THE EFFICACY OF *Moringa oleifera* PLANT EXTRACTS AGAINST SELECTED FUNGAL AND BACTERIAL PLANT PATHOGENS INFECTING SELECTED VEGETABLE CROPS IN ZIMBABWE.

By

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215081065

Submitted in fulfillment of the academic requirements for the degree of

DOCTOR OF PHILOSOPHY

In the

Discipline of Crop Science

School of Agricultural, Earth and Environmental Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

Pietermaritzburg

South Africa

March 2018

PREFACE

The research contained in this thesis was completed while based in the discipline of Crop Science, School of Agricultural, Earth and Environmental Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa. The research was supported by the National Research Foundation of South Africa and the University of Zimbabwe Research Board Funds.

The content of this work has not been submitted in any form to any other university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

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(Professor Augustine Gubba: Co-supervisor)

DECLARATION

I, Maria Goss, declare that:

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(Maria Goss)

As research supervisor I agree to submission of this thesis for examination

Signed…… Date……..
(Professor Paramu L. Mafongoya: Main supervisor)

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ACKNOWLEDGEMENTS

I would like to express deepest gratitude and appreciation to my supervisors Prof P. L. Mafongoya and Prof A. Gubba for their immeasurable guidance, support, dedication and encouragement throughout the course of this study. Their positive contributions, constructive criticisms and suggestions helped to improve the focus of this research study. I would also like to acknowledge the unwavering support rendered by my daughters (Rufaro and Ruvimbo Mujuru) and the twins (Makomboreroashe and Tungamiraishe). Their participation, patience, tolerance, endurance during my absence from home on field trips within the country and to South Africa ensured the success of this study.

I am also extremely thankful to Dr David Icishahayo (posthumously) for sharing his expertise with me on biometry, experimental designs for agriculture and statistical analysis. Deepest gratitude go to Engineer Jonah Chimusoro for availing his farm and resources. I further express sincere thanks to my friends and colleagues, Dr Obert Jiri, Rumbidzai Debra Kasauware Chapoto, Denver Maghadi, Martin Sanyamuwera, Rose Tafadzwa Masekesa, Ania Matikiti, and Gaudencia Mahenga Kujeke who have in one way or the other through their motivation, encouragement and prayers, contributed to the success of this study. In addition, I would like to appreciate the support rendered by Dr Sam Tesfaye, Dr Stan Mabasa and Prof Upenyu Mazarura throughout this study.

Sincere gratitude is sincerely extended to the National Research Fund for funding this research, the University of Zimbabwe Research Board Fund for funding part of the field research, Pedstock Nurseries for raising seedlings for this study, the Central Analytical Facilities, Stellenbosch University in the Mass spectrometry Unit, South Africa for Moringa extract phytochemical analysis, the University of KwaZulu-Natal Fee Remission and the smallholder commercial farmers in the 3 agro ecological regions of Zimbabwe for providing valuable information and trial sites. I also want to thank my mother (Miriam Goss) for the golden opportunity (education) she gave me which laid a firm and strong foundation for where I am right now. To you dear mom, your desire has been fulfilled! Above all, I would like to acknowledge and thank the wonderful Almighty God for His grace, mercy and love which have taken me this far. The strength you gave me during the course of this study is incalculable. I Thank You Lord!

ABSTRACT

Diseases and pests are among the major constraints to horticultural production worldwide, and this has been further exacerbated by mono-cropping production systems in response to increased food demands of an ever-expanding population. As a result, farmers have resorted to excessive chemical use in order to manage diseases and pests, and maintain high yields. However, excessive chemical use has been associated with negative environmental and health effects. Numerous studies have been carried out to determine antifungal and antibacterial properties in traditional medicinal plants aimed at developing bio-pesticides which can be utilized together with synthetic pesticides in integrated disease management strategies. One such alternative is the use of *Moringa oleifera* extracts. Currently, *in-vitro* studies carried out on Moringa antimicrobial action have mainly focused on controlling human enteric pathogens. It is against this background that this study was conducted during the 2014 – 2016 cropping seasons. The main objectives of the study were to: 1) determine farmers' perceptions on vegetable disease incidence, prevalence and disease control methods in relation to seasonality and prevailing climactic conditions. 2) Evaluate effectiveness of Moringa leaf and seed plant extracts in suppressing growth and development of bacterial (*Pectobacterium atrosepticum,* Hall, *Pectobacterium carotovorum subspp. brasilienses,* Bur., *Dickeya dadantii,* Dick.) and fungal (*Pythium ultimum,* Trow., *Rhizoctonia solani,* Kuhn., *Fusarium solani,* Mart., *Phytophthora infestans*, Mont.) plant pathogens *in-vitro*. 3) Determine whether Moringa aqueous extract concentration influences the efficacy of its antimicrobial activity. 4) Determine whether Moringa bark aqueous extracts could exhibit antibacterial activity against four different pathovars of the *Xanthomonas campestris,* Pammel., pathogen. 5) Determine the antifungal and antibacterial efficacy of Moringa leaf, seed and bark aqueous extracts against selected fungal and bacterial pathogens infecting two crops Lettuce (*Lactuca sativa,* L.) and Cabbage (*Brassica olearacea,* L.) grown under greenhouse and open field conditions respectively.

Initially, a survey was carried out to determine the perceptions of horticulture farmers on crop disease incidence and control methods in the sub-humid areas of Zimbabwe. Survey results revealed that farmers face more outbreaks of fungal diseases compared to bacterial diseases. In addition, farmers have noted an increase in disease incidence over the past 5 – 10 years. The survey further revealed that the majority of the respondents depend on chemicals to control diseases in

their crops. Only a small percentage of the farmers use cultural or mechanical alternative disease control methods. However, none of the respondents utilize botanical or bio-pesticide disease control strategies to manage these diseases. There is need to raise awareness among farmers regarding the negative health and environmental effects of increased chemical use and the potential of using bio-pesticide strategies in plant disease management. Field and greenhouse trials were conducted over three growing seasons using the completely randomized block and split plot experimental designs set up as factorial trials.

The laboratory results exhibited the efficacy of *Moringa* leaf, bark and seed aqueous extracts in significantly controlling the growth of fungal (*Pythium ultimum, Rhizoctonia solani, Fusarium solani* and *Phytophthora infestans*) and bacterial (*Pectobacterium carotovorium subspp. brasiliensis, Pectobacterium atrosepticum* and *Xanthomonas campetris pv campestris)* pathogens. There were significant interactions between Moringa aqueous extract source and concentration which influenced the antimicrobial action of the extracts ($P = 0.001$). The results from the greenhouse and field studies revealed that *Moringa* leaf, seed and bark aqueous extracts significantly controlled bottom rot (*Rhizoctonia solani*) and stem/root rot (*Fusarium solani*) diseases in lettuce and black rot disease (*Xanthomonas campestris pv campestris*) in cabbages ($P < 0.05$). Disease suppression was more effective at higher concentrations of the *Moringa* aqueous extracts, whilst the highest disease severity occurred at the lowest *Moringa* aqueous concentration levels. However, Moringa seed aqueous extract demonstrated higher antibacterial activity against black rot disease and antifungal activity against test pathogens ($P < 0.05$). Moringa seed and leaf aqueous extracts also significantly enhanced head weight and diameter in lettuce.

