

**EFFICACY OF PLANT EXTRACTS FOR MANAGEMENT OF POTATO BACTERIAL
WILT (*Ralstonia solanacearum* Smith) IN RWANDA**



MARIE CHANTAL MUTIMAWURUGO



**A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for
the Doctor of Philosophy Degree in Horticulture of Egerton University**



EGERTON UNIVERSITY

APRIL, 2021

2021/114340
f

ABSTRACT

Potato (*Solanum tuberosum* L.) is one of staple foods for food security program and income generation in Rwanda. However, potato yield in Rwanda is still below the potential yield. Bacterial wilt caused by *Ralstonia solanacearum* (Smith) is one of the major pathogens limiting potato production. The use of plant extracts with antibacterial activities, which are locally available and environmental friendly, could be an alternative in the management of the disease. Thus, the aim of this study was to: (i) To screen different plant extracts for their efficacy in the control of potato bacterial wilt; (ii) To evaluate the effective concentration of plant extracts in management of *R. solanacearum*; (iii) To determine the effective application frequency of plant extracts in management of *R. solanacearum*, potato growth, yield, tuber quality, post-harvest infection and post-harvest yield losses; and (iv) To identify the bioactive compounds in effective plant extracts. An *in vitro* screening of the antibacterial activity of methanol, water and chloroform extracts of ten local plant materials against the pathogen was performed. Higher antibacterial activity was found in tobacco, wild marigold and garlic extracts (19.6, 18.6, and 18.3 mm inhibition zones, respectively). An *in vivo* study was also conducted to determine the effective concentration and frequency of application of selected bioactive plant extracts over potato bacterial wilt, potato growth, tuber yield, quality, and post-harvest-infection and yield loss due to the pathogen under field conditions in two growing seasons (Season A and B). The field experiments, were laid out in a Randomized Complete Block Design (RCBD) with factorial arrangements. In field experiments, weekly and bi-weekly application of plant extracts showed higher biological control efficacy (BCE) against pathogen in potato plants (58.3 and 57.4 %, respectively) than monthly application (47.6 %) at $p \leq 0.05$. Weekly and bi-weekly application of tobacco and wild marigold extracts at 50 mg mL^{-1} performed better all analysed parameters. Identification of bioactive compounds in methanol extract of tobacco was performed through high performance liquid chromatography (HPLC) technique with standards. It was detected that methanol extract of tobacco contains four bioactive compounds with activity against *R. solanacearum* (chlorogenic acid, rutin, unknown flavonoids, and 5-caffoylquinic acid). It is concluded and recommended that methanol extract from tobacco or wild marigold once applied weekly or bi-weekly at 50 mg mL^{-1} can be used in management of potato bacterial wilt and improvement of potato production and tuber quality in sustainable and organic horticulture.

TABLE OF CONTENT

DECLARATION AND RECOMMANDATION	ii
COPYRIGHT	iii
DEDICATION	iv
ACKNOWLEDGMENTS	v
ABSTRACT	vi
LIST OF TABLES	xiii
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS AND ACRONYMS/SYMBOLS	xix
CHAPTER ONE	1
INTRODUCTION	1
1.1 Background Information	1
1.2 Statement of the Problem	2
1.3 Objectives	3
1.3.1 Overall Objective	3
1.3.2 Specific Objectives	3
1.4 Hypotheses	3
1.5 Justification of the Study	4
1.6 Scope and Limitations	4
1.7 Definition of Terms	5
CHAPTER TWO	6
LITERATURE REVIEW	6
2.1 Origin and Description of Potato	6
2.2 Economic Importance of Potato	6
2.3 Constraints to Potato Production	7
2.4 Bacterial Wilt of Potato	9
2.4.1 Causal Agent	9
2.4.2 Races and Biovars of <i>Ralstonia solanacearum</i> and their Geographical Distribution	10

2.4.3	Pathogen Cycle, Survival and Dispersal	12
2.4.4	Mechanisms of Infection and Symptoms.....	14
2.4.5	Economic Importance of Bacterial Wilt	16
2.5	Methods of Controlling Potato Bacterial Wilt.....	16
2.5.1	Chemical Control Methods.....	16
2.5.2	Cultural Practices	18
2.5.3	Use of Transgenic Potato Plants	20
2.5.4	Biological Control Measures	22
2.6	Modes of Action of Plant Antimicrobial Compounds	32
2.7	Gaps in Knowledge.....	34
CHAPTER THREE		35
VIRULENCE AND CHARACTERIZATION OF ISOLATES OF POTATO		
BACTERIAL WILT CAUSED BY <i>Ralstonia solanacearum</i> (Smith) IN RWANDA....		35
	Abstract	35
3.1	Introduction	35
3.2	Materials and Methods.....	37
3.2.1	Origin of Bacterial Isolates	37
3.2.2	Isolation, Identification and Storage of Isolates	39
3.2.3	Pathogenicity Test.....	40
3.2.4	Biovar Identification	42
3.3	Results and Discussion	42
3.3.1	Bacterial Streaming Test.....	42
3.3.2	Cultural and Morphological Characteristics of Colonies on Growth Media .	43
3.3.3	Morphological Characteristics of Bacterial Cells through Gram Staining	45
3.3.4	Pathogenicity and Virulence of the Bacterial Isolates	45
3.3.5	Biovar Identification	49
3.4	Conclusion	51

