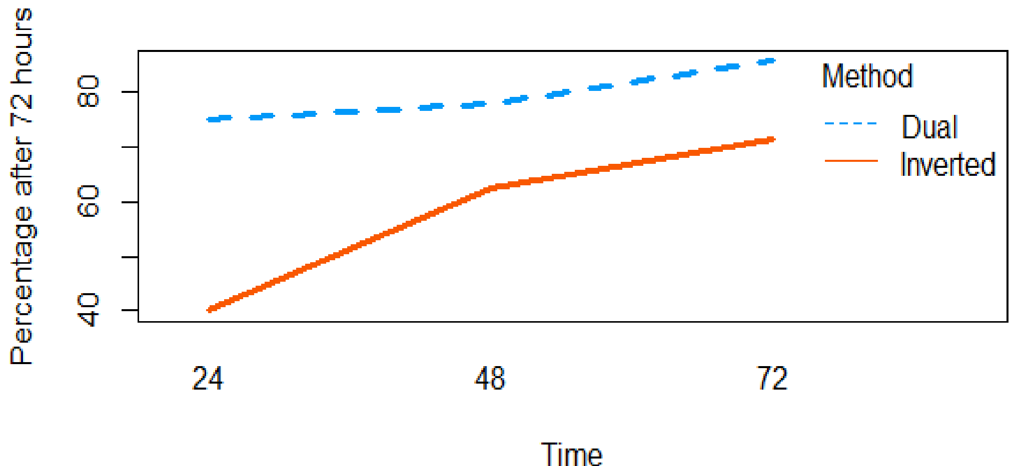

Figure 10: Interaction of time of exposure and method of screening in the inhibition of *Xanthomonas axonopodis* pv. *vignicola* by *Bacillus subtilis*

4.5.2 Antibacterial activity of *Bacillus amyloliquefaciens* against *Xanthomonas axonopodis* pv. *vignicola* through dual culture and inverted plate techniques

Bacillus amyloliquefaciens displayed varying levels of percentage inhibition against *X. axonopodis* pv. *vignicola,* based on the method of screening and the period of exposure (Figure 11). *Bacillus amyloliquefaciens* showed a statistically significant ($P \leq 0.05$: Appendix XIV) antibacterial activity of between 45.00% and 76.12% through the dual

culture technique and a percent inhibition of between 45.00% and 73.89% through the inverted plate method (Figure 11) over an exposure period of seventy-two hours. Both techniques displayed an increasing trend in inhibition over time. As time progressed, the variation in inhibition levels between the two methods decreased. By 72 hours, the inhibition percentages were similar, indicating that both techniques might have similar results over extended periods. When Tukey multiple comparison of means was done at 95% family-wise confidence level for dual culture and inverted plate methods, it was noted that the period of exposure had a significant effect, while the method of screening was not statistically significantly different in its effect on *B. amyloliquefaciens* at P≤0.05 (Appendix XV).

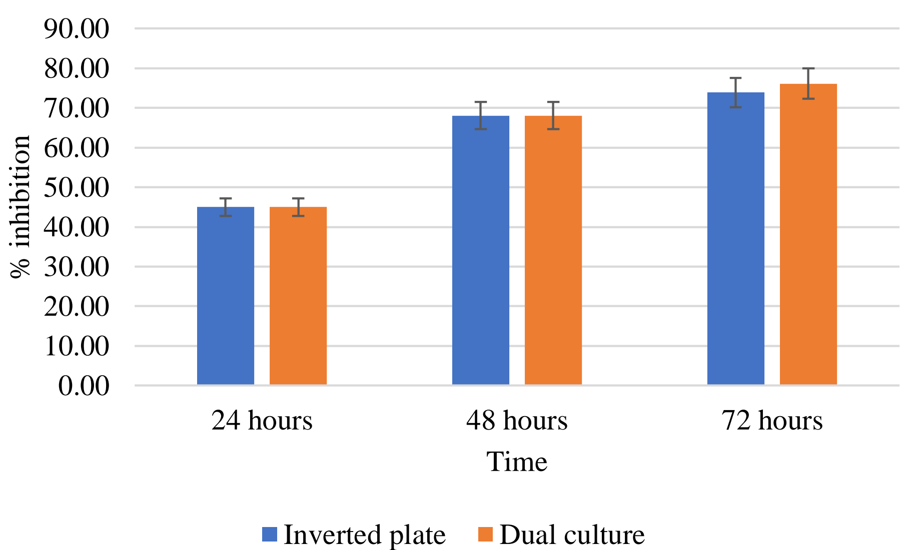

Figure 11: Percent inhibition of *Xanthomonas axonopodis* pv. *vignicola* by *Bacillus amyloliquefaciens* using inverted plate and dual culture techniques

The interaction plot (Figure 10) clearly illustrated the significant factor in the *B. amyloliquefaciens* inhibition activity. The interaction plot demonstrated the level of inhibition of *X. axonopodis* pv. *vignicola* by *B. amyloliquefaciens* over varied time intervals (24 hours, 48 hours, and 72 hours), dual culture and inverted plate methods.

For the initial 24 hours, both methods showed similar levels of inhibition (45%). From 24 to 48 hours, both methods showed an equal rise in inhibition of approximately 23%. Further, from 48 to 72 hours, the increase was more gradual for both methods, with dual culture increasing by approximately 8% and the inverted plate method increasing by approximately 5%. The plot lines for the two methods converge at 48 hours, suggesting that both methods have similar efficacy at this time point. The slight deviation at 72 hours between the two methods might indicate that the dual culture method might be slightly more efficacious than the inverted plate method. However, the interaction between time and method was statistically not significantly different (Appendix XVI).

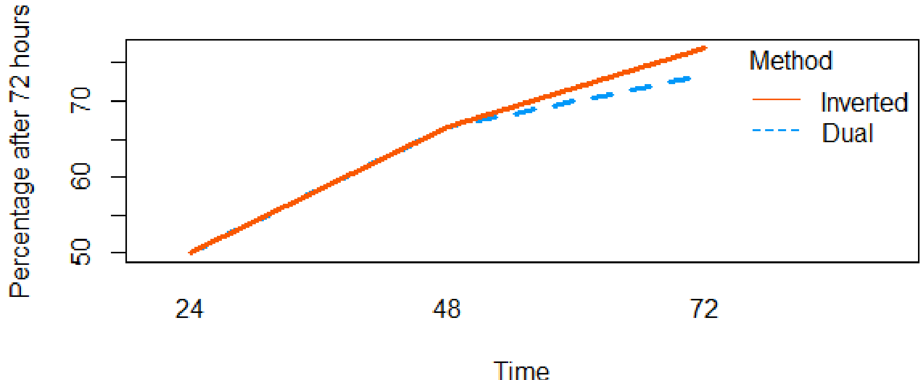

Figure 10: Interaction of time of exposure and method of screening in the inhibition of *Xanthomonas axonopodis* pv. *vignicola* by *Bacillus amyloliquefaciens*

4.5.3 Comparison of antibacterial activities of *Bacillus subtilis* and *Bacillus amyloliquefaciens*

Figure 11 below displays the percentage inhibition of *X. axonopodis* pv. *vignicola* by the two bio-agents, *B. amyloliquefaciens* and *B. subtilis,* over the three varied exposure

time intervals (24 hours, 48 hours, and 72 hours). For the inverted plate technique, *B. amyloliquefaciens* displayed higher inhibition compared to *B. subtilis* at all-time intervals, while in the dual culture technique, *B. subtilis* showed higher inhibition compared to *B. amyloliquefaciens*. Both *Bacillus subtilis* and *B. amyloliquefaciens* showed a comparable trend of increasing inhibition over time. Both bio-agents displayed a significant increase in inhibition from 24 to 48 hours, with a decreasing marginal effect noted at 72 hours. The slight deviation at 72 hours between the two methods might indicate that the dual culture method might be slightly more efficacious than the inverted plate method (Figure 12).

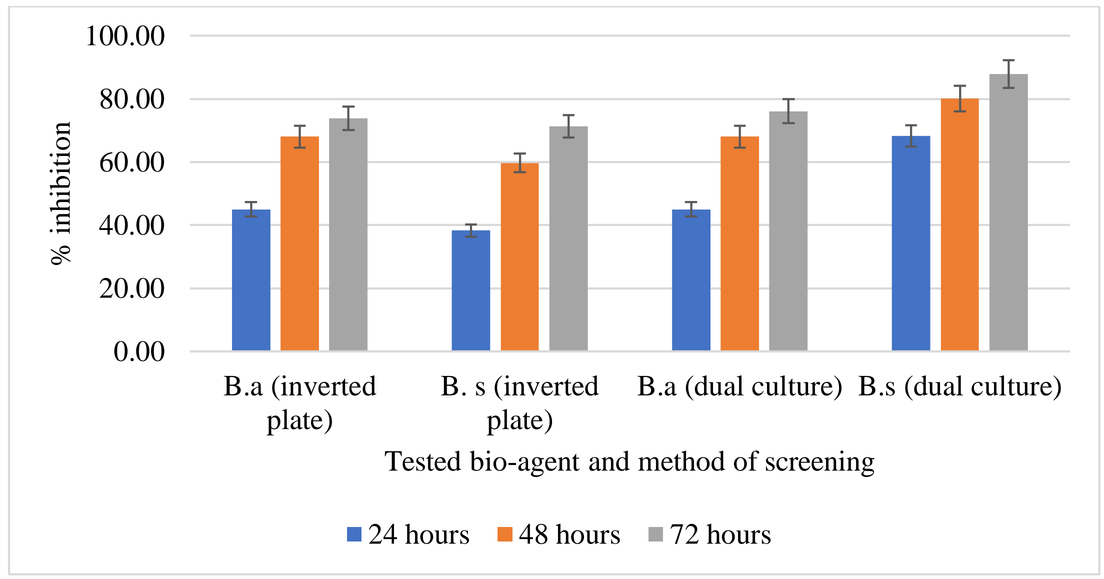

Figure 11: Comparison of antibacterial activities of *Bacillus subtilis* and *Bacillus amyloliquefaciens*

Key; - B.a = *Bacillus amyloliquefaciens* and B.s = *Bacillus subtilis*

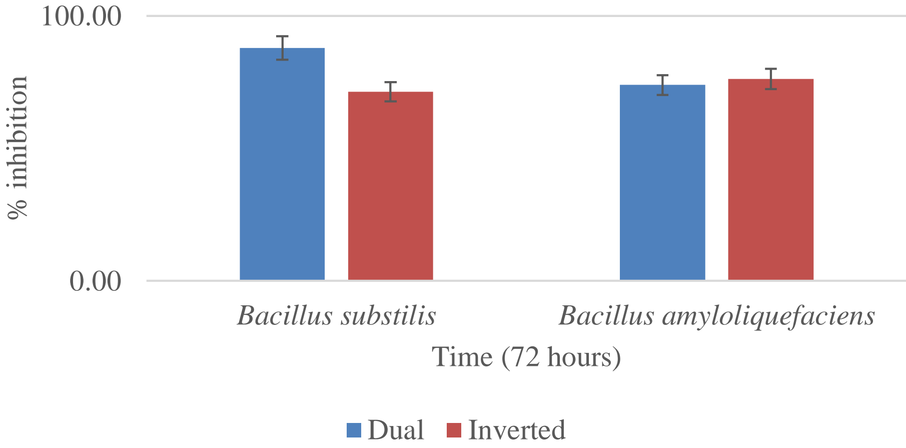

Figure 12: Comparison of antibacterial activities of *Bacillus subtilis* and *Bacillus amyloliquefaciens* after 72 hours of exposure in both dual culture and inverted plate methods

4.5.4 *In vitro* evaluation of *Salvia nilotica*, neem, garlic and ginger extracts against *Xanthomonas axonopodis* pv. *vignicola*

The tested plant extracts showed varying levels of inhibition to *X. axonopodis* pv. *vignicola*. The levels of inhibition varied depending on the type of extract and level of concentration. *The leaf extracts of S. nilotica did not affect Xanthomonas axonopodis pv. vignicola* at both levels of extract concentration. *Xanthomonas axonopodis* pv. *vignicola* showed the highest susceptibility to the ciprofloxacin antibiotic (55.70 mm), while ginger extracts showed the lowest inhibition (18.5 mm) at the concentration levels of 12.5 mg/ml (Figure 13). There was a significant difference in the performance of the different bioagents at $P \leq 0.05$ (Appendix XVII). When Tukey multiple comparison of means was done at 95% family-wise confidence level for botanical inhibition, it was noted that the type of the botanical had a significant effect on the inhibition of *X. axonopodis* pv. *vignicola* (Appendix XVIII).