Moringa aqueous extracts can therefore be considered as bio-pesticides in Integrated Crop Disease Management strategies, and these can be a viable, and environmentally friendly alternative to chemical use. Based on our findings, it is recommended that further *in-vivo* studies to improve extraction protocols, and determine ideal application methods be carried out to improve Moringa aqueous extract bio-pesticide efficacy. These should be carried out in selected crop pathogens of economic importance. Currently, there is very little literature regarding *in-vivo* crop, pathogen and bio-pesticide interaction studies with Moringa. Training workshops and demonstration plots to impart knowledge and skills to farmers on preparation and utilization is key to enhance uptake.

TABLE OF CONTENTS

PREFACE ..... **ii**
DECLARATION ..... **iii**
ACKNOWLEDGEMENTS ..... **iv**
ABSTRACT ..... **v**
LIST OF FIGURES ..... **xii**
LIST OF TABLES ..... **xv**

CHAPTER 1 ..... **1**
INTRODUCTION TO THESIS **1**
1.1 Rational of the study 1
1.2 Justification for using *Moringa oleifera* as a biocontrol agent 3
1.3 Importance of *Xanthomonas campestris*, *Rhizoctonia solani*, Kuhn. and *Fusarium solani* pathogens 5
1.4 Research objectives 6
1.5 Hypotheses 7
1.6 General methodology and study approach 7
1.7 Organization of the thesis 8
References **9**

CHAPTER 2 ..... **14**
LITERATURE REVIEW **14**
2.1 Vegetable diseases and chemical control **14**
2.2 General concerns over uptake of bio-pesticides **15**
2.3 Alternatives in plant disease management **15**
2.4 *Moringa oleifera* antimicrobial research trends **17**
2.5 Strengths and weaknesses in Moringa botanical research **19**
2.6 Concluding observations **20**
References 21

CHAPTER 3 ..... **27**

FARMER PERCEPTIONS ON VEGETABLE DISEASES AND THEIR CONTROL IN SUB-HUMID AREAS IN ZIMBABWE **27**

Abstract **27**

3.1 Introduction **28**

3.2 Materials and methods **30**

3.2.1 Description of the study area 30

3.2.2 Data collection and analysis 30

3.3 Results **31**

3.3.1 Farmers Demographics and Crop Preferences 31

3.3.2 Frequently observed disease symptoms of the 33

3.3.3 Farmer perceptions on seasonal disease incidence 33

3.3.4 Rate of disease incidence and crop losses experienced by farmers 34

3.3.5 Most common plant disease control methods practised by Domboshava farmers 35

3.3.6 Farmer perceptions on efficacy of chemical use and application frequency 36

3.4 Discussion **37**

3.5 Conclusion **42**

References 43

CHAPTER 4 ..... **47**

EFFICACY OF *Moringa oleifera* LEAF AND SEED AQUEOUS EXTRACTS AGAINST SELECTED PLANT PATHOGENS. **47**

Abstract **47**

4.1. Introduction **48**

4.2 Importance of selected pathogens **49**

4.3. Materials and methods **50**

4.3.1 Experimental site and sources of pathogens and plant aqueous extracts 50

4.3.2 Experimental design and treatments 51

4.3.3 Media preparation and sub-culturing 51

4.3.4 Preparation of the Moringa aqueous extracts 52

4.3.5 Pathogen growth measurements and data analysis 53

4.4. Results **53**

4.4.1 Antimicrobial action of *Moringa oleifera* aqueous extracts on fungal and bacterial growth 53

4.5 Discussion **58**

4.5.1 Antimicrobial action of phytochemicals in local Moringa accessions 58
4.5.2 Antibacterial action of Moringa leaf aqueous extracts 59
4.5.3 Effect of Moringa seed and leaf aqueous extracts on *Dickeya dadantii* 60
4.6 Conclusions **61**
References 62

CHAPTER 5 ..... **68**
MORINGA OLEIFERA EXTRACTS EFFECT ON FUSARIUM SOLANI AND RHIZOCTONIA SOLANI GROWTH **68**
Abstract **68**
5.1 Introduction **69**
5.2. Materials and methods **72**
5.2.1 Experimental Design 72
5.2.2 Preparation of medium and sub-culturing 72
5.2.3 Preparation of Moringa oleifera seed and leaf extracts 73
5.2.4 Mycelia growth of the fungal pathogen 74
5.2.5 Data Analysis 74
5.3. Results **75**
5.3.1. Effect of concentration levels of Moringa extracts on radial growth of *R. solani* and *F. solani* 75
5.3.2 Interaction of Moringa extracts and concentration levels on radial growth of *F. solani* 75
5.3.3 Effect of concentration levels of Moringa extracts on percent inhibition of *R. solani* .76
5.3.4 Effect of Moringa extracts and concentration levels on percent inhibition *of F. solani* 77
5.4. Discussion **78**
5.5. Conclusion **80**
References 81

CHAPTER 6 ..... **86**
IN-VITRO EVALUATION OF *Moringa oleifera* BARK EXTRACTS IN SUPPRESSING FOUR *Xanthomonas campestris* pathovars **86**
Abstract **86**
6.1 Introduction **87**
6.2 Materials and methods **89**
6.2.1 Preparation of the Moringa bark aqueous extracts 89

6.2.2 Preparation of *Xanthomonas campestris pathovars* 90
6.2.3 Media Preparation and Pathogen Inoculation 91
6.2.4 Data Collection and Analysis 93
6.3 Results **94**
6.3.1 Effect of Moringa bark extract concentration on bacterial colony growth 94
6.3.2 Effect of time on antibacterial activity of the Moringa bark aqueous extracts 95
6.4 Discussion **96**
6.4.1 Effect of Moringa aqueous extracts concentration of the on colony length 96
6.4.2 Effect of Moringa bark extract concentration on antibacterial activity 97
6.5 Conclusion **99**
References 100

CHAPTER 7 104

***Fusarium solani* AND *Rhizoctonia solani* CONTROL USING Moringa *oleifera* PLANT EXTRACTS IN GREENHOUSE LETTUCE (*Lactuca sativa* L.) 104**

Abstract **104**
7.1 Introduction **105**
7.2 Materials and methods **106**
7.2.1 Experimental site and trial management 106
7.2.2 Fungal inoculum preparation and application 107
7.2.3 Moringa leaf and seed aqueous preparation 108
7.2.4 Data collection and analysis 108
7.3 Results **109**
7.4 Discussion **121**
7. 5 Conclusion **125**
References 126

CHAPTER 8 132

USE OF *Moringa oleifera* EXTRACTS TO CONTROL BLACK ROT (*Xanthomonas campestris pv. campestris*) IN FIELD GROWN CABBAGE (*Brassica oleracea*), BEATRICE, ZIMBABWE. 132

Abstract **132**
8.1. Introduction **133**
8.2. Materials and Methods **136**
8.2.1 Experimental Site and Methodology 136