CHAPTER FOUR	52
IN VITRO ANTIBACTERIAL ACTIVITY OF SELECTED PLANT EXTRACTS AGAINST POTATO BACTERIAL WILT (<i>Ralstonia solanacearum</i> Smith) IN RWANDA	52
Abstract	52
4.1 Introduction	52
4.2 Materials and Methods	54
4.2.1 Plant Extracts	54
4.2.2 Extraction of Bioactive Compounds	55
4.2.3 Preparation of Bacterial Inoculum	56
4.2.4 Screening for Antibacterial Activity of Plant Extracts	56
4.2.5 Determination of Minimal Inhibitory Concentration (MIC)	57
4.3 Results and Discussion	58
4.3.1 Screening of Plant Extracts against <i>Ralstonia solanacearum</i>	58
4.3.2 Minimal Inhibitory Concentration (MIC) of Plant Extracts	63
4.4 Conclusion	66
 CHAPTER FIVE	 67
EFFECT OF FREQUENCY OF APPLICATION OF SELECTED PLANT EXTRACTS ON POTATO GROWTH, YIELD, QUALITY, POST-HARVEST INFECTION AND YIELD LOSS	67
Abstract	67
5.1 Introduction	68
5.2 Materials and Methods	69
5.2.1 Description of Study Area	69
5.2.2 Preparation of Bacterial Inoculum	73
5.2.3 Preparation of Potato Seeds and Planting	73
5.2.4 Experimental Layout	74
5.2.5 Data Collection	76
5.3 Results and Discussion	80

5.3.1	Effect of Plant Extracts on Potato Growth Parameter	80
5.3.2	Effect of Plant Extracts on Yield Parameters	105
5.3.3	Effect of Plant Extracts on Potato Tuber Quality Parameters	114
5.3.4	Effect of Plant Extracts on Control of DI and their BCE in Potato Plants and Tubers.....	131
5.3.5	Effect of Plant Extracts on Potato Tuber Post-harvest Infection (PHI) and Post-harvest Yield Losses (PHL) due to <i>Ralstonia solanacearum</i>	155
5.4	Conclusion	163
CHAPTER SIX		165
IDENTIFICATION OF BIOACTIVE COMPOUNDS AGAINST POTATO BACTERIAL WILT IN SELECTED PLANT EXTRACTS.....		165
	Abstract	165
6.1	Introduction	166
6.2	Materials and Methods.....	168
6.2.1	Phytochemical Screening of Bio-compounds in Plant Extracts	169
6.2.2	Fractionation of Plant Extracts and Test of Antibacterial Activity of Fractions.....	172
6.2.3	Identification of Bioactive Compounds.....	174
6.3	Results and discussion	175
6.3.1	Phytochemical Screening of Bio-compounds.....	175
6.3.2	Fractionation of Plant Extracts and their Antibacterial Activity	182
6.3.3	Identification of Bioactive Compounds in Tobacco Plant Extract against <i>Ralstonia solanacearum</i>	193
6.4	Conclusion	202
CHAPTER SEVEN		204
GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS		204
7.1	General Discussion	204
7.2	Conclusions	209
7.3	Recommendations	210

7.4	Areas for Further Studies	210
REFERENCES	212
APPENDICES	229
Appendix A:	Published paper 1	229
Appendix B:	Published paper 2.....	230
Appendix C:	Collected plant species from Rwanda for in vitro antibacterial screening Against <i>Ralstonia solanacearum</i>	231
Appendix D.	Raw data of effect of plant extracts (S), solvent extracts (E) and concentrations (C) on disease incidence (DI %) in greenhouse experiment	232
Appendix E.	Raw data and ANOVA tables of effect of different concentrations of plant extracts on control of disease incidence (DI) under greenhouse conditions..	234
Appendix F.	Mean separation for effect of different concentrations of plant extracts on number of days to 50% sprouting and flowering in greenhouse experiment	238
Appendix G.	Mean separation for effect of different concentrations of plant extracts on yield (t ha ⁻¹) in greenhouse experiment	239
Appendix H.	Mean separation of effect of different concentrations of plant extracts, on tuber size (quality parameter) in greenhouse experiment	240
Appendix I.	Mean separation of effect of different concentrations of plant extracts on potato tuber post-harvest infection (PHI) and yield loss (PHL) in greenhouse experiment	241
Appendix J.	Raw data of effect of different application frequency of plant extracts on total yield (t ha ⁻¹) in season B	242
Appendix K.	Data of interactions between analysed factors in days to 50 % sprouting and flowering in field experiment	243
Appendix L.	Data of interactions between analysed factors in plant height (28, 56, and 70 AP) in field experiment	245
Appendix M.	Data of interactions between analysed factors in number of stems per plant in field experiment	248
Appendix N.	Data of interactions between analysed factors in yield parameters (number of tubers per plant and total yield t ha ⁻¹) in field experiment	251

Appendix O.	Data of interactions between analysed factors in quality parameters (tuber size, DM, SG, and RS content) in field experiment	253
Appendix P.	Data of interactions between analysed factors in disease incidence (DI) and biological control efficacy (BCE) in plants in field experiment	258
Appendix Q.	Data of interactions between analysed factors in disease incidence (DI) and biological control efficacy (BCE) in tubers in field experiment	263
Appendix R.	Data of interactions between analysed factors in post-harvest infection and post-harvest yield loss in stored potato tubers from field experiment ...	265
Appendix S.	Comparison between chromatograms of different extracts from wild marigold through HPLC recorded at 230 nm	267
Appendix T.	Comparison between chromatograms of different extracts from garlic through HPLC recorded at 230 nm	268
Appendix U.	Greenhouse experiment	269
Appendix V.	Comparison between potatoes treated with plant extracts and potatoes treated with controls under field conditions	270
Appendix W.	Isolation of bioactive compounds from methanolic extract of tobacco through open column chromatography and Thin Layer Chromatography (TLC)	271
Appendix X.	Research permit	272