At the extract concentration level of 25 mg/ml, it was observed that the inhibition levels varied depending on the plant. Ginger and ciprofloxacin displayed the lowest and the highest inhibition levels, respectively (Figure 13), for the effective products. There was a significant difference in the performance of the different plant extracts at P≤0.05 (Appendix XIX). When Tukey multiple comparison of means was done at 95% family-wise confidence level for bio-agent inhibition, it was noted that the type of the plant extract had a significant effect on the inhibition of *X. axonopodis* pv. *vignicola* (Appendix XX). Among the plant extracts, neem showed a higher inhibition potential (12.5 mg/ml, 34.8 mm, and 25 mg/ml, 38.5 mm) at both concentrations compared to garlic. Comparing the two concentration levels, no significant difference was found at P≤0.05 (Appendix XXI).

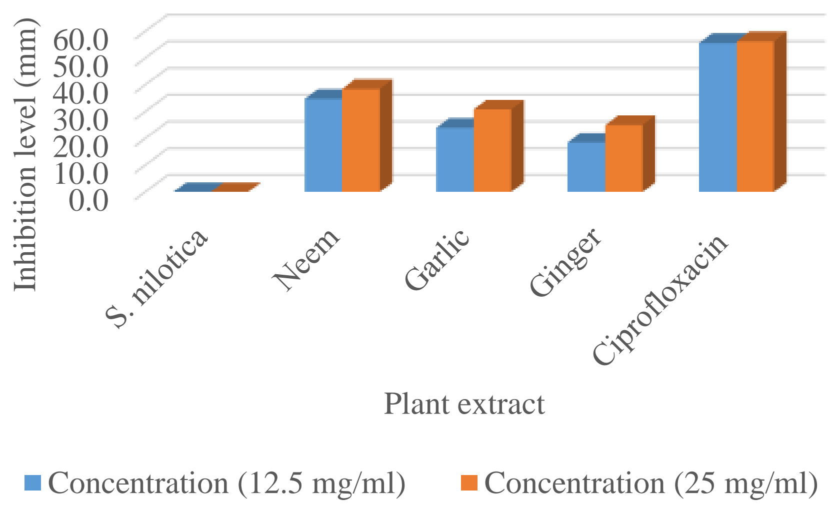

Figure 13: Comparison of antibacterial activities of the tested bio-agents at the two levels of concentrations

CHAPTER FIVE

DISCUSSION

5.1 The incidence and prevalence of bacterial blight on cowpea grown in different regions of Kenya

The cowpea bacterial blight disease symptoms recorded during the survey included stunted growth; necrotic lesions on the leaves that extended to the stems; light yellow round spots on the leaf surface, with the larger spots showing a brown necrotic centre with crimson veins; and drying of leaves. The mature infected cowpea crops that had produced the pods had their pods shrunk and turned yellow, with smaller, wrinkled, and poorly developed seeds from the sick pods. These leaf symptoms were similar to the typical bacterial blight symptoms as noted on the cowpea crops by Agbicodo et al. (2010), Omomowo et al. (2021) and Praneetha et al. (2022).

Out of eighty (80) cowpea farms sampled, 48 (60%) farms tested positive for the cowpea bacterial blight disease caused by the pathogen *X. axonopodis* pv. *vignicola*. The cowpea bacterial blight disease incidence varied among the sites surveyed. In this study, we observed that the highest incidence of bacterial blight occurred in Kakamega County, while the lowest was in Uasin Gishu County. Cowpea bacterial blight was prevalent in all the six regions surveyed. Similarly, the cowpea bacterial blight prevalence was higher in Makueni and followed by Kakamega, Isiolo, Uasin Gishu and Laikipia counties, but lowest in Meru county. The variation may be linked to regional climatic differences, as previously reported by Girma et al. (2022), who found lower disease levels at higher altitudes. Our findings align with the studies in Uganda (Nantale et al., 2023b) and Nigeria (Amodu et al., 2017), though incidence rates in Kenya appear

somewhat lower. One potential factor is seed source, where a significant proportion of farmers in Makueni and Makindu used uncertified seeds, a practice shown to increase disease risk (Njonjo et al., 2019). Girma et al. (2022) reported that the high-altitude regions that grow cowpea are less prone to bacterial blight disease development. Ochichi (2015), from a survey done on cowpea bacterial blight in various sites in Western Kenya, reported a disease incidence of between 3 and 70% during the short rainy season when farmers planted drought-resistant crops. In a replica of research done in Bangladesh (Saha et al., 2022), cowpea bacterial blight incidence was between 4 and 57% in Bangladesh, which was close to the incidence and prevalence recorded in the current study. In Uganda, Nantale et al. (2023a) reported a disease prevalence of 95% for all the cowpea genotypes they tested for disease susceptibility. Further, Horn and Shmelis (2020) and Makonnen et al. (2022) noted that the bacterial blight of cowpea is capable of inflicting damage to approximately 53% of cowpea leaves, 68% of the seeds, and 71% of the pods. Ganiyu et al. (2017) reported a common bacterial blight incidence ranging from 20 to 43% in the Abeokuta region of Nigeria, which is in tandem with the results obtained from the different regions of Kenya. Similarly, Amodu et al. (2017) noted a bacterial blight incidence of between 60 and 94% from a survey done at selection sites in Northern Nigeria.

In the current study, the differences in the incidence of cowpea bacterial blight in the sampled sites could be attributed to differences in cowpea cultivars farmed, the growth stage of the crop during the survey, the cropping system used, disease control and management measures deployed by farmers, and the handling of the crop waste after harvest. In the study by Njonjo et al. (2019), 76% of the farmers in Wote, Makueni County, Kenya, were using local cowpea cultivar seeds. In Mwatate, 75% of farmers were using certified cowpea seeds, while in Makindu, all the sampled farmers reported

using uncertified cowpea seeds. These findings had earlier been reported by Njonjo (2018) from a study done in the Makueni and Taita Taveta regions in Kenya and noted that up to 82% of the cowpea farmers use previous-season, farm-saved, uncertified seeds, hence could have acted as the primary inoculum source for the bacterial blight disease in the studied areas. Further, Njonjo et al. (2019) noted that the bacterial blight disease incidence and severity were positively correlated with the seed source. The current study, however, has provided the disease status in at least six regions of Kenya, representing the traditional cowpea production zones in comparison to the new cowpea production zones.

5.2.1 Morphological and biochemical features of *Xanthomonas axonopodis* pv. *vignicola* isolates

The *X. axonopodis* pv. *vignicola* isolates displayed slight variance in morphological and cultural characteristics on nutrient agar. The dominant pigmentation was typical yellow, followed by creamy yellow pigmentation, and the least common was a light yellow colour. All the *X. axonopodis* pv. *vignicola* isolates produced xanthomonadin and had rod arrangements, an entire margin, a mucoid surface, and convex elevation. In terms of form, the majority (89.58%) of the isolates were circular, while others were irregular. Further, most isolates were opaque, while a few were translucent. These typical characteristics are identified with the *X. axonopodis* pv *vignicola* as noted by previous research (Andrews et al., 1976; Nauman et al., 2023; Suke et al., 2022; and Van den Mooter and Swings, 1990).

Upon analysis of the biochemical features of the *X. axonopodis* pv *vignicola* isolates, it was noted that all the isolates were Gram-negative rods, hydrolysed starch, lacked the ability to break down mannitol, synthesise indole, and produce sufficient acid during

the fermentation of glucose. Further, the isolates were able to break down citrate, lactose, and ferment glucose, which confirmed morphologically and biochemically the presence of *X. axonopodis* pv. *vignicola* as the cause of bacterial blight disease, as also reported by Oguntate et al. (2021) and Olatunde et al. (2024) from their study on the morphological, biochemical, and virulence features of *X. axonopodis* pv. *vignicola* isolates. Similarly, Chormale et al. (2021) and Madavi (2022) noted that *X. axonopodis* pv. *vignicola* isolates showed positive reactions for the catalase test, starch hydrolysis, gelatin liquification, the potassium hydroxide solubility test, and hydrogen sulphite production, and a negative reaction to indole generation, Gram staining, and the methyl red test – features which were prominently shown by the current isolates. Further, *X. axonopodis* pv. *vignicola* produced yellow colonies on nutrient broth, catalase-positive, gram-negative rods, and oxidase-negative rods, which run in tandem with the reports of Opara and Abengowe (2020). Upon observation under magnifications of x400, it was noted that the bacterial cells had a single flagellum and were rod-shaped, which are the typical morphological features of bacterial blight pathogen *X. axonopodis* pv. *vignicola*. However, Olatunde et al. (2024) noted that there was a correlation between the colony colour intensity and the virulence of the isolates.

The findings are also in tandem with the observations of Bhagat et al. (2023), who noted that when *X. axonopodis* isolates are grown on nutrient agar media, they produce circular, mucoid, convex, light yellow to typically yellow pigmented colonies with a smooth surface that is opaque in contrast to transmitted light. The morphological and biochemical features of the forty-eight isolates in the current study were in agreement with the observations reported by Fatima et al. (2019), Ferraz et al. (2018) and Ganiyu et al. (2022). Nurcahyanti et al. (2021) reported *X. axonopodis* pv. *vignicola* isolates as yellow in pigment, gram negative and reacting positively to starch hydrolysis, thereby

affirming our findings despite the analysis having been done on nutrient agar media. Anmod et al. (2022) similarly cultured *X. axonopodis* pv. *malvacearum* on nutrient agar media, and despite the pathogen being pathogenic to cotton crops, it displayed similar morphological and biochemical features to *X. axonopodis* pv. *vignicola.* Further, Adila et al. (2021) and Ferraz et al. (2024) reported similar results when characterising *X. axonopodis* pv. *phaseoli* and *X. axonopodis* pv. *eucalyptorum,* respectively. The 48 isolates from the different regions, which showed the typical *X. axonopodis* pv. *vignicola* characteristics, confirm the widespread nature of this pathogen with little differentiation in terms of morphological and biochemical characters, despite the wide variety of areas of origin and the adaptations in the genotype and variety of the host.