8.2.2 *Xanthomonas campestris* Pathogen and Moringa aqueous extract preparation 136
8.2.3 Cabbage bacterial inoculation and data collection 137
8.3. Results **.....138**
8.4. Discussion **.....143**
8.5. Conclusion **.....146**
References 148

CHAPTER 9 152
CONCLUSIONS AND RECOMMENDATIONS 152
9.1 Major findings regarding antifungal and antibacterial activity of Moringa aqueous extracts **.....152**
9. 2 Implications of findings for commercial development of Moringa bio-pesticides **.....153**
9.3 Recommendations for improved validation and efficacy as a Moringa bio-pesticide **.....154**
9.4 Areas for future research to stabilize Moringa bio-pesticide formulations **.....154**

LIST OF FIGURES

Figure 2.1: Examples of medicinal plant utilization and reliance continuum in the communities. 16
Figure 3.1: Preferences in crop types grown as indicated by Domboshava farmers. 32
Figure 3.2: Commonly observed deviation from normal plant growth (disease symptoms) according to farmers' responses in Domboshava 33
Figure 3.3: Farmers response disease incidence in relation to season type 34
Figure 3.4: Farmers response on experienced disease type and their occurrence 34
Figure 3.5: Crop losses due disease experienced by farmers in Domboshava. 35
Figure 3.6: The most common disease control methods being practiced by farmers in Domboshava..... 36
Figure 3.7: Farmers' Perceptions on chemical control efficacy on disease control and spraying frequencies 37
Figure 4.1 Antifungal activity of Moringa leaf (a) and Moringa seed (b) aqueous extracts on the growth *of Pythium ultimum*..... 55
Figure 4.2. Antifungal activity of Moringa leaf (a) and Moringa seed (b) aqueous extracts on the growth *of R. solani* 56
Figure 4.3. Antifungal effects of Moringa leaf (a) and Moringa seed aqueous extracts (b) on *P. infestans* growth 56
Figure 4.4. Antibacterial action of Moringa leaf (a) and Moringa seed (b) aqueous extracts on *P. atrosepticum* growth 57
Figure 4.5. Antibacterial Effect of Moringa leaf (a) and Moringa seed aqueous extracts (b) on *P. c. brasiliensis* growth 57
Figure 4.6. Antibacterial effects of Moringa leaf (a) and Moringa seed (b) aqueous extracts on *D. Dadantii* growth 58
Figure 5.1: Interaction effects between Moringa extracts and radial growth of F. solani, June 2015..... 76
Figure 5.2: Effect of Moringa extracts and concentration levels on percentage inhibition of Fusarium solani, June 2015..... 78_Toc508137703

Figure 6.1: Plant leaves infected four different strains of *X. campestris*: a. *pv campestris* – cabbage *(Brassica oleracea var. capitata)* b. *pv vesicatoria* – pepper *(Capsicum group*) c. *pv malvacearum* – cotton *(Gossypium hirsutum*) and d. *pv phaseoli* – beans (*Phaseolus vulgaris).* ..... 90_Toc508137705
Figure 6.2: Strains of *X. campestris* bacterial colonies: a. *pv vesicatoria* b. *pv campestris* c. *pv malvacearum* and d. *pv phaseoli* 91
Figure 6.3: Effect of Moringa bark aqueous concentration level on growth of *Xanthomonas campestris* pathovars. 95
Figure 6.4: Effect of time on colony length of *Xanthomonas campestris* pathovars 96
Figure 7.1: Interaction effects of Moringa leaf aqueous concentration level and stage at which lettuce was inoculated with *F. solani* on disease severity of *F. solani*. 111
Figure 7.2: Interaction effect of Moringa seed aqueous extract concentration and lettuce inoculation stages on disease severity of *R. solani* 112
Figure 7.3: Interaction effect of Moringa leaf aqueous concentration and growth stage at which lettuce was inoculated with *F. solani* on disease incidence 113
Figure 7.4: Effect of Moringa seed aqueous concentration level and stage at which lettuce was inoculated with *F. solani* on disease incidence 114
Figure 7.5: Interaction effects of Moringa seed aqueous concentration and lettuce inoculation stages on lettuce head weight inoculated with *R. solani* 115
Figure 7.6: Interaction effects of Moringa leaf aqueous concentration and lettuce inoculation stages on lettuce head weight of *F. solani*. 116
Figure 7.7: Interaction effects of Moringa seed aqueous concentration and lettuce inoculation stages on lettuce head weight of *F. solani* 117
Figure 7.8: Effect of Moringa leaf aqueous concentration and stage of inoculation of lettuce with *R. solani* on lettuce head diameter 118
Figure 7.9: Effect of Moringa seed aqueous concentration and stage of inoculation of lettuce with *F. solani* on lettuce head diameter 119
Figure 7.10: Effects of Moringa seed aqueous concentration levels and stage of lettuce inoculation on head diameter of lettuce 120
Figure 7.11: Interaction Effects of Moringa aqueous treatment concentration levels, lettuce inoculation stages and lettuce head diameter 121

Figure 8.1. Effect of Moringa seed, leaf and bark aqueous extracts on mean cabbage leaf defoliation at 7 - 10 weeks after emergence. P = 0.009 141

LIST OF TABLES

Figure 3.2: Commonly observed deviation from normal plant growth (disease symptoms) according to farmers' responses in Domboshava. 33
Table 5.1: Effect of concentration levels on radial growth of R. solani and F. solani growth 75
Table 5.2: Effect of concentration levels on percent inhibition of R. solani 77
Table 6.1: Medium modifications 91
Table 6.2: P-values from Day 1 – Day 7 95
Table 7.1: Effect of Moringa aqueous extract concentration on disease severity of *R. solani* and *F. solani*. 110
Table 8.1. Disease Severity scoring table for black rot disease in this study 138
Table 8.2. Means showing effect of Moringa aqueous extracts on black rot disease severity in cabbages 140

LIST OF APPENDICES

APPENDIX A156: RESULTS OF THE GC/MS ANALYSIS CARRIED OUT ON LOCAL ZIMBABWEAN Moringa oleifera ACCESSIONS USED IN THE STUDY 156

APPENDIX B: SURVEY QUESTIONNAIRE..... 157

CHAPTER 1

INTRODUCTION TO THESIS

1.1 Rational of the study

Vegetable farmers are facing major challenges brought about by the prevalence and incidence of crop diseases and the lack of resistant cultivars to soil-borne fungal pathogens (Altizer *et al*., 2006). This inability of farmers to effectively manage disease outbreaks has caused them to resort to extensive and indiscriminate use of chemicals to manage these disease outbreaks (Aktar *et al.*, 2009). Farmers have also adopted intensified agricultural practices and increased pesticide and fertilizer use to cater to the increased food demands (Potts *et al*., 2015). Intensified agricultural practices not only pollute the environment, they also disrupt the normal functioning of ecosystems and have detrimental effects on animal and human life (Potts *et al.*, 2015; Ogle, 2016). Thus holistic, sustainable and environmentally friendly strategies have to be developed, especially in the face of the negative impact of climate change on crop productivity and yield. However, rising demands on agricultural products due to population explosion world-wide have caused a majority of farmers to resort to intensive and indiscriminate use of synthetic chemicals to manage disease and pest pressures (Ogle, 2016). This has resulted in build up of crop pathogens to chemicals (Aktar *et al.*, 2009).