5.2.2 Pathogenicity and virulence of *Xanthomonas axonopodis* pv. *vignicola* isolates on susceptible cowpea variety

Of the forty-eight (48) *X. axonopodis* pv. *vignicola* isolates, forty-one (85.42%) of them were virulent to the Ken Kunde cowpea variety, while seven isolates were avirulent and therefore did not ignite the disease symptoms. The bacterial blight symptoms on the susceptible cowpea were observed from the ninth day of inoculation. The symptoms observed were small brownish water-soaked lesions that with time grew and coalesced to produce larger chlorotic patches on the leaves. Yellowing and falling of the lower leaves was also observed. Opara and Abengowe (2020), from their pathogenicity study, noted that *X. axonopodis* pv. *vignicola* caused the susceptible cowpea cultivars to produce disease symptoms after seven days from the inoculation date. The observed disease symptoms include large brown necrotic lesions on the centre and margins of the leaves, with the pathogen infecting the stem as the disease progressed, resulting in cracks, cankers, and the death of leaves, which was mostly manifested through leaf

falling. As the crop matured and produced pods, the pods became infected, generating symptoms such as circular, sunken, and red-brown spots. Similar findings were reported by Lin et al. (2020), who noted that virulent *X. axonopodis* pv. *vignicola* isolates produced disease symptoms on a susceptible crop seven days after inoculation. They documented the disease symptoms as small, irregular, water-soaked brown necrotic lesions on the infected leaves, with the necrotic areas growing in size as the infection progressed. The current results agree with this previous work; however, the *X. axonopodis* pv. *vignicola* isolates in the current study caused the disease symptoms on the 9th day, which indicated the aggressiveness of the isolates from the different regions of Kenya.

Similarly, Madavi (2022) noted the existence of four pathogenic groups of *X. axonopodis* pv. *citri*: highly pathogenic, moderately pathogenic and poorly pathogenic, based on their ability to cause the disease. Members of the *Xanthomonas* genus frequently undergo horizontal transfer of virulence genes responsible for adaptation to the environment as well as acquiring pathogenicity potential (Sharma et al., 2022). The findings could correspond to the reports which were confirmed by Ariute et al. (2022) when they noted the role of pathogenicity islands in the virulence of *X. axonopodis*. Sharma et al. (2022) noted the existence of variation in the pathogenicity of *X. axonopodis* isolates based on their genetic make-up, which can influence the proliferation of the isolates in the various hosts, leading to the isolates expressing avirulence, low and high pathogenicity. The findings in the current study showed a high percentage of virulent *X. axonopodis* pv. *vignicola* isolates, with only a few being poor pathogens on susceptible cowpea plants. These findings are, however, the first to be reported in Kenya on the pathogenicity levels of *X. axonopodis* pv. *vignicola* isolates from the different regions.

5.2.3 Molecular characterization of *Xanthomonas axonopodis* pv. *vignicola* using 16S rDNA

In the current study, the molecular characterisation of the *X. axonopodis* pv. *vignicola* isolates was done using the 16S rDNA technique. When the sequencing was done and the sequences blasted at the NCBI gene bank for comparison with already deposited sequences, it was noted that the percentage identity of *X. axonopodis* pv. *vignicola* isolates ranged between 94% and 99%. The findings from the current study are in tandem with the findings of Bhardwaj (2024), who used a similar technique to identify *Xanthomonas cucurbitae* from cucumber. Similar results had earlier been reported by Singh et al. (2024) when they used the same molecular technique to identify *Xanthomonas campestris* pv. *campestris*. Goncalves et al. (2002) noted that the 16S rDNA molecular technique is highly effective in discriminating different members of *X. axonopodis* when the correct primers are used. Ferraz et al. (2017) and Jyostha (2019) used the similar technique on *X. axonopodis* and *X. campestris*, respectively, and they noted similar findings to the current study.

5.3 Genetic diversity of *Xanthomonas axonopodis* pv. *vignicola* using inter-simple sequence repeat

In the current study we illustrate the analysis of the genetic diversity among the *X. axonopodis* pv. *vignicola* isolates through the inter-simple sequence repeat. The sample sizes varied across the populations, with some populations having significantly more *X. axonopodis* pv. *vignicola* isolates (Kakamega and Makueni with 14) and others having fewer (Meru with 2). A high level of amplification was noted with the use of ISSR molecular markers. Generally, the 48 *X. axonopodis* pv. *vignicola* isolates showed polymorphism in ISSR analysis using six primers. In this study, it was noted through

ISSR analysis that great genetic variation exists among the forty-eight isolates of *X. axonopodis* pv. *vignicola*. Some primers produced more common banding patterns, while others produced patterns crucial for demonstrating polymorphism. Further, the current study clearly demonstrates high levels of genetic variation between the 48 *X. axonopodis* pv. *vignicola* isolates from the six counties in Kenya.

The isolates of *X. axonopodis* pv. *vignicola* showed intraspecific variation due to the production of both unique and locally common bands. An analysis of molecular variance (AMOVA) performed on *X. axonopodis* pv. *vignicola* isolates from six different regions and populations in Kenya showed Kenya showed that among *X. axonopodis* pv. *vignicola* populations, only 5% of the total genetic variance was attributed to differences between the six regions. This indicated that the genetic differences among the populations of *X. axonopodis* pv. *vignicola* isolates are relatively small. Within the populations, a significant 95% of the total genetic variance was attributed to differences within the populations. This suggests that individual populations, rather than different populations, contain the majority of the genetic diversity in the *X. axonopodis* pv. *vignicola* isolates. The higher percentage of polymorphic loci observed in Makueni and Kakamega could be attributed to the high number of sampled sites that turned positive for *X. axonopodis* pv. *vignicola* pathogens. This could also be attributed to variance in the agro-ecological conditions of the sampled sites as well as varying cropping systems. The low percentage of polymorphic loci in Meru County could be attributed to the low number of sampled sites that tested positive for *X. axonopodis* pv. *vignicola* pathogens. Furthermore, these zones are high-altitude areas which are recently expanded regions for cowpea production, indicating the pathogen has only been introduced through seeds. The simple sequence repeat (SSR) primers were used by Duche et al. (2015) to evaluate the genetic diversity of *X.*

axonopodis pv. *vignicola* isolates, and they noted that genetic variation did exist among the isolates. Whereas their analysis used SSR, SSR, the findings are largely similar to the current results. They linked the geographical zone of the isolates to molecular variation that existed.

Nei's genetic identity (I) ranges from 0 to 1, where values closer to 1 indicate populations that are more genetically similar, and those closer to 0 indicate populations that are less genetically similar. In regard to Nei genetic distance, lower values indicate populations are genetically similar, while higher values suggest greater genetic divergence. The low genetic distances between certain populations (e.g., Kakamega and Uasin Gishu) may suggest possible gene flow or historical connections. Generally, high genetic identities suggest possible gene flow or recent common ancestry, which is evident between Kakamega, Uasin Gishu, and Makueni. Similarly, Fatima et al. (2012) used three ISSR primers which were also used in the current study, A-16, A-31 and D-3, to assess the genetic variability among *X. axonopodis* pv. *vignicola* isolates from varied geographical areas. The three ISSR primers revealed the existence of intraspecific variation among the species studied.

The UPGMA dendrogram generated three main clusters; cluster A comprised the highest number (45) of strains, which were from different areas, while cluster B had a single member (MK05). The isolates from Kakamega showed substantial distribution among the clusters, suggesting significant genetic divergence within the Kakamega population, followed by the isolates from Laikipia, which indicated moderate genetic variation, but most of the *X. axonopodis* pv. *vignicola* isolates from Makueni grouped together, indicating low genetic divergence within the Makueni isolates, as also noted by the shorter branching. However, the isolates from the Uasin Gishu cluster tightly,

indicating low genetic divergence. In relation to geographic origin, the dendrogram revealed a strong correlation between genetic clustering and geographic origin. The *X. axonopodis* pv. *vignicola* isolates from the same county tended to form distinct clusters, demonstrating that geographic proximity influences genetic similarity. For example, Kakamega and Laikipia showed more divergent branching, suggesting higher genetic divergence compared to Makueni and Uasin Gishu, with lower divergence and thus higher genetic similarity.

The PCoA generated three main groups of *X. axonopodis* pv. *vignicola* isolates similar to the UPGMA-based dendrogram, and a dissimilarity coefficient of between 0.056 and 1.14 strongly affirms this. Samanta and Mandal et al. (2014) recorded in their UPGMA-based dendrogram four main clusters, with the majority of the strains clustering in one main cluster group. This largely corroborates the current study. They associated the nature of strains' diversity to manual community spread of the pathogens by farmers rather than the location; this can explain the findings in that isolates of a particular geographical region mostly clustered within cluster A. The findings by Kharde et al. (2018) were corroborated in their report on *X. axonopodis* pv. *citri.* The analysis by Sharma et al. (2022) on X. *axonopodis* pv. *puniciae* showed that they clustered into two key groups when their genetic diversity was analysed using SSR markers, with cluster one consisting of five (5) isolates while the second cluster contained 15 isolates based on the UPGMA dendrogram. Despite the differences in the markers, this *X. axonopodis* pathovar showed a similar pattern with one main cluster and few isolates in the other cluster, as also reported in the current report, indicating a tendency of clustering among the isolates of this bacterium. Further, there was no link between the isolates' geographical region and the genetic diversity. This could indicate the pathogen's ability to adapt to wide agro ecological conditions. Similar to the findings from the current

study, there was no strong link between the geographical region and the *X. axonopodis* pv. *vignicola* isolates in the genetic variation despite having been analysed using ISSR markers.

In the current study, the six molecular markers amplified a total of 140 scored bands ranging from 100 bp to 3.5 kb in size, of which 124 bands were polymorphic and sixteen were private and unique. Samanta and Mandal et al. (2014) used five ISSR markers for genetic analysis of forty-three isolates of *X. axonopodis* pv. *vignicola* and noted a total of 91 scored bands ranging from 275 base pairs to 3600 base pairs in size. Fatima et al. (2012) used three ISSR primers which were also used in the current study, A-16, A-31 and D-3, to assess the genetic variability among *X. axonopodis* pv. *vignicola* isolates from varied geographical areas. The three ISSR primers revealed the existence of intraspecific variation among the species studied. They noted that amplification bands ranged in size from 200 bp to 1000 bp with the demonstration of high levels of polymorphism among the analysed isolates. However, the band sizes were 100 bp to 3500 bp in the current research. Further, they noted that there was no association between the diversity of the *X. axonopodis* pv. *vignicola* isolates and the geographical region of origin.

As per the PCoA results, the first axis accounted for 13.14% of the total variation, while the second axis accounted for 23.30%, with an entire influence of 32.74%. The present differences could be due to the number and the difference in the ISSR markers used in the study. In regard to clustering of populations, the Isiolo isolates were grouped together, indicating that populations from Isiolo are genetically similar to each other, similar to the Kakamega *X. axonopodis* pv. *vignicola* isolates. The points are less clustered in Laikipia, indicating more genetic diversity within Laikipia populations. In

the Meru populations, a moderate clustering was observed, suggesting moderate genetic diversity, which also existed in Makueni, indicating genetic similarity within Makueni populations; whereas in Uasin Gishu region, these points are closely clustered, showing high genetic similarity within Uasin Gishu populations. Ullah et al. (2021) analysed the population structure and diversity of *X. axonopodis* isolates in Peshawar, Pakistan, and they noted a very low diversity across regions but high diversity across sampled sites. When they carried out principal coordinate analysis, they noted that isolates from varied regions were grouped together with low overlap across regions. The first factor contributed 11.37% of the difference, while the second factor explained 9.83%, with an entire influence of 21.2%. These findings are in agreement with the results for the current study, where very low variation was attributed to the various locations, while 95% of the variation was attributed to the differences within the population.

5.4 Cross pathogenicity of *Xanthomonas axonopodis* pv. *vignicola* on common beans, lentils, soybean, pigeon peas and green grams

Xanthomonas axonopodis pv. *vignicola* caused infection in all the cowpea varieties tested, including soybeans and lentils. However, it was observed that the green grams and all the common bean varieties were not susceptible to *X. axonopodis* pv. *vignicola*. The pathogenic bacteria, when it was re-isolated from inoculated crops, showed similar typical morphological and cultural characteristics of *X. axonopodis* pv. *vignicola* and the disease symptoms. The cross-infection research findings demonstrated that *X. axonopodis* pv. *vignicola* can cause bacterial disease in some of the crops in the same family. The percentage disease severity was higher in cowpea varieties but lower in the soya and lentils, with severity levels differing significantly ($P \leq 0.001$). In other parts of the world, such as in the findings of Chen et al. (2021), they noted that *X. axonopodis*

pv. *vignicola* has a wide host range capable of undergoing cross-infection of crop members of the same family. Nantale et al. (2023a) observed that *X. axonopodis* pv. *vignicola* was able to infect some of the tested cowpea varieties, while other genotypes were resistant to *X. axonopodis* pv. *vignicola*.

However, in the current study, all the tested cowpea varieties, including the local landrace variety, were susceptible. Although there is congruence in the current study on the host range, which is critical in informing the crop rotation system for disease management by breaking and reducing primary inoculum in the soil. Saha et al. (2022) tested ten cowpea varieties for susceptibility to cowpea bacterial blight disease in Bangladesh. They reported that seven of the ten tested cowpea varieties were susceptible to *X. axonopodis* pv. *vignicola* with an average disease severity of over 50%, while only three genotypes were resistant. Similarly, earlier findings had been reported by Agbicodo et al. (2010), who investigated eleven selected cowpea varieties in Nigeria for their resistance to cowpea bacterial blight. It was observed that all eleven tested varieties were susceptible to cowpea bacterial blight, with the severity varying significantly and ranging from 0.33 to 3 based on the score scale of 0 to 4 used in the study, which to a large extent shows similarity.

Further, Deo-Donne et al. (2018) assessed 31 genotypes of cowpeas for resistance to bacterial blight and reported that 12.9% of the genotypes were susceptible, 64.5% of the genotypes were moderately resistant, and 22.6% of the genotypes were resistant. The disease severity ranged from 1.0 to 3.3 based on the score scale of 0 to 4 employed during the study. Similarly, Durojaye et al. (2019) evaluated 103 cowpea landraces from America, the Philippines, and Africa for their resistance to *X. axonopodis* pv. *vignicola*. They noted that two accessions were resistant to *X. axonopodis* pv. *vignicola,*

while the rest were susceptible, with the variations in susceptibility being highly significant among the tested landraces. They reported a mean disease severity of 2.0 on a disease rating scale of 0-4. Whereas the above studies were testing for the resistance across a wide range of varieties and genotypes of cowpea, our current study only focused on the commonly released or grown varieties and landrace cultivars in the farmers' possession. However, all these showed a high degree of susceptibility, but other legumes such as common bean, pigeon pea and green gram showed resistance bordering on immunity. Lentils (*Lens culinaris* ssp. *culinaris*) and soybeans (*Glycine max*) showed susceptibility, indicating possible cross-infectivity of *X. axonopodis* pv. *vignicola* isolates and perhaps agreeing with the findings of Gena et al. (2009), who reported cross-infectivity with French beans and field beans. In the current study common beans were not susceptible, which makes this report partly not in tandem with Gena et al.'s (2009) report. It is, however, noted that the current host range study could provide the source of the *X. axonopodis* pv. *vignicola* resistance genes in the Fabaceae family. This report may be the first report to study the extent of variation and cross-infectivity of *X. axonopodis* pv. *vignicola* as a pathogen and a major disease of cowpea in Kenya.

5.5 The antibacterial potential of *Bacillus* sp. and selected botanicals against *Xanthomonas axonopodis* pv. *vignicola*

5.5.1 The antibacterial activities of *Bacillus subtilis* and *Bacillus amyloliquefaciens* against *Xanthomonas axonopodis* pv. *vignicola*

Both *B. subtilis* and *B. amyloliquefaciens* showed slightly varying levels of inhibition against *X. axonopodis* pv. *vignicola,* ranging between 38.33% and 87.79%. Both bio-agents displayed a significant increase in inhibition from 24 to 48 hours, with a plateau

effect noted at 72 hours. The findings from the current study are in agreement with the findings of Medeot et al. (2020), who opined that fengycins from *B. amyloliquefaciens* have antibacterial properties against *X. axonopodis,* resulting in alterations of cell wall structure and reduced cell size. Similarly, Ke et al. (2023) and Sampathkumar et al. (2023) noted that *B. amyloliquefaciens* was able to inhibit the growth of *X. citri* through the production of palmitic acid, hydrocarbons, fengycin, bacillomycin, phytosphingosine, phenols and surfactin secondary metabolites in the culture medium. Further, Nurcahyanti et al. (2021) and Widjayanti et al. (2023) did isolate *B. amyloliquefaciens*, *B. subtilis* and *B. siamensis* from soybean phyllosphere and then assessed them for their potential in controlling *X. axonopodis*. They observed that all the *Bacillus* sp. isolates were able to inhibit the growth of *X. axonopodis* under *in vitro* tests.

Though the metabolites were not isolated in the current study, it is widely known that *Bacillus* sp. produces polyketide and lipopeptide metabolites that have a wide range of inhibitory effects against a wide range of plant pathogens, especially bacteria and fungi (Wang et al., 2024). Qian et al. (2020) noted that *B. amyloliquefaciens* have strong inhibitory effects against *X. axonopodis* pathovars*,* with the inhibitory potential being affected by environmental conditions such as the pH, inoculation amounts and the type and amount of growth media. Wang et al. (2022), from their study, noted that *B. amyloliquefaciens* has a wide inhibitory activity against a wide range of pathogenic bacteria and fungi, where they mostly act through inhibiting the production of extracellular enzymes, inducing cell wall damage and releasing cell wall contents.

The report of Nargund et al. (2022), who extracted and purified zinc oxide nanoparticles from culture media of *B. subtilis* and exposed it to *X. oryzae* pv. *oryzae* and *X.*

axonopodis pv. *punicae.* They noted that zinc oxide nanoparticles inhibited the growth of these pathogens with inhibition zones of 12.67 ± 0.24 mm and 23 ± 0.4 mm, respectively. The findings of Nargund et al. (2022) and the current study are consistent with each other because the observed effect by the *B. subtilis* in the current research could be through the same mechanisms.

Similar to our findings, Ambadkar et al. (2015) and Singh et al. (2022) noted the inhibitory activity of *B. subtilis* against *X. axonopodis* pv. *punicae,* causing bacterial blight disease of pomegranate. The biocontrol agent caused inhibition levels of 12.71%. Further, from the current report, the inverted plate method inhibits the bacterial growth through the production of metabolites, which was observed. It has largely been reported by Djatmiko et al. (2022) that the inhibitory activity of the nano-suspension formulation of *B. subtilis* is responsible for the effect against *X. oryzae* pv. *oryzae,* causing bacterial blight disease of rice. Fengycin and lipopeptides are the active inhibitory secondary metabolites produced by *B. subtilis* which act against a wide range of plant pathogens (Gao et al., 2022 & Mahapatra et al., 2022). Similar findings were also reported by Baard et al. (2023). *Bacillus subtilis* produces inhibitory factors, including lipopeptides such as iturins, fengycins, surfactins, and kurstakins; siderophores; gageopeptides; and ribosomal peptides that act against a wide range of plant pathogens (Akinsemolu et al., 2024 and Iqbal et al., 2023).

5.5.2 The antibacterial activity of crude extracts of *Salvia nilotica*, garlic, neem and ginger against *Xanthomonas axonopodis* pv. *vignicola*

The tested plant extracts showed varying levels of inhibition to *X. axonopodis* pv. *vignicola*. The levels of inhibition varied depending on the type of extract and level of concentration. *Xanthomonas axonopodis* pv. *vignicola* was resistant to *S. nilotica*

extracts at both levels of extract concentration but susceptible to garlic, neem and ginger. Chepkwony et al. (2021) noted methanolic leaf extracts of *Salvia nilotica* were active against *Trichophyton mentagrophytes*. In the current study *S. nilotica* had no effect on the growth of *X. axonopodis* pv. *vignicola,* which to a large extent can be due to the differences in the type of microorganism tested.

The inhibitory levels of ciprofloxacin showed a relatively greater effect compared to the tested plant extracts and were significantly different, indicating the inability of these plant extracts to totally inhibit this bacterium. However, the inhibition levels, though not significantly different, corroborate with the findings of Ambadkar et al. (2015), who noted that neem, garlic and ginger inhibited the growth of *X. axonopodis* pv. *punicae*. Neem oil extracts have antibacterial properties against *X. axonopodis* pv. *citri,* responsible for citrus canker disease (Gurav et al., 2022 and Iftikhar et al., 2025). The findings from the current study confirm the research results of Haq et al. (2021), who noted that neem and ginger had antibacterial properties against *Xanthomonas axonopodis* pv. *mangiferaeindicae* and *Agrobacterium tumefaciens*. Neem leaves are rich in bioactive antimicrobial compounds, including azadirachtin, nimbin, quercetin and dihydronimocinol (Adusei et al., 2022).

Tariq et al. (2023) evaluated the antimicrobial activities of garlic and ginger extracts against *X. axonopodis* pv. *citri* and observed that the pathogen was susceptible to garlic, while ginger had no effect on its growth. Their findings on garlic agree with the findings from the current study, but those on ginger differ and may be linked to the difference in pathovars. The allicin from *Allium sativum* is the main active ingredient conferring antibacterial properties against *X. axonopodis* (Wang et al., 2022 & Zhu et al., 2022). Pereira et al. (2025) reported the efficacy of ginger essential oil against *Xanthomonas*

sp., and they noted that the extract was effective against the tested bacterial species, with the α-zingiberene being the active ingredient.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Cowpea bacterial blight was detected in all six counties surveyed, with the highest incidence in Kakamega and lowest in Meru. These differences may reflect variations in agro ecological conditions, cultivar susceptibility, or farm-level disease management.
2. The identity of the pathogen was confirmed as *Xanthomonas axonopodis* pv. *vignicola* using cultural, morphological, biochemical tests, and 16S rDNA molecular analysis. The isolates showed high sequence similarity to reference *X. axonopodis* pv. *vignicola* strains.
3. Although the *Xanthomonas axonopodis* pv. *vignicola* isolates shared many cultural, morphological, and biochemical characteristics, ISSR analysis revealed genetic variability among populations. However, this variability did not correlate strongly with geographical origin but rather to the differences in the individual populations across the regions, suggesting gene flow or shared seed sources across regions.
4. Cross-pathogenicity tests demonstrated that *Xanthomonas axonopodis* pv. *vignicola* can infect soybeans and lentils under controlled conditions, but not common common bean, green gram and garden pea. This has implications for crop rotation practices involving legumes so as to avoid build-up of disease inoculum in the field.
5. *In vitro* assays showed that *Bacillus subtilis* and *B. amyloliquefaciens* were effective in inhibiting *X. axonopodis pv. vignicola*. Among botanicals, ethanolic neeme extracts exhibited the highest antibacterial activity, whereas *S. nilotica* showed

minimal effect.

6.2 Recommendations

1. Agricultural extension services should train farmers on early identification and management of cowpea bacterial blight to reduce spread and crop loss.

2. Given the observed genetic diversity among isolates, routine monitoring and further molecular characterization using high-resolution markers (e.g., SSRs, SNPs) or whole genome approaches is recommended.

3. a) Crop rotation should exclude lentils and soybeans to reduce inoculum carry over between seasons.

    b) Screening of diverse cowpea genotypes for resistance to *X. axonopodis* pv. *vignicola* should be prioritised to identify and deploy resistant cultivars.

4. *Bacillus subtilis* and *B. amyloliquefaciens,* as well as ethanolic extracts of neem, garlic and ginger, can be used as biocontrol agents against *X. axonopodis pv. vignicola*. However, further investigation is recommended to isolate and characterize the active phytochemicals in neem, ginger, and garlic responsible for antibacterial activity, and to validate their efficacy under field conditions.

5. Given their inhibitory potential, the tested bioagents could be developed as safer alternatives to synthetic bactericides, pending field validation and formulation development.