Chemical use to control vegetable pests and diseases has not only resulted in extensive health hazards to animal and human life, it has also contributed immensely to decline in the quality and yield of pollinator dependent horticultural crops (Klein *et al*., 2007; Potts *et al.*, 2015). Chemical use not only negatively impacts yield indirectly through decreased pollinator activity, but it also influences other sectors of our communities, such as through contamination of drinking water sources and also further enhances pesticide resistance by crop pathogens (Clough *et al*., 2016).

The need to respond to food demand has caused farmers to adopt mono-cropping which inherently results in increased disease outbreaks. This practice has led to pathogens developing resistance to chemicals which are being continually applied to crops making chemicals less efficient in

controlling crop diseases (Ogle, 2016). Farmers respond to such challenges by intensifying chemical use resulting in large quantities of residual chemicals being perpetually present in the environment, and further compounding the pesticide resistant pathogenic strains. This results in health hazards to human and animal life, including fish, other fauna and soil inhabiting micro-organisms; and pesticide resistant-pathogens (Aktar *et al.,* 2009; Davis *et al.,* 2015). However, it is not all the farmers who are able to respond to increased food demands by intensifying chemical use. Small scale, resource poor farmers cannot afford any increase in input costs (Pramanik *et al.,* 2016). Such farmers, therefore, suffer huge losses due to recurrent disease epidemics resulting in resistance build up, and their fields become sources of disease inoculum, providing a disease continuum every season.

Bio-pesticides also play a key role as they provide the opportunities for organic farmers. As the global population becomes more informed on detrimental impacts of pesticide use to health and the environment, the demand for organically produced, chemical free produce is on the increase (Hjelmar, 2011). It becomes important that the body of knowledge regarding preparation and utilization guidelines for application of bio-pesticides be enhanced to encompass all practical issues.

The problem of pesticide-resistant pathogens has also been excarcabated by climate change and variability which has caused diseases which were once classified as minor diseases and pests, to become of major concern, thus requiring more frequent and diversified chemicals (Ifejika, 2010). The resultant problems of reduced crop yield, quality and productivity, therefore, need to be effectively addressed and sustainable solutions to manage these problems devised. Climate change and variability causes fluctuations in occurrence, duration and intensity of rainfall and fluctuating temperature ranges are experienced in different locations (Bernstein *et al.,* 2000). Not only does climate change influence weather patterns, it also results in increases in disease incidence. Evans *et al*., (2008) indicated an increase in incidence and severity of phoma (*Leptosphaeria maculans,* Sacc.) disease across fluctuating weather patterns over 2 seasons. Yáñez-López (2012) states that extreme weather conditions were causing 14.1% crop losses, that is, approximately US$220 billion losses globally. Climate change has also resulted in changes in disease dynamics by altering and disrupting disease epidemiology and distribution (Yáñez-López, 2012). These disruptions in

epidemiology may affect plant pathogen survival, vigour and its rate of multiplication and infection across all the crop production systems. Smallholder vegetable producers are the most vulnerable to disease epidemics. This is because they are not only financially challenged, but they also lack the knowledge and skills of chemical use, thus further enhancing pesticide-resistant strains through improper utilisation (Nyamupingidza and Machakaire, 2003; Pahla *et al*., 2014).

There is need to identify alternative sustainable disease management strategies to reduce the current overreliance on chemical control. To this end, biological methods using plant aqueous extracts must also be evaluated as feasible alternatives to crop disease management. This view is shared by Ifejika (2010), who calls for further validation and documentation of utilization of traditional, medicinal plants as botanicals in plant disease management strategies. Gautam *et al.*, (2013) argues that the changing disease scenario due to climate change calls for better, sustainable agricultural practices and the use of eco-friendly disease management strategies involving natural biocontrol mechanisms. Not only are bio-pesticides environmentally friendly and cheaper compared to chemicals, they utilize indigenous knowledge systems of farmers (Aktar, *et al.,* 2009). They are the only effective way to address the plethora of challenges in disease and pest epidemiology caused by the effects of climate change whilst research is being carried out (Ifejika, 2010).

Bio-pesticides have been used in several studies to successfully suppress, manage and control several pathogens across the globe (Bailey *et al.*, 2004; Aliyu *et al*. 2008; Akinbode and Ikotun, 2008). To this end they have great potential in providing viable solutions to the challenges being posed by the continued over reliance on synthetic pesticides.

1.2 Justification for using *Moringa oleifera* as a biocontrol agent

Moringa is a multipurpose tree, acclaimed for its medicinal and nutritional value. Based on these properties, it is one of the most traditionally adopted trees which has been utilized for long periods of time to treat several ailments in the form of tonic, powder, concoction or can be eaten fresh (Fahey, 2005). It is considered one of the world's most useful trees because almost every part of it is utilized in some way (Ashfaq *et al*., 2012). It is mostly grown for its leaves, seeds, flowers

and roots (Ashfaq *et al*., 2012; Anwar *et al*., 2007; Maroyi, 2006; Bukar *et al*., 2010; Das *et al*., 2010; Adedapo *et al*., 2009). Recent studies have shown that Moringa seed aqueous extracts cotain active antibactericidal substances such as *pterygospermin, moringine* and *glycosides* (Abalaka *et al*., 2012). These substances have been observed to inhibit the growth of *Bacillus subtilis,* Cohn., *Mycobacterium, Escherichia coli,* Mig., *Psuedomonas aeruginosa, Psuedomonas shigella* and *streptococcus pyogenes* which are commonly associated with food contamination (Viera *et al*., 2010). Other studies have shown that Moringa leaf aqueous extracts can successfully control root-knot nematode and *Sclerotium,* a pathogen causing damping off and root rot diseases in cowpea (Ashfaq *et al*., 2012) *in-vitro* studies. The plant has properties which make it of high scientific interest and it is a subject of extensive research worldwide (Das *et al*., 2010; Chollom, 2012; Adedapo *et al*., 2009). In the tropics and subtropics, researchers have reported that the tree is grown as a backyard tree for leaf and pod consumption, for medicinal purposes and for fiber (Abalaka *et al*., 2012; Bukar *et al*., 2010; Fahey, 2005). The tree is also used as animal fodder, fertilizer, live fence, water purification, wood fuel and as a natural pesticide (Foidl *et al*., 2001, Basra *et al*., 2011).

Moringa has been utilized to enhance crop growth and quality in several crops as it is said to possess plant growth regulating properties (Foidl *et al.,* 2001). However, recent laboratory-based studies have revealed that the plant posseses antimicrobial properties (Abiodun *et al*., 2012). It has been successfully used to suppress several fungal and bacterial pathogens such as *Fusarium solani,* Mart., *Fusarium oxysporum,* Sacc., (Gifoni *et al*., 2012), *Shigella shinga,* Kiy., *Pseudomonas aeruginosa,* Schourso., and *Shigella sonnei,* Carl., in *in-vitro* studies (Rahman *et al*., 2009).

However, most of the studies done are yet to validate laboratory based observations under field conditions, and the majority of these studies have dealt with human enteric pathogens and not with crop pathogens (Abalaka *et al*., 2012). In addition, the mechanism used by the bioactive compounds in suppressing pathogen activity are not fully understood (Freire *et al*., 2015). Some authors state that the bioactive phytochemicals cause structural plasma membrane disarrangement to effect antimicrobial action (Gifoni *et al*., 2012), while others argue that these bioactive components work by enhancing inhibitory spore germination and growth of specific pathogens (Kooltheat *et al.,* 2014). Park *et al.,* (2011) argues that the Moringa antimicrobial activity is

achieved through interference with critical pathogen pathways. This points to the need for further studies to provide answers to questions which are arising as additional research is being carried out on Moringa antimicrobial properties and how to harness them to improve crop productivity.
It is therefore, the focus of this current study to validate these *in-vitro*, laboratory based studies by carrying out field and greenhouse trials. The pathogens used in this study are of economic importance in terms of their negative impacts on crop productivity (yield and quality) in Zimbabwe (Nyamupingidza and Machakaire, 2003). In addition, the vegetable crops (cabbage and lettuce) under study form an integral part of the livelihoods of a majority of Zimbabwean horticultural market farmers. These market gardeners grow horticultural crops solely to meet market demand, as a critical source of income for the family (Pahla *et al.*, 2014).

1.3 Importance of *Xanthomonas campestris, Rhizoctonia solani,* Kuhn. and *Fusarium solani* pathogens

The family *Xanthomonadacea*, is listed as being among the most problematic pathogens in brassica plants, with the *Xanthomonas* species causing tremendously devastating diseases (Vorster *et al.*, 2002). Black rot (*Xanthomonas campestris pv. campestris*. Pam.*)* specifically renders a larger percentage of crops incapable of producing marketable produce, with the harvested produce also being unfit for processing or value addition (Seebold *et al*., 2008). In Zimbabwe, black rot is a common problematic disease in all the five agro-ecological regions, with the disease incidence levels ranging from 10% to 80% (Wulff *et al.,* 2002). *Xanthomonas campestris* is one of the species that has raised great concern among vegetable farmers. In Zimbabwe, brassicas are grown all year round except in the rainy season when cultivation of brassicas almost becomes impossible due to black rot disease caused by the *Xanthomonas campestris* pv *campestris* bacterial pathogen (Wulff *et al.,* 2002). Commercial and smallholder farmers growing brassicas are incurring huge economic losses of horticultural crops due to *Xanthomonas campestris* pv *campestris.* The control of the disease progression has proved quite ineffective to contain by relying solely on synthetic pesticides (Culver *et al*., 2012). Copper based chemical control methods result in pathogenic resistance buildup, rendering chemical control ineffective especially when applied after symptoms appearance (Culver *et al*., 2012).

Rhizotonia solani is a very common soil inhabitant which survives between lettuce crops as sclerotia or mycelium in soil and crop debris. It not only survives pathogenically on alternate hosts, but can also be introduced into a vegetable field by wind- or water-disseminated spores (Nuez, 2001). The fact that the fungus is both seed and soil borne and is present in most soils, makes it difficult to control. Not only does the fungus cause Rhizoctonia root rot, but it is also a key part of a group of fungi responsible for damping off which is a devastating disease of brassica crops globally (Agrios, 2004). Many small scale brassica farmers in Zimbabwe, incur huge seedling losses due to damping off disease (Pahla *et al*., 2014).

Fusarium solani is a widely distributed soil-borne fungus pathogenic to at least 111 plant species spanning 87 genera, causing wilt and rot diseases on a wide variety of crops (Naik and Rani, 2008). The *Fusarium solani* pathogen can survive in soil for many years as resistant spores and leads to incidence of root rot diseases which can cause 10 to 80% losses in different species of vegetables (Gonzalez *et al*., 2006).

1.4 Research objectives

The overall objective of this study was to evaluate the antibacterial and antifungal action of *Moringa* aqueous extracts against selected plant pathogens in two vegetable crops. The study also investigated the local smallholder farmers' perception on brassica and solanaceous vegetable diseases and their control.

The specific objectives were:

1. To determine farmers' perceptions on vegetable disease incidence, prevalence and disease control methods in relation to seasonality and prevailing climactic conditions.
2. To evaluate if *Moringa* leaf and seed plant aqueous extracts can effectively suppress the growth and development of bacterial (*Pectobacterium atrosepticum*, Hall., *Pectobacterium carotovorum subspp. brasilienses,* Bur., *Dickeya dadantii,* Dick.*)* and fungal (*Pythium ultimum,* Trow. *R. solani, F. solani Phytophthora infestans,* Mont.) plant pathogens *in-vitro*.

3. To determine whether *Moringa* bark aqueous extracts at different concentration levels could exhibit antibacterial activity against four different pathovars of the devastating *Xanthomonas campestris,* Pammel., pathogens.
4. To determine the antifungal and antibacterial efficacy of *Moringa* leaf, seed and bark aqueous extracts against selected fungal and bacterial pathogens infecting two crops (Lettuce - *Lactuca sativa,* L. and Cabbage - *Brassica olearacea,* L.) grown under greenhouse and field conditions respectively.

1.5 Hypotheses

1. Farmers' perceptions on disease incidence and disease control methods are influenced by their own knowledge, practices, exposure and experiences.
2. *Moringa* leaf and seed aqueous extracts can effectively suppress the growth and development of bacterial and fungal pathogens.
3. The antifungal efficacy of *Moringa* leaf and seed aqueous extracts against *F. solani* and *R. solani* pathogens is influenced by the concentration levels of the plant aqueous solution.
4. The four *Xanthomonas campestris* pathovars react differently in their resistance to the antibacterial activity exhibited against their growth by *Moringa* bark aqueous and concentration level.
5. Moringa leaf, bark and seed aqueous extracts can effectively suppress bottom/stem rot and black rot in greenhouse and field grown lettuce and cabbage respectively.

1.6 General methodology and study approach

The study used participatory research approach using structured questionnaires and key informant interviews to obtain information on the objective. Laboratory, greenhouse and field trials were conducted to obtain information on antimicrobial action of the different *Moringa* plant aqueous extracts against selected crop pathogens. Data was subjected to statistical analyses using various appropriate statistical packages such as Statistical Package for Social Sciences (SPSS), GenStat Version 14 and analysis of variances were developed at $P < 0.05$.

A field study was carried out within the farmers' own operating systems and environments with full farmer participation, without altering their normal agronomic practices. This approach was based on the fact that disease epidemiology, virulence and spread is influenced by the prevailing environment in which it infects its host plant within a given crop species (Agrios, 2004).