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APPENDICES

Appendix I: Bacterial leaf blight (BLB) field survey questionnaire

Section I: Background information

Farmer ID: ---------------- Name of farmer: ----------------------------- Date: -----/-----/2022 Age: --------Sex: (M) (F) Village: ------------------- Agro-Ecological Zone: -------------------Latitude: ---------- Longitude: ------------------Altitude (m): --------------------

Head of household (M/F): Highest level of education: ----------------------

Section II: Information on production practices

I. How many years have you practiced cowpea production? ----------------------------

II. Area under cowpea production (acres): --

III. Varieties of cowpea grown: ---

IV. Sources of seeds: a) Own b) Neighbour c) Market------------- d) Agro-shop----------

V. Other crops grown on the farm (beans, maize, potatoes, bananas, kales, cabbages) --

VI. What method (s) of field preparation do you practice? -------------------------

VII. Pre-season practices ---

VIII. Do you mix cowpea crop with other crops? (Yes) (No)

IX. If yes, with which crops? ---

X. Do you practice crop rotation in cowpea production? (Yes) (No)

XI. If yes, with what crops? --

XII. Do you use any soil amendments in cowpea production? (Yes) (No)

XIII. If yes, which ones? --

XIV. What are the most common diseases of cowpea in your cowpea field? --

XV. What methods of pest and disease control do you employ? --------------------------

XVI. (a) Do you know this disease? (Show farmer a photo of cowpea leaf showing symptoms of bacterial leaf blight) a) (Yes) (No)

(b) Occurrence- (Presence) or (Absence) in the farm ……………………….

XVII. What method (s) do you use to control it? ---

XVIII. How do you handle the crop residues after harvest? --------------------------------

XIX. Yield per harvest (kg)? --

XX. Disease incidence (%) of Bacterial leaf blight ---

Other observations --

Incidence; Q_1…………… Q_2………………………… Q_3………………………

Appendix II: ANOVA table for the cowpea bacterial blight incidence from the different sites

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Sites	47	12785	272.01	5.516	8.02e-11 ***
Residuals	96	4734	49.31		

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '' 1 - The main effect of Site is statistically significant and large ($F(47, 96) = 5.52$, $p < .001$; Eta2 = 0.73, 95% CI [0.59, 1.00]).

Appendix III. Tukey multiple comparison of means for disease incidence by sampled site with 95% family-wise confidence level

```
$Site
                    diff         lower        upper     p adj
IS02-IS01     4.83742103 -18.72517253  28.40001459 1.0000000
IS04-IS01     3.99540284 -19.56719072  27.55799640 1.0000000
IS05-IS01     7.16766617 -16.39492739  30.73025973 1.0000000
IS06-IS01    17.35215725  -6.21043630  40.91475081 0.5893666
IS06-2-IS01   9.56387547 -13.99871809  33.12646903 0.9999457
IS08-IS01    11.91681665 -11.64577691  35.47941021 0.9944442
IS09-IS01     3.15508912 -20.40750444  26.71768268 1.0000000
KK01-IS01    13.36609201 -10.19650155  36.92868557 0.9666881
KK02-IS01     5.45480732 -18.10778624  29.01740088 1.0000000
KK03-IS01     6.46160330 -17.10099026  30.02419686 1.0000000
KK04-IS01    11.04713384 -12.51545972  34.60972740 0.9986569
KK05-IS01     9.70405237 -13.85854119  33.26664593 0.9999227
KK06-IS01    26.63923215   3.07663859  50.20182571 0.0083403
KK07-IS01    15.85427380  -7.70831975  39.41686736 0.7827919
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KK09-IS01    28.87572191   5.31312835  52.43831547 0.0019401
KK11-IS01    21.33789587  -2.22469769  44.90048943 0.1491254
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KK14-IS01    14.40999095  -9.15260261  37.97258451 0.9147608
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LK04-IS01     3.73851993 -19.82407362  27.30111349 1.0000000
LK05-IS01    -1.85963500 -25.42222856  21.70295856 1.0000000
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MK03-IS01    25.60420980   2.04161624  49.16680336 0.0157148
MK04-IS01    12.53124513 -11.03134843  36.09383869 0.9871561
MK05-IS01    19.18165917  -4.38093439  42.74425273 0.3486022
MK06-IS01    24.93787536   1.37528180  48.50046891 0.0232572
MK07-IS01    17.10799930  -6.45459426  40.67059286 0.6228780
MK08-IS01    16.41640718  -7.14618638  39.97900074 0.7146445
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MK12-IS01     1.84191774 -21.72067582  25.40451129 1.0000000
MK13-IS01    -6.69042310 -30.25301666  16.87217046 1.0000000
MK14-IS01     2.09068950 -21.47190406  25.65328306 1.0000000
MK15-IS01    16.90012476  -6.66246880  40.46271832 0.6510851
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MU02-IS01    -4.94610968 -28.50870324  18.61648388 1.0000000
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LK05-IS02     -6.69705603 -30.25964959  16.86553753 1.0000000
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MK07-IS02     12.27057827 -11.29201529  35.83317183 0.9908605
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MK12-IS02     -2.99550330 -26.55809686  20.56709026 1.0000000
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MU02-IS02     -9.78353071 -33.34612427  13.77906284 0.9999061
UG02-IS02      1.20927784 -22.35331572  24.77187140 1.0000000
UG04-IS02     -9.77666437 -33.33925793  13.78592918 0.9999076
UG05-IS02     -4.31476569 -27.87735925  19.24782786 1.0000000
UG07-IS02     -9.44480852 -33.00740207  14.11778504 0.9999601
UG08-IS02    -13.71794872 -37.28054228   9.84464484 0.9529463
UG09-IS02     -6.05038237 -29.61297593  17.51221119 1.0000000
IS05-IS04      3.17226333 -20.39033023  26.73485689 1.0000000
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IS06-2-IS04    5.56847263 -17.99412093  29.13106619 1.0000000
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IS09-IS04     -0.84031371 -24.40290727  22.72227984 1.0000000
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KK03-IS04      2.46620047 -21.09639309  26.02879403 1.0000000
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KK07-IS04     11.85887097 -11.70372259  35.42146453 0.9949004
KK08-IS04     10.77282813 -12.78976543  34.33542169 0.9991953
KK09-IS04     24.88031908   1.31772552  48.44291264 0.0240433
KK11-IS04     17.34249303  -6.22010053  40.90508659 0.5906979
KK12-IS04      8.33981452 -15.22277904  31.90240808 0.9999986
KK13-IS04      6.69990398 -16.86268958  30.26249754 1.0000000
KK14-IS04     10.41458811 -13.14800544  33.97718167 0.9996087
KK15-IS04     11.34072936 -12.22186420  34.90332292 0.9977575
LK01-IS04      1.34256816 -22.22002540  24.90516172 1.0000000
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LK02-IS04	-7.84705340	-31.40964696	15.71554016	0.9999998
LK04-IS04	-0.25688290	-23.81947646	23.30571066	1.0000000
LK05-IS04	-5.85503783	-29.41763139	17.70755573	1.0000000
MK01-IS04	21.56571211	-1.99688145	45.12830567	0.1345832
MK02-IS04	12.37275986	-11.18983370	35.93535342	0.9895293
MK03-IS04	21.60880696	-1.95378660	45.17140052	0.1319625
MK04-IS04	8.53584229	-15.02675126	32.09843585	0.9999972
MK05-IS04	15.18625633	-8.37633722	38.74884989	0.8524642
MK06-IS04	20.94247252	-2.62012104	44.50506608	0.1772157
MK07-IS04	13.11259647	-10.44999709	36.67519003	0.9745362
MK08-IS04	12.42100434	-11.14158921	35.98359790	0.9888481
MK09-IS04	4.04404966	-19.51854390	27.60664322	1.0000000
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MK13-IS04	-10.68582594	-34.24841950	12.87676762	0.9993208
MK14-IS04	-1.90471333	-25.46730689	21.65788023	1.0000000
MK15-IS04	12.90472193	-10.65787163	36.46731549	0.9798375
MU01-IS04	-1.81720430	-25.37979786	21.74538926	1.0000000
MU02-IS04	-8.94151252	-32.50410608	14.62108104	0.9999902
UG02-IS04	2.05129604	-21.51129752	25.61388960	1.0000000
UG04-IS04	-8.93464618	-32.49723974	14.62794738	0.9999904
UG05-IS04	-3.47274750	-27.03534106	20.08984606	1.0000000
UG07-IS04	-8.60279032	-32.16538388	14.95980324	0.9999966
UG08-IS04	-12.87593052	-36.43852408	10.68666304	0.9804978
UG09-IS04	-5.20836417	-28.77095773	18.35422939	1.0000000
IS06-IS05	10.18449109	-13.37810247	33.74708465	0.9997617
IS06-2-IS05	2.39620930	-21.16638426	25.95880286	1.0000000
IS08-IS05	4.74915048	-18.81344308	28.31174404	1.0000000
IS09-IS05	-4.01257704	-27.57517060	19.55001651	1.0000000
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KK02-IS05	-1.71285885	-25.27545241	21.84973471	1.0000000
KK03-IS05	-0.70606286	-24.26865642	22.85653069	1.0000000
KK04-IS05	3.87946767	-19.68312589	27.44206123	1.0000000
KK05-IS05	2.53638620	-21.02620736	26.09897976	1.0000000
KK06-IS05	19.47156598	-4.09102758	43.03415954	0.3152842
KK07-IS05	8.68660764	-14.87598592	32.24920120	0.9999955
KK08-IS05	7.60056480	-15.96202876	31.16315836	0.9999999
KK09-IS05	21.70805575	-1.85453781	45.27064931	0.1260811
KK11-IS05	14.17022970	-9.39236386	37.73282326	0.9297781
KK12-IS05	5.16755119	-18.39504237	28.73014475	1.0000000
KK13-IS05	3.52764065	-20.03495291	27.09023421	1.0000000
KK14-IS05	7.24232478	-16.32026878	30.80491834	1.0000000
KK15-IS05	8.16846603	-15.39412753	31.73105959	0.9999992
LK01-IS05	-1.82969517	-25.39228873	21.73289838	1.0000000
LK02-IS05	-11.01931673	-34.58191029	12.54327683	0.9987230
LK04-IS05	-3.42914623	-26.99173979	20.13344733	1.0000000
LK05-IS05	-9.02730116	-32.58989472	14.53529239	0.9999874
MK01-IS05	18.39344878	-5.16914478	41.95604234	0.4475149
MK02-IS05	9.20049653	-14.36209703	32.76309008	0.9999794
MK03-IS05	18.43654363	-5.12604993	41.99913719	0.4418439
MK04-IS05	5.36357896	-18.19901460	28.92617252	1.0000000
MK05-IS05	12.01399300	-11.54860056	35.57658656	0.9936028
MK06-IS05	17.77020919	-5.79238437	41.33280275	0.5317360
MK07-IS05	9.94033314	-13.62226042	33.50292670	0.9998634
MK08-IS05	9.24874101	-14.31385254	32.81133457	0.9999765
MK09-IS05	0.87178633	-22.69080723	24.43437989	1.0000000
MK10-IS05	-0.43090990	-23.99350346	23.13168366	1.0000000
MK12-IS05	-5.32574843	-28.88834199	18.23684513	1.0000000
MK13-IS05	-13.85808927	-37.42068283	9.70450429	0.9464614
MK14-IS05	-5.07697666	-28.63957022	18.48561690	1.0000000
MK15-IS05	9.73245860	-13.83013496	33.29505215	0.9999171
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MU02-IS05	-12.11377585	-35.67636941	11.44881771	0.9926313
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UG04-IS05	-12.10690951	-35.66950307	11.45568405	0.9927018
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UG07-IS05	-11.77505365	-35.33764721	11.78753991	0.9955044
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KK04-IS06     -6.30502341 -29.86761697  17.25757014 1.0000000
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KK07-IS06     -1.49788345 -25.06047701  22.06471011 1.0000000
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LK04-IS06    -13.61363732 -37.17623088   9.94895624 0.9573888
LK05-IS06    -19.21179225 -42.77438581   4.35080131 0.3450539
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MK02-IS06     -0.98399456 -24.54658812  22.57859900 1.0000000
MK03-IS06      8.25205255 -15.31054101  31.81464610 0.9999989
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MK05-IS06      1.82950192 -21.73309164  25.39209547 1.0000000
MK06-IS06      7.58571810 -15.97687546  31.14831166 0.9999999
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MK08-IS06     -0.93575007 -24.49834363  22.62684349 1.0000000
MK09-IS06     -9.31270476 -32.87529832  14.24988880 0.9999720
MK10-IS06    -10.61540099 -34.17799455  12.94719257 0.9994094
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MK13-IS06    -24.04258036 -47.60517392  -0.47998680 0.0385498
MK14-IS06    -15.26146775 -38.82406131   8.30112581 0.8453268
MK15-IS06     -0.45203249 -24.01462605  23.11056107 1.0000000
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MU02-IS06    -22.29826694 -45.86086049   1.26432662 0.0953192
UG02-IS06    -11.30545838 -34.86805194  12.25713518 0.9978875
UG04-IS06    -22.29140060 -45.85399415   1.27119296 0.0956377
UG05-IS06    -16.82950192 -40.39209547   6.73309164 0.6605697
UG07-IS06    -21.95954474 -45.52213830   1.60304882 0.1121142
UG08-IS06    -26.23268494 -49.79527850  -2.67009138 0.0107332
UG09-IS06    -18.56511859 -42.12771215   4.99747497 0.4250804
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KK03-IS06-2   -3.10227217 -26.66486573  20.46032139 1.0000000
KK04-IS06-2    1.48325837 -22.07933519  25.04585193 1.0000000
KK05-IS06-2    0.14017690 -23.42241666  23.70277046 1.0000000
KK06-IS06-2   17.07535668  -6.48723688  40.63795024 0.6273318
KK07-IS06-2    6.29039833 -17.27219522  29.85299189 1.0000000
KK08-IS06-2    5.20435549 -18.35823806  28.76694905 1.0000000
KK09-IS06-2   19.31184644  -4.25074712  42.87444000 0.3334117
KK11-IS06-2   11.77402040 -11.78857316  35.33661396 0.9955115
KK12-IS06-2    2.77134188 -20.79125167  26.33393544 1.0000000
KK13-IS06-2    1.13143134 -22.43116222  24.69402490 1.0000000
KK14-IS06-2    4.84611548 -18.71647808  28.40870904 1.0000000
KK15-IS06-2    5.77225673 -17.79033683  29.33485029 1.0000000
LK01-IS06-2   -4.22590448 -27.78849804  19.33668908 1.0000000
LK02-IS06-2  -13.41552603 -36.97811959  10.14706753 0.9649657
LK04-IS06-2   -5.82535554 -29.38794909  17.73723802 1.0000000
LK05-IS06-2  -11.42351047 -34.98610403  12.13908309 0.9974251
MK01-IS06-2   15.99723948  -7.56535408  39.55983303 0.7661834
MK02-IS06-2    6.80428722 -16.75830634  30.36688078 1.0000000
MK03-IS06-2   16.04033433  -7.52225923  39.60292789 0.7610731
MK04-IS06-2    2.96736966 -20.59522390  26.52996322 1.0000000
MK05-IS06-2    9.61778370 -13.94480986  33.18037726 0.9999377
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Appendix IV: ANOVA table for the cowpea bacterial blight disease incidence by county

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
County	5	1978	395.7	7.278	5.49e-05 ***
Residuals	42	2283	54.4		

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '' 1. - The main effect of County is statistically significant and large ($F(5, 42) = 7.28$, $p < .001$; Eta2 = 0.46, 95% CI [0.23, 1.00]).

Appendix V: Multiple comparison of mean disease incidence among the counties

County	**Difference**	**Lower**	**Upper**	**p adjustment**
Kakamega-Isiolo	7.4861386	-2.26897649	17.2412538	0.2205261
Laikipia-Isiolo	-6.4072522	-19.88588074	7.0713763	0.7154579
Makueni-Isiolo	6.0819601	-3.67315503	15.8370752	0.4394316
Meru-Isiolo	-8.6325091	-26.03334373	8.7683254	0.6778986
Uasin Gishu-Isiolo	-9.4270145	-21.31404752	2.4600185	0.1911562
Laikipia-Kakamega	-13.8933909	-26.37217606	-1.4146057	0.0213057
Makueni-Kakamega	-1.4041785	-9.72336867	6.9150116	0.9957559
Meru-Kakamega	-16.1186478	-32.75702804	0.5197325	0.0624116
Uasin Gishu-Kakamega	-16.9131531	-27.65318142	-6.1731249	0.0003805
Makueni-Laikipia	12.4892123	0.01042712	24.9679975	0.0497001
Meru-Laikipia	-2.2252569	-21.28691616	16.8364023	0.9992719
Uasin Gishu-Laikipia	-3.0197623	-17.22748423	11.1879597	0.9877099
Meru-Makueni	-14.7144692	-31.35284949	1.9239110	0.1099613
Uasin Gishu-Makueni	-15.5089746	-26.24900287	-4.7689463	0.0012722
Uasin Gishu-Meru	-0.7945054	-18.76601005	17.1769993	0.9999940

Appendix VI: Per cent identity of *Xanthomonas axonopodis pv. vignicola* isolates

Isolate	Per cent identity
IS01	99.75
IS02	99.90
IS04	99.55
IS05	98.35
IS06	97.55
IS06-2	96.85
IS09	98.97
IS08	99.50
KK01	99.65
KK02	96.83
KK03	95.90
KK04	94.99
KK05	98.75
KK06	95.65
KK07	99.85
KK08	95.89
KK09	99.99
KK10	97.78
KK12	98.20
KK13	99.35
KK14	98.45
KK15	97.55
LK01	99.87
LK02	99.65
LK04	95.89
LK05	97.75
MU01	99.95
MU02	98.56
MK01	97.80
MK02	97.50
MK03	99.25
MK04	98.75
MK05	99.56
MK06	97.25
MK07	98.80
MK08	99.95
MK09	95.88
MK10	99.85
MK12	95.75
MK13	99.50
MK14	97.56
MK15	96.75
UG02	97.72
UG04	99.98
UG05	96.60
UG07	99.59
UG08	99.54
UG09	95.55

Appendix VII: Summary of analysis of molecular variance (AMOVA)

Source	df	SS	MS	Est. Var.	%
Among populations	5	3.060	0.612	0.024	5%
Within populations	42	18.190	0.433	0.433	95%
Total	47	21.250		0.457	100%

Key: df-degrees of freedom; SS-sum of squares; MS-mean squares; Est.Var.-estimated variance and %- percentage of molecular variance.

Appendix VIII: The *Xanthomonas axonopodis* pv. *vignicola* diversity matrix data

```
Output: NTSYSpc 2.10e, (C) 2000, Applied Biostatistics Inc.
Date & time: 31/05/2024 09:59:18
----------------------------------------
Input parameters
Read input from file: C:\Users\kirarei\Documents\ISSR 33.NTS
Format: width=9 decimals=4
Page width: 80
Field width: 9
Decimal places: 4
Page width: 80
Comments:
  SIMGEND: input=C:\Users\kirarei\Documents\ISSR 32.NTS, coeff=NEI72, dir=Cols
Matrix type = 2, size = 48 by 48, missing value code = "none"(dissimilarity)
```

	IS01	KK02	MK08	IS02	KK01	IS04	KK09	KK06
IS01	0.0000							
KK02	0.8524	0.0000						
MK08	0.7085	0.3262	0.0000					
IS02	0.9639	0.8047	0.4377	0.0000				
KK01	0.9051	1.1513	0.6020	0.5697	0.0000			
IS04	0.9113	0.7520	0.6082	0.3040	0.2939	0.0000		
KK09	0.6174	1.1513	0.6020	0.5697	0.9163	0.5170	0.0000	
KK06	0.7885	0.8524	0.7085	0.5585	1.3105	0.9113	0.6174	0.0000
MK05	0.9639	1.0924	0.6609	0.9163	1.2629	1.5568	1.9560	0.7408
KK04	0.8524	0.2877	0.3262	0.8047	1.1513	1.0397	1.1513	1.1401
IS05	0.6881	1.0397	0.6082	0.3040	0.2939	0.2513	0.8047	0.9113
MK04	0.6292	0.4700	0.3262	0.8047	0.7458	1.0397	0.7458	0.8524
KK05	1.0733	0.9141	0.4825	0.5148	0.6791	0.4621	0.6791	0.7856
IS06	0.7935	0.6343	0.6727	0.7458	0.8047	0.8755	1.0924	0.6393
MK09	0.7085	0.8370	0.4055	0.6609	0.6020	0.8959	1.0075	0.4854
MK03	1.1989	1.7329	0.8959	1.1513	0.8047	1.0986	1.4979	1.8921
MK06	1.3105	0.7458	1.0075	1.9560	0.0000	0.0000	1.6094	0.6174
MK13	0.9962	0.8370	1.0986	0.9486	1.7006	1.3013	0.6020	0.7085
MK01	0.7935	0.6343	0.4904	0.7458	0.8047	0.6931	0.8047	0.7935
ISO8	0.5355	0.7128	0.7921	0.6420	0.9886	0.7716	0.4778	0.5355
KK08	1.0116	0.8524	0.7085	0.5585	0.6174	0.6881	0.9051	0.7885
KK07	0.6496	0.3363	0.3466	0.6020	0.4377	0.3952	0.6609	0.4955
MK14	1.4017	0.8370	0.6931	0.6609	1.7006	0.8959	1.0075	0.9962
KK03	0.7085	1.2425	1.0986	0.9486	0.6020	0.8959	1.0075	1.4017
KK12	1.6044	1.0397	1.3013	0.4581	1.2102	0.8109	1.9033	0.9113
KK13	1.0551	0.4904	0.3466	1.0075	1.3540	1.6479	0.0000	1.0551
KK10	0.9113	0.7520	0.6082	0.4581	0.8047	0.8109	0.8047	0.3516
IS06-2	0.6496	0.6727	0.7520	0.4478	0.4377	0.2616	0.4377	0.6496
MK07	1.1401	0.9808	1.2425	0.8047	1.8444	1.0397	0.7458	0.4469
MK15	1.2516	0.8047	0.6609	0.6931	0.8574	0.8636	1.2629	0.5585
IS09	0.6174	0.7458	0.6020	0.5697	0.5108	0.5170	0.5108	1.3105
UG02	0.4955	0.4904	0.3466	0.4478	0.9486	0.7316	0.4377	0.3620
UG04	0.9962	0.3262	0.4055	0.6609	1.7006	0.8959	1.7006	0.7085
UG05	0.7408	0.5816	0.4377	0.3567	0.5697	0.6405	0.5697	0.5585
UG07	1.3105	0.7458	1.0075	0.8574	1.6094	0.8047	0.9163	1.3105

UG08	0.7085	0.8370	1.0986	1.3540	1.0075	1.3013	1.7006	0.7085
UG09	1.5455	0.6931	1.2425	1.4979	1.1513	1.4452	0.0000	0.0000
MK02	1.1401	0.9808	1.2425	1.4979	0.0000	0.0000	1.8444	0.8524
MK10	0.6393	0.8166	0.4904	0.4581	0.8047	0.5390	0.5816	0.6393
MK12	1.0116	0.6292	0.4854	0.5585	0.6174	0.6881	0.6174	0.7885
KK14	0.6897	0.5304	0.5689	0.6420	0.7009	0.4352	0.7009	0.6897
KK15	0.7085	0.5493	0.4055	0.6609	0.6020	0.6082	1.0075	0.4854
LK01	0.9113	0.7520	0.8959	0.6405	1.2102	0.5878	0.8047	0.5058
LK02	0.9090	0.7498	0.6060	0.5249	1.0256	0.6264	1.0256	0.4390
LK04	0.8720	0.7128	0.7921	1.0475	0.7009	0.7716	1.3940	0.6897
LK05	0.8524	1.3863	1.2425	0.5816	1.1513	1.0397	0.7458	0.4469
MU01	0.8320	0.6727	0.7520	0.4478	1.3540	0.9548	0.6609	0.2442
MU02	0.8320	0.6727	0.7520	0.6020	0.6609	0.3952	0.6609	0.6496

	MK05	KK04	IS05	MK04	KK05	IS06	MK09	MK03
MK05	0.0000							
KK04	0.8047	0.0000						
IS05	0.8636	0.7520	0.0000					
MK04	0.8047	0.4700	0.7520	0.0000				
KK05	1.0256	0.9141	0.4621	0.6264	0.0000			
IS06	0.9281	0.6343	0.6931	0.4801	0.5675	0.0000		
MK09	0.6609	0.8370	0.6082	0.3262	0.4825	0.4904	0.0000	
MK03	1.1513	1.7329	1.0986	1.0397	0.9730	0.9808	0.8959	0.0000
MK06	1.2629	0.7458	1.9033	0.4581	1.0845	0.8047	0.6020	0.0000
MK13	2.0472	1.2425	1.9945	0.8370	0.7702	0.4904	1.0986	1.5890
MK01	1.1513	1.0397	1.0986	1.0397	0.5675	0.6931	0.8959	0.6931
ISO8	1.3352	0.9359	0.9948	0.7128	0.8691	0.8770	1.0797	0.8770
KK08	0.9639	0.8524	0.5058	0.6292	1.0733	0.7935	0.7085	0.7935
KK07	1.0075	0.6727	0.5493	0.4904	0.4236	0.4315	0.3466	0.8370
MK14	1.3540	0.8370	0.8959	0.8370	0.2594	0.6727	0.6931	0.8959
KK03	1.3540	0.8370	0.6082	0.8370	1.1757	0.8959	0.6931	1.5890
KK12	1.5568	1.4452	1.0986	2.1383	0.9730	0.6931	1.3013	1.0986
KK13	0.6020	0.4904	0.9548	0.4904	0.8291	0.8370	0.3466	1.2425
KK10	0.8636	1.0397	0.8109	0.7520	0.4621	0.4055	0.3851	0.6931
IS06-2	1.0075	0.6727	0.3952	0.6727	0.6060	0.5493	0.7520	1.2425
MK07	1.4979	0.9808	1.4452	0.9808	1.3195	0.6343	0.8370	0.0000
MK15	0.9163	1.0924	0.8636	0.8047	0.7380	0.4581	0.6609	0.7458
IS09	1.2629	0.7458	0.5170	0.4581	1.0845	1.0924	1.0075	0.8047
UG02	0.7843	0.6727	0.7316	0.4904	0.6060	0.5493	0.5289	0.5493
UG04	0.9486	0.5493	0.8959	0.8370	0.4825	0.6727	0.6931	1.5890
UG05	1.2040	0.8047	0.6405	0.5816	0.5148	0.3404	0.4377	0.7458
UG07	1.9560	0.7458	1.2102	0.7458	0.3914	0.8047	1.0075	1.4979
UG08	1.3540	1.2425	1.3013	0.8370	1.1757	0.4904	0.4055	0.8959
UG09	1.4979	0.6931	1.4452	0.6931	1.3195	1.0397	1.2425	1.0397
MK02	1.0924	0.6931	1.4452	0.6931	1.3195	0.4801	0.8370	0.0000
MK10	0.9281	0.6343	0.4055	0.4801	0.5675	0.3747	0.4904	0.9808
MK12	1.2516	0.6292	0.6881	0.4469	0.5625	0.6393	0.4854	0.7935
KK14	1.3352	0.9359	0.7716	0.7128	0.6460	0.5893	0.5689	0.5893
KK15	0.9486	0.8370	0.8959	0.8370	0.7702	0.4904	0.4055	0.8959
LK01	1.5568	1.4452	1.0986	1.4452	1.3784	0.5390	0.8959	1.0986
LK02	0.8614	0.9730	0.6264	0.9730	0.6830	0.7599	0.6060	0.9141
LK04	1.0475	1.2236	0.9948	0.7128	1.5623	0.7229	0.7921	0.5893
LK05	0.8047	0.9808	0.5289	0.6931	0.9141	0.6343	0.5493	1.7329
MU01	1.0075	0.8959	0.9548	0.6727	0.8291	0.4315	0.5289	1.9356
MU02	1.2951	1.1836	0.7316	1.1836	1.1168	0.8370	1.0397	0.8370

	MK06	MK13	MK01	ISO8	KK08	KK07	MK14	KK03
MK06	0.0000							
MK13	1.0075	0.0000						
MK01	0.0000	0.4904	0.0000					
ISO8	0.9886	0.5689	0.5893	0.0000				
KK08	1.3105	1.4017	1.1989	0.6897	0.0000			
KK07	0.9486	0.7520	0.5493	0.5790	0.6496	0.0000		
MK14	1.0075	1.0986	0.8959	1.0797	1.4017	0.7520	0.0000	
KK03	1.7006	1.7918	1.5890	1.0797	0.9962	0.7520	1.0986	0.0000
KK12	0.0000	0.8959	0.6931	0.9948	1.6044	0.9548	0.6082	1.3013
KK13	0.6609	0.0000	1.2425	1.8318	1.0551	0.6931	0.7520	1.4452
KK10	1.2102	0.6082	0.4055	0.5893	0.9113	0.3952	0.3851	0.8959
IS06-2	1.3540	0.7520	0.8370	0.4455	0.6496	0.4055	1.0397	0.5289
MK07	0.7458	0.5493	1.7329	0.9359	0.8524	0.8959	1.2425	1.2425
MK15	1.9560	0.9486	0.4581	1.0475	0.7408	0.6020	0.6609	1.3540
IS09	1.6094	1.0075	0.8047	0.4778	0.3942	0.9486	1.7006	1.0075
UG02	0.9486	0.7520	0.5493	0.3277	0.6496	0.2877	0.5289	1.0397
UG04	1.0075	1.0986	0.4904	1.0797	1.4017	0.5289	0.4055	1.7918
UG05	1.2629	0.4377	0.4581	0.6420	0.7408	0.3143	0.4377	0.6609