1.7 Organization of the thesis

Chapter 1 provides the background to the problem of pesticide use, disease resistance development and benefits of bio-pesticide usage to sustainable agriculture and greening the environment. Chapter 2 provides a synthesis of the literature which has been carried out using Moringa as a natural biological agent in pathogen control. It also provides an insight into the challenges, and the areas in this work which are yet to be clarified, improved upon and validated through further studies. Chapter 3 provides an overview of the farmers' perceptions on vegetable diseases incidence and their control in the semi-humid regions of Zimbabwe. The farmers were involved from planning, planting to full implementation of the extract evaluation trials. Chapter 4 provides an insight into the antimicrobial activity of Moringa plant leaf and seed aqueous extracts efficacy against selected fungal and bacterial crop pathogens *in-vitro* as a screening exercise. Chapter 5 provides a description of how the concentration levels of Moringa leaf and seed aqueous solutions influences its efficacy as an antifungal agent against *R. solani* and *F. solani* pathogens. Chapter 6 describes the antibacterial activity of Moringa bark aqueous against the devastating *Xanthomonas campestris pv campestris* pathovars notorious for black rot diseases in cabbages, rape and lettuce. Chapter 7 provides an assessment of the ability of Moringa seed and leaf aqueous extracts to suppress damping off and root rot diseases caused by *Rhizoctonia solani* and *Fusarium solani* in greenhouse grown lettuce. Chapter 8 looks at the efficacy of Moringa seed, leaf and bark plant aqueous extracts in controlling black rot disease caused by *Xanthomonas campestris pv campestris* in field grown cabbage. Chapter 9 provides main conclusions, recommendations and areas of future research.

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CHAPTER 2

LITERATURE REVIEW

2.1 Vegetable diseases and chemical control

Vegetables refer to any edible herbaceous plant part and vegetable production constitutes approximately 2.88 million metric tons/year globally and this figure continues to raise significantly (Hanif *et al.*, 2006). As consumers become more health-conscious, the global market for fresh vegetables imported from Kenya and Zimbabwe has increased in volume and product variety (Dole and Humphoursey, 2010). This increase in demand for fresh vegetable produce has resulted in a shift from off-season supply towards an increasing year-round supply, further expanding the market for imported fresh vegetables (Dole and Humphoursey, 2010). These conditions provide an all year-round source of alternate pathogen hosts, introducing further challenges into disease management dynamics.

Among all the pathogens which affect horticultural crops, fungal pathogens such as *Rhizoctonia solani* and *Fusarium solani*, cause major economic losses worldwide (Palou *et al.*, 2016). Vegetable diseases reduce the quality and quantity of harvested produce, thus increasing the production costs for the farmer by lowering price of the commodity (Jayne *et al.*, 2003). Fungal plant pathogens are more prevalent and are known to cause huge losses in vegetable crops. As a strategy to manage these losses, farmers have resorted to heavy applications of conventional fungicides. This has, however, resulted in the buildup and proliferation of single, double and triple-resistant pathovors against several fungicides, which has seriously compromised the effectiveness of these chemicals (Palou *et al.*, 2016; Droby *et al.*, 2016). Development of these fungicide resistant pathogen biotypes, coupled with the environmental and health concerns raised, has resulted in the withdrawal of key fungicides from the market. The main drive, therefore, is focused on developing alternative disease management technologies which are safe and effective (Droby *et al.*, 2016). Furthermore, food concerns, chemical residues and negative environmental impact have brought about strict regulatory changes in the use and registration of pesticides, resulting in further withdrawal of substantial amounts of fungicides from the market (Droby *et al.*, 2016). This

fact further emphasizes the need for intensified study into bio-pesticide alternatives (Khan and Hamid, 1986). The need, therefore, for alternative bio-pesticide methods to synthetic pesticides is key.

Bio-pesticide uptake and adaptation is on the increase as evidenced by the registration and approval of 22 biocontrol agents compared to 20 synthetic chemical pesticides in the past 5 years in the European Union alone (Droby *et al.,* 2016). However, despite the awareness of the need for bio-pesticides as alternatives to chemical pesticides, there are still some drawbacks to the effective and enhanced uptake of these technologies which need to be addressed.

2.2 General concerns over uptake of bio-pesticides

Natural bio-pesticides are a good alternative to synthetic chemicals in disease management, and these medicinal plants have been utilized to fulfill this role since time immemorial (Goswami *et al.*, 2016). However, Tsouh-Fokou *et al.*, (2015) and da Silva Oliveira *et al.*, (2016), recommended the need for further efficacy and safety studies of medicinal plant utilization as bio-pesticides in food crops. Droby *et al.*, (2016), studies evaluated the effectiveness of bio-pesticides in controlling postharvest diseases in horticultural crops, argues that there is very little literature on formulation of bio-pesticides for purposes of up-scaling, stabilization and commercialization. It is also important that bio-pesticides perform favourably in comparison to synthetic pesticides when applied as stand-alone treatments commercially in field conditions. Economic formulation of large quantities of aqueous bio-pesticides with reasonable shelf-life and constant efficacy under commercial testing are critical if a commercial bio-pesticide is to be developed and adopted (Droby *et al.,* 2016). This current research is aimed at building the body of knowledge towards these mechanisms under organic, commercial and small scale field vegetable farming systems.

2.3 Alternatives in plant disease management

Over 80% of the world population is heavily reliant on medicinal plant use in one form or another. This statistic translates to approximately over 2 billion people in both developing and developed countries (Smith-Hall *et al*., 2012). The documented indigenous utilization of medicinal plants has

mainly been of their application as home remedies for common ailments (Figure 2.1). Very few cases in the past are documented regarding medicinal plant utilization as bio-pesticides in pest and disease control (Smith-Hall, *et al.*, 2012). This fact inadvertently creates a need to identify new approaches and alternatives to disease management. The fact that 80% of the world's population utilizes medicinal plants in home based remedies brings out the importance of such plants and the ease of acceptance of such plants into mainstream crop disease management strategies by local communities (Smith-Hall, *et al*, 2012). This, therefore, emphasizes the need for further studies to enable ease of application and incorporation of these bio-pesticides into mainstream agricultural activities in both small scale and commercial production systems.

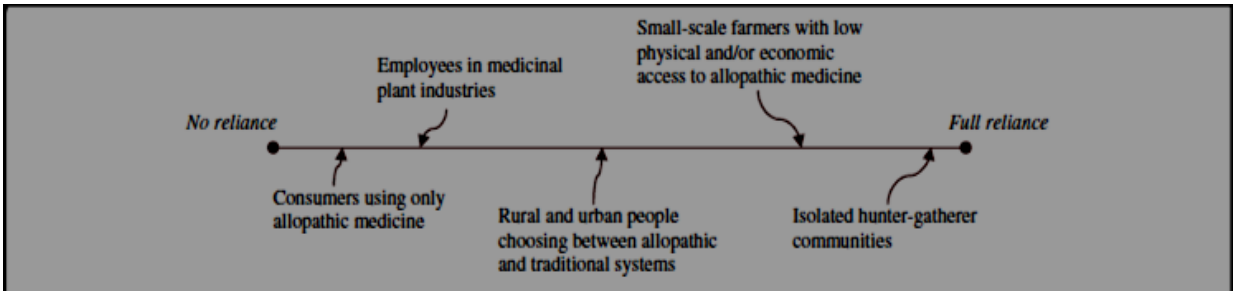

Figure 2.1: Examples of medicinal plant utilization and reliance continuum in the communities. Source: Smith-Hall *et al*. (2012)

Although there is no evidence based research on the safety and efficacy standards for herbal and bio-pesticide utilization (Smith-Hall *et al.*, 2012), there still is urgent need for farmers to understand the main drivers and factors which would enhance adoption of this practice. Such adaptations would lead to climate-smart and environmentally friendly, sustainable agricultural disease management practices which embrace bio-pesticides (Davis, 2011). It, therefore, becomes important to identify important medicinal plants and carry out studies regarding their antimicrobial properties. These can then be efficiently harnessed to develop biocontrol agents for incorporation into mainstream integrated disease and pest management strategies in agriculture.