```
UG07   |  0.9163   0.6020   0.8047   0.7009   1.3105   0.9486   0.3143   1.0075
UG08   |  1.0075   1.0986   0.8959   1.0797   1.4017   0.5289   0.6931   0.6931
UG09   |  1.1513   1.2425   1.0397   0.9359   0.8524   1.5890   1.2425   1.2425
MK02   |  0.2350   0.8370   0.0000   1.2236   1.1401   1.1836   1.2425   1.2425
MK10   |  1.0924   1.1836   0.9808   0.8770   0.6393   0.5493   0.4904   0.6727
MK12   |  0.9051   0.7085   0.7935   0.5355   0.3185   0.3620   0.7085   0.7085
KK14   |  1.3940   0.7921   0.5893   0.6190   0.8720   0.2223   0.5689   0.7921
KK15   |  1.7006   0.6931   0.2027   0.7921   0.9962   0.3466   1.0986   1.0986
LK01   |  1.9033   0.6082   0.6931   0.9948   1.1989   0.5493   1.3013   1.9945
LK02   |  1.4311   1.1168   0.6264   0.8102   0.5726   0.4825   0.8291   1.5223
LK04   |  1.3940   1.4852   0.8770   0.7732   0.6897   0.4455   1.4852   1.0797
LK05   |  0.7458   0.8370   1.7329   0.7128   0.6292   0.8959   1.2425   0.8370
MU01   |  0.6609   0.3466   0.8370   0.5790   0.8320   0.5390   1.0397   1.4452
MU02   |  0.0000   1.0397   0.5493   0.7332   0.6496   0.5390   1.0397   1.0397

          KK12     KK13     KK10     IS06-2   MK07     MK15     IS09     UG02
       --------------------------------------------------------------------------
KK12   |  0.0000
KK13   |  1.6479   0.0000
KK10   |  0.4055   0.9548   0.0000
IS06-2 |  0.9548   1.7918   0.5493   0.0000
MK07   |  1.0397   1.5890   1.0397   0.6727   0.0000
MK15   |  0.6405   1.0075   0.3040   1.0075   1.0924   0.0000
IS09   |  1.9033   1.3540   1.2102   0.4377   1.1513   1.2629   0.0000
UG02   |  0.7316   0.6931   0.2616   0.5390   0.8959   0.6020   0.6609   0.0000
UG04   |  0.6082   0.3466   0.6082   1.4452   1.2425   0.6609   1.7006   0.5289
UG05   |  0.4581   1.0075   0.1705   0.6020   0.8047   0.3567   0.8574   0.3143
UG07   |  0.8047   1.3540   0.8047   0.6609   0.7458   1.2629   0.9163   0.9486
UG08   |  0.6082   0.7520   0.3851   1.0397   0.8370   0.6609   1.7006   0.5289
UG09   |  1.4452   0.8959   1.4452   0.8959   1.3863   1.4979   0.4581   1.5890
MK02   |  2.1383   0.8959   1.4452   1.1836   0.6931   2.1910   1.8444   1.1836
MK10   |  0.8755   0.8370   0.5390   0.5493   0.8166   0.4581   0.8047   0.4315
MK12   |  1.1989   1.0551   0.5058   0.4955   0.8524   0.5585   0.6174   0.4955
KK14   |  0.4352   1.1386   0.4352   0.5790   0.9359   0.6420   0.9886   0.3277
KK15   |  0.6082   0.7520   0.3851   0.7520   0.8370   0.4377   1.0075   0.5289
LK01   |  0.5878   1.6479   0.5878   0.7316   0.5289   0.6405   1.2102   0.5493
LK02   |  0.8087   0.7702   0.6264   0.9525   0.7498   0.5249   1.0256   0.4825
LK04   |  0.9948   1.1386   0.7716   0.9155   1.2236   0.4879   0.9886   0.5790
LK05   |  1.4452   1.5890   0.5289   0.3363   0.6931   1.0924   0.7458   0.6727
MU01   |  0.7316   1.0986   0.3952   0.5390   0.3363   0.7843   0.9486   0.4055
MU02   |  0.7316   1.7918   0.5493   0.5390   0.8959   0.3143   0.6609   0.5390

          UG04     UG05     UG07     UG08     UG09     MK02     MK10     MK12
       --------------------------------------------------------------------------
UG04   |  0.0000
UG05   |  0.6609   0.0000
UG07   |  0.6020   0.8574   0.0000
UG08   |  0.6931   0.4377   1.0075   0.0000
UG09   |  1.2425   1.4979   0.4581   1.2425   0.0000
MK02   |  1.2425   1.0924   1.1513   1.2425   1.3863   0.0000
MK10   |  0.6727   0.4581   0.8047   0.6727   1.7329   0.8166   0.0000
MK12   |  0.9962   0.4043   0.6174   0.9962   0.8524   1.1401   0.5058   0.0000
KK14   |  0.5689   0.3543   0.7009   0.3866   1.6290   1.6290   0.4715   0.5355
KK15   |  0.4055   0.4377   1.0075   0.4055   1.2425   1.9356   0.6727   0.7085
LK01   |  0.8959   0.4581   1.9033   0.6082   0.0000   1.4452   0.6931   1.1989
LK02   |  0.4236   0.6791   1.0256   0.8291   1.6661   1.6661   0.6264   0.7267
LK04   |  1.0797   0.6420   2.0872   0.5689   1.6290   1.6290   0.7229   0.8720
LK05   |  1.9356   0.8047   1.1513   1.2425   1.3863   0.6931   0.6343   0.6292
MU01   |  0.7520   0.4478   0.9486   0.7520   1.5890   0.6727   0.6828   0.6496
MU02   |  1.0397   0.4478   1.3540   0.7520   1.5890   0.0000   0.5493   0.6496

          KK14     KK15     LK01     LK02     LK04     LK05     MU01     MU02
       --------------------------------------------------------------------------
KK14   |  0.0000
KK15   |  0.3866   0.0000
LK01   |  0.4352   0.3851   0.0000
LK02   |  0.5226   0.4236   0.6264   0.0000
LK04   |  0.3677   0.5689   0.5893   0.6561   0.0000
LK05   |  1.2236   1.2425   1.0397   0.9730   1.6290   0.0000
MU01   |  0.7332   0.5289   0.3952   0.6161   1.1386   0.3363   0.0000
MU02   |  0.4455   0.5289   0.3952   0.4825   0.4455   1.1836   0.8755   0.0000

Ending date & time: 31/05/2024 09:59:19
```

Appendix IX: ANOVA table for the tested crops bacterial blight disease severity levels

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Crop	6	2138.2	356.4	16.93	1.1e-05 ***
Residuals	14	294.8	21.1		

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 - The main effect of crop is statistically significant and large ($F(6, 14) = 16.92$, $p < .001$; Eta2 = 0.88, 95% CI [0.73, 1.00]).

Appendix X: Multiple comparison of mean disease severity levels of the tested legume crops

Crop	Difference	Lower	Upper	p adjustment
Cowpea Brown-Bumala Brown	-2.5000000	-15.293402	10.293402	0.9925218
Ken Kunde-Bumala Brown	-3.3333333	-16.126736	9.460069	0.9683517
Lentils-Bumala Brown	-23.3333333	-36.126736	-10.539931	0.0003448
Local cowpea-Bumala Brown	2.5000000	-10.293402	15.293402	0.9925218
M66-Bumala Brown	-1.2500000	-14.043402	11.543402	0.9998437
Soya-Bumala Brown	-22.5000000	-35.293402	-9.706598	0.0004990
Ken Kunde-Cowpea Brown	-0.8333333	-13.626736	11.960069	0.9999855
Lentils-Cowpea Brown	-20.8333333	-33.626736	-8.039931	0.0010633
Local cowpea-Cowpea Brown	5.0000000	-7.793402	17.793402	0.8253278
M66-Cowpea Brown	1.2500000	-11.543402	14.043402	0.9998437
Soya-Cowpea Brown	-20.0000000	-32.793402	-7.206598	0.0015649
Lentils-Ken Kunde	-20.0000000	-32.793402	-7.206598	0.0015649
Local cowpea-Ken Kunde	5.8333333	-6.960069	18.626736	0.7091756
M66-Ken Kunde	2.0833333	-10.710069	14.876736	0.9971868
Soya-Ken Kunde	-19.1666667	-31.960069	-6.373264	0.0023144
Local cowpea-Lentils	25.8333333	13.0399313	8.626736	0.0001179
M66-Lentils	22.0833333	9.289931	34.876736	0.0006017
Soya-Lentils	0.8333333	-11.960069	13.626736	0.9999855
M66-Local cowpea	-3.7500000	-16.543402	9.043402	0.9455400
Soya-Local cowpea	25.0000000	-37.793402	-12.206598	0.0001676
Soya-M66	-21.2500000	-34.043402	-8.456598	0.0008783

Appendix XI. ANOVA for *Bacillus subtilis* inhibition by the dual culture and inverted plate methods

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Time	2	2118	1059.3	11.70	0.001030**
Method	1	2240	2239.6	24.74	0.000204***
Residuals	14	1268	90.5		

Significant codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘’ 1 - The main effect of time is statistically significant and large (F (2, 14) = 11.70, p = 0.001; Eta2 (partial) = 0.63, 95% CI [0.29, 1.00]). The main effect of method is statistically significant and large (F (1, 14) = 24.74, p < .001; Eta2 (partial) = 0.64, 95% CI [0.34, 1.00]).

Appendix XII: Tukey multiple comparison of means for *Bacillus subtilis* inhibition of *Xanthomonas axonopodis* pv. *vignicola*

Time (hours)	Difference	Lower	Upper	p adjustment
48-24	16.574074	2.196195	30.95195	0.0235334
72-24	26.275946	11.898067	40.65383	0.0007977
72-48	9.701872	-4.676007	24.07975	0.2166151
Method	difference	lower	upper	p adjustment
Inverted-Dual	-22.30905	-31.92923	-12.68887	0.0002042

Appendix XIII: ANOVA table for the interaction of time of exposure and method of screening in the inhibition of *Xanthomonas axonopodis* pv. *vignicola* by B. subtilis.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Time	2	2118	1059.3	11.314	0.001030**
Method	1	2240	2239.6	23.922	0.000204***
Time: Method	2	144	72.0	0.769	0.485010
Residuals	12	1124	93.6		

Significant codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘’ 1 - The main effect of time is statistically significant and large (F (2, 12) = 11.31, p = 0.002; Eta2 (partial) = 0.65, 95% CI [0.29, 1.00]). - The main effect of method is statistically significant and large (F (1, 12) = 23.92, p < .001; Eta2 (partial) = 0.67, 95% CI [0.34, 1.00]). - The interaction between time and method is statistically not significant and medium (F (2, 12) = 0.77, p = 0.485; Eta2 (partial) = 0.11, 95% CI [0.00, 1.00]).

Appendix XIV: ANOVA table for *Bacillus amyloliquefaciens* percent inhibition through both dual culture and inverted plate methods

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Time	2	2960.5	1480.2	13.631	0.000518***
Method	1	2.5	2.5	0.023	0.882291
Residuals	14	1520.3	108.6		

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '' 1 - The main effect of time is statistically significant and large (F (2, 14) = 13.63, p < .001; Eta2 (partial) = 0.66, 95% CI [0.34, 1.00]). - The main effect of method is statistically not significant and very small (F (1, 14) = 0.02, p = 0.882; Eta2 (partial) = 1.62e-03, 95% CI [0.00, 1.00]).

Appendix XV: Tukey multiple comparison of means for *Bacillus amyloliquefaciens* percentage inhibition

Time (hours)	Difference	Lower	Upper	p adjustment
48-24	23.055556	7.309078	38.80203	0.0048719
72-24	30.006105	14.259627	45.75258	0.0005467
72-48	6.950549	-8.795929	22.69703	0.4976934
Method	difference	lower	upper	p adjustment
Inverted-Dual	0.7407407	-9.795159	-11.27664	0.8822908

Appendix XVI: ANOVA table for the interaction of time of exposure and method of screening in the inhibition of *Xanthomonas axonopodis* pv. *vignicola* by *Bacillus amyloliquefaciens*

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Time	2	2960.5	1480.2	11.72	0.00151**
Method	1	2.5	2.5	0.02	0.89111
Time: Method	2	4.9	2.5	0.02	0.98067
Residuals	12	1515.3	126.3		

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '' 1 - The main effect of time is statistically significant and large (F (2, 12) = 11.72, p = 0.002; Eta2 (partial) = 0.66, 95% CI [0.31, 1.00]). - The main effect of method is statistically not significant and very small (F (1, 12) = 0.02, p = 0.891; Eta2 (partial) = 1.63e-03, 95% CI [0.00, 1.00]). - The interaction between Time and Method is statistically not significant and very small (F (2, 12) = 0.02, p = 0.981; Eta2 (partial) = 3.25e-03, 95% CI [0.00, 1.00])

Appendix XVII: ANOVA table for the inhibition of *Xanthomonas axonopodis* pv. *vignicola* by the different botanicals at concentration level of 12.5 mg/ml

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Extracts	4	5078	1269.4	369.7	8.15e-11 ***
Residuals	10	34	3.4		

Significant codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1->The ANOVA suggests that: - The main effect of extracts is statistically significant and large (F(4, 10) =369.74, p < .001; Eta2 = 0.99, 95% CI [0.98, 1.00])

Appendix XVIII: Tukey multiple comparison of means for the action of the different botanical extracts against the growth of *Xanthomonas axonopodis* pv. *vignicola* at extract concentration of 12.5 mg/ml.

Bio-agent adjustmen	Difference	Lower	Upper	p
Garlic-Ciprofloxacin	-31.66667	-36.645769	-26.6875640	0.0000000
Ginger-Ciprofloxacin	-37.16667	-42.145769	-32.1875640	0.0000000
Neem-Ciprofloxacin	-20.83333	-25.812436	-15.8542306	0.0000006
S. nilotica-Ciprofloxacin	-55.66667	-60.645769	-50.6875640	0.0000000
Ginger-Garlic	-5.50000	-10.479103	-0.5208973	0.0293004
Neem-Garlic	10.83333	5.854231	15.8124360	0.0002301
S. nilotica-Garlic	-24.00000	-28.979103	-19.0208973	0.0000002
Neem-Ginger	16.33333	11.354231	21.3124360	0.0000061
S. nilotica-Ginger	-18.50000	-23.479103	-13.5208973	0.0000019
S. nilotica-Neem	-34.83333	-39.812436	-29.8542306	0.0000000

Appendix XIX: ANOVA table for the inhibition of *Xanthomonas axonopodis* pv. *vignicola* by the different botanicals at concentration level of 25 mg/ml

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Extract	4	5072	1268.1	90.16	8.38e-08 ***
Residuals	10	141	14.1		

Significant codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘’ 1.> The ANOVA suggests that: - The main effect of extract is statistically significant and large (F (4, 10) = 90.16, p < .001; Eta2 = 0.97, 95% CI [0.93, 1.00])

Appendix XX: Tukey multiple comparison of means for the action of the different botanical extracts against the growth of *Xanthomonas axonopodis* pv. *vignicola* at extract concentration of 25 mg/ml.

Bio--agent adjustment	Difference	Lower	Upper	p
Garlic-Ciprofloxacin	-25.500000	-35.577378	-15.422622	0.0000630
Ginger-Ciprofloxacin	-31.333333	-41.410711	-21.255956	0.0000100
Neem-Ciprofloxacin	-17.866667	-27.944044	-7.789289	0.0012019
S. nilotica-Ciprofloxacin	-56.333333	-66.410711	-46.255956	0.0000000
Ginger-Garlic	-5.833333	-15.910711	4.244044	0.3736098
Neem-Garlic	7.633333	-2.444044	17.710711	0.1681566
S. nilotica-Garlic	-30.833333	-40.910711	-20.755956	0.0000115
Neem-Ginger	13.466667	3.389289	23.544044	0.0091534
S. nilotica-Ginger	-25.000000	-35.077378	-14.922622	0.0000749
S. nilotica-Neem	-38.466667	-48.544044	-28.389289	0.0000015

Appendix XXI: ANOVA table for the comparison of the two levels of extract concentration for the inhibition of *Xanthomonas axonopodis* pv. *vignicola.*

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Concentration	1	93	93.3	0.253	0.619
Residuals	28	10325 368.7			

> The ANOVA suggests that: - The main effect of concentration is statistically not significant and very small (F (1, 28) = 0.25, p = 0.619; Eta2 = 8.95e-03, 95% CI [0.00, 1.00]

Appendix XXII: Similarity Report

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