One such plant is the Moringa tree which has been used in traditional and therapeutic applications for decades (Foidl *et al.*, 2001). It is renowned for its medicinal and nutritional properties worldwide (Foidl *et al*., 2001). Several studies have revealed its antimicrobial properties and as a result, quite a number of studies have been carried out to validate these claims (Torres-Castillo *et al*., 2013; Anwar *et al*., 2007).

2.4 *Moringa oleifera* antimicrobial research trends

There has been a lot of research into evaluating and validating the antimicrobial properties of the Moringa tree. However, most of these studies have been *in-vitro* and laboratory based as summarized by Torres-Castillo *et al.,* (2013); Anwar *et al*., (2007); Adandonon *et al*., (2006), as follows:

1. Spiliotis *et al.,* (1998) carried out an *in-vitro* investigation to determine the antibacterial properties of Moringa seed aqueous extracts against *Bacillus cereus,* Fran., *C. albicans, Streptococcus faecalis,* Andre., (now known as *Enterococcus faecalis,* Schl.*), S. aureus* and *Staphylococcus epidermidis,* Evans. The results of these studies showed that Moringa seed aqueous extracts were able to significantly suppress the growth of these test human pathogens.
2. Nikkon *et al.,* (2003), evaluated the antibacterial action of Moringa root aqueous extracts in an *in-vitro* study against *Shigella boydii,* Ewing., *Shigella dysentriae,* Shiga., and *S. aureus.* The results showed that Moringa root aqueous significantly suppressed growth of the test pathogens.
3. Doughari *et al*., (2007) reported on the effectiveness of Moringa as an antibiotic and compared its effects to those of conventional antibiotic drugs such as Ciprofloxacin, Cotrimoxazole and Chloramphenicol. This particular study further emphasizes the focus of research on efficacy of the Moringa antimicrobial properties against human enteric pathogens.
4. Jamil *et al.,* (2007) carried out an *in-vitro* study to determine the antimicrobial properties of Moringa seed aqueous against bacterial (*Pasteurella multocida,* Past., *E. coli, B. subtilis* and *S. aureus*) and fungal (*F. solani* and *Rhizopus stolonifer,* Enrenb.) pathogens. The antibacterial action of the extracts was more effective against bacterial pathogens compared to the fungal pathogens in this particular study. The study also focused on plant pathogenic strains of agricultural importance such as *F. solani* which is included in the group of pathogens which are responsible for a number of soil-borne diseases, such as damping off. This particular group of soil-inhabiting fungal pathogens, is viewed as difficult to manage and has the ability to live for prolonged years in organic matter in the soil, causing root rots in plants.

5. Abdulkadir *et al.,* (2015) reported antibacterial action of Moringa leaf juice against four Gram negative bacteria (*S. shinga, P. aeruginosa, S. sonnei* and *Pseudomonas spp.*) and six Gram-positive bacteria (*S. aureus, B. cereus, Streptococcus-B- haemolytica,* Fry., *B. subtilis, Sarcina lutea,* Good., *and Bacillus megaterium,* de Bary.).
6. Mishoursa *et al*., (2011) summarized some of the major studies done to evaluate the antifungal and antibacterial properties of Moringa against disease causing pathogens. These studies validated the potential of Moringa as a reliable and viable disease management option. Moringa can be an ideal alternative bio-pesticide which can offset the adverse chemical impacts on the environment and the continued buildup of pathogen resistance to chemicals. These *in-vitro* studies showed that Moringa aqueous extracts possess antimicrobial properties in significant amounts to inhibit pathogen growth.
7. Raza *et al.,* (2015) reported good *in-vivo* antiviral activity against new castle disease in poultry. The results further indicated that an increase in aqueous concentration was directly proportional to the death of the new castle virus. These studies are among the few documented *in-vivo* trials.
8. Younus *et al.,* (2016) evaluated the ability of Moringa plant aqueous extracts in exhibiting antiviral activity against foot and mouth disease in an *in-vitro* study. The results showed great antiviral activity against foot and mouth disease by inhibiting viral growth at l-50ug/ml. They attributed this antiviral activity to the presence of glycosides, flavonoids, isothiocynates and caffeoyi acids present in the Moringa aqueous extracts.
9. Abbas *et al.,* (2016) evaluated the antifungal activity of Moringa plant aqueous extracts against *Colletotrichum falcatum,* Corda., which causes red rot disease in sugar cane. The results showed the ability of Moringa aqueous extracts as having inhibited pathogen growth by 57.43%. The efficacy of inhibition was influenced by the concentration level of the aqueous solution. This was an *in-vitro* study.

All these studies point to the potential of using Moringa to manage bacterial, fungal and viral diseases. There is need to harness this potential and incorporate Moringa as part of an integrated disease management programme for crop plants.

2.5 Strengths and weaknesses in Moringa botanical research

The interest generated by the antimicrobial properties of Moringa among researchers prompted a lot of research undertakings. However, the majority of the studies mainly targeted human pathogens. Additionally, the mechanisms involved in the antimicrobial action against plant pathogenic strains need to be determined and understood as there exist mixed arguments regarding its mode of action within plant tissue (Freire *et al.,* 2015). For instance, one study used the inhibition halo technique to determine and validate the antimicrobial activity of essential Moringa oil extracted from the leaves. The important point to note in this particular study is that, it has diverted from the majority of the *in-vitro* studies whose focus was mainly on human pathogenic strains (Mishoursa *et al.,* 2011). The researcher also focused on pathogenic strains which are notorious as postharvest diseases in food storage and processing.

Lürling and Beekman (2010) reported the effectiveness of Moringa plant aqueous extracts against the cynobacterium *Microcystis earuginosa,* Kützing., which is a water inhabiting bacterium. Moyo (2012), Khojali *et al.*,(2014) and Ndhlala *et al*., (2014) also reported successful inhibition of human bacterial pathogenic strains by using Moringa plant aqueous extracts as the control agent. However, Ndhlala *et al*., (2014) raised concerns over the lack of actual scientific research to validate all these findings and claims. He further argues that despite the existence of much evidence regarding the antimicrobial properties of Moringa plant aqueous extracts, it all remains anecdotal because of the presence of very little actual scientific research. This remains the greatest knowledge gap existing in Moringa research undertaken so far.

Despite the intensive research demonstrating the antimicrobial properties of Moringa, there is an apparent lack of scientifically validated field trials in agricultural production systems. However, some limited, yet thorough studies by very few chemists have elaborately identified the phytochemical and nutritional properties of some Moringa accessions (Fahey, 2005b). Studies by Adandonon *et al*., (2006) are among the earliest studies involving crop pathogens carried out under field and greenhouse conditions where the antifungal activity of Moringa plant aqueous extracts against damping off disease (*Sclerotium rolfsii,* Sacc.) were evaluated. However, the effectiveness of the antifungal action cannot be attributed to Moringa plant aqueous extracts alone, since a

combination of different medicinal plants plus Moringa were used in his study. Further studies by Kuri *et al*., (2011) assessing the antifungal activity of Moringa plant aqueous extracts on seed-borne pathogens *Phomopsis vexans,* Sacc. and Roum., *Fusarium oxysporum,* Schlecht., and *Aspergillus flavus,* Link., might also be listed as being among the first scantily documented studies involving actual crop pathogens.

Other studies involving agricultural interest in Moringa plant aqueous extracts have focused on other aspects of its composition for improved crop productivity and quality, since it contains a natural plant growth hormone zeatin (Iqbal, 2014). Moringa has exhibited the ability to enhance germination and early seedling development and establishment of major cereal crops (Phiri, 2010). While other studies have explored the effectiveness of Moringa as an organic fertilizer (Emmanuel and Zaku, 2011), further studies have evaluated its allopathic effect on growth and productivity of mungbean (Hossain *et al.*, 2012). Moringa seed has also been used extensively in groundwater treatment (Mangale *et al*., 2012). The majority of studies on Moringa, however, have been on its medicinal, nutritional and phytochemical compositions (Ogbunugafor *et al*., 2011; Nweze and Nwafor, 2014; Dahiru *et al.* 2006; Valdez-Solana *et al.*, 2015; Karthika *et al*., 2013).

The overwhelming antimicrobial efficacy reports of Moringa plant aqueous extracts in suppressing pathogens is not supported by placebo, controlled and randomized scientific trials (Fahey, 2005a), a fact which raises concern. There is, therefore, need for validation through application of scientific research.

2.6 Concluding observations

Studies which have been clearly validated and documented involve Moringa plant aqueous extracts against human enteric pathogens, nutritional and phyto-chemical constituents (Valdez-Solana *et al*., 2015; Nweze and Nwafor, 2014), and more recently animal studies. Not much data exists on Moringa extract efficacy against crop pathogens *in-vivo* (Harvell, 2002; Smith-Hall *et al.*, 2012; Berger, 1977). In order for natural biological disease and pest control strategies to become a reality, there is need to carry out scientific, controlled and calculated field studies.

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CHAPTER 3

FARMER PERCEPTIONS ON VEGETABLE DISEASES AND THEIR CONTROL IN SUB-HUMID AREAS IN ZIMBABWE

Abstract

Horticultural farmers in Zimbabwe grow a variety of vegetable crops for their livelihoods and as a source of household income. Given the monoculture cropping system over large tracts of cropping area, they face major problems in disease outbreaks. These diseases are a major constraint to the viability of their horticultural enterprises. As a result of these disease outbreaks, farmers have resorted to alternating a number of chemicals to achieve disease control. There is need to determine the levels at which alternative disease management strategies are being adopted by practicing farmers. This study was carried out using 250 randomly selected vegetable farmers by administering questionnaires. The main objective of the study was to determine farmers' perceptions on vegetable disease incidence and severity in relation to prevailing weather conditions and to determine the control methods practiced by vegetable growers to manage fungal and bacterial diseases. The study results indicated significant increases in fungal and bacterial disease incidence of 84.6% (within the community cropping fields) and severity of 73.1% (within individual farmer fields /plant field per crop species) over the past 5-10 years ($P < 0.05$). Fungal diseases had the highest incidence of 84.6% compared to the low incidence of bacterial diseases at 15.4%. The study also revealed that disease incidence was highest [30.8%] during the winter months of May – July and the rainy months [23.1%] of November – February. Lower disease incidence and severity percentages were experienced during summer months of August - October [19.2%] and spring months of March – April [3.8%]. The study further indicated that 96.2% of the respondents relied on chemical methods of disease control, 53.8% used cultural control, and 11.5% relied on natural control methods. However, none of the farmers used bio-pesticides or biological control methods. The study concludes that although farmers are aware of the disease shifts in response to different climate change and variability, they are unaware of the effects of extensive chemical use to the environment. The farmers are also not aware of the existence of alternative disease management methods involving use of natural botanicals or bio-pesticides.

Key words: Disease incidence and severity, farmers' perspective, natural botanicals

3.1 Introduction

Leafy vegetables such as brassicas are an important part of the human diet and are sources of various nutrients such as vitamins A, C, dietary fibre and minerals (Kader, 2003). These nutrients protect the human body from disease infection by boosting the immune system. Green leafy vegetables, such as lettuce and spinach, have the capacity to reduce the risk of certain types of cancer due to carotenoid compounds present in them (Kader *et al*, 2003; Hanif *et al*., 2006; Adams, 2013). In addition to cancer prevention, vegetables act as antioxidants in the system whose role is to improve processes such as metabolism through metabolic activation as well as detoxification processes and disposal of possibly carcinogenic compounds (Horrigan, 2002). In Zimbabwe, vegetables also play important socio-economic roles of providing a reliable food source as well as generate income for basic family needs after selling their produce within the community (Chipurura, 2010; Brown *et al.*, 2012).

Vegetables are defined as the fresh portions of herbaceous plants and are susceptible to fungal, bacterial, viral and physiological diseases (Hanif *et al*. 2006). The incidence and severity of these diseases is influenced by biotic or abiotic factors, among which is the phenomenon of climate change which is yet to be understood by the majority of farmers (Jiri and Mafongoya, 2015). Climate change and variability has resulted in the creation of new invasion niches for fungi and bacteria in areas where environmental conditions were previously unfavorable for the proliferation of these disease causing micro-organisms to flourish (Mina and Dubey 2010; Agrios, 2004). This would lead to high yield losses, both qualitatively and quantitatively, due to increased disease pressure (Altizer *et al*. 2006). In response to these new disease outbreaks, farmers are resorting to the use of increased synthetic pesticide applications with potential negative impacts on the environment (Ngowi *et al.* 2007); and reduced returns per unit area due to the increased production costs (Deuter, 2008). Diseases, however, vary due to the ambient growing conditions and climate, since the climate influences the type of disease causing microorganisms that exist in a certain area (Luck *et al*. 2011).

Abiotic stresses predispose plants to diseases by weakening their defenses and influencing the type of organisms which survive in certain types of environments (Ngowi *et al*. 2007). Knowledge about diseases and the negative environmental effects of chemical use, will influence the choice of control methods, with the overall cost of chemicals limiting many farmers to mechanical, natural and cultural control methods (Tibugari *et al.* 2012). Reports show a marked increase in the use of pesticides as more natural habitats are converted to agricultural landscapes (Ngowi *et al.* 2007). This development has disrupted natural ecosystem functionality and introduced imbalances in the pathogen-host dynamics (Horrigan, 2002). Disease causing micro-organisms have specific conditions that favour their growth, spread and spore formation. (Agrios, 2004). Changes in temperature and rainfall patterns have resulted in creation of new niches for disease causing micro-organisms (Mina *et al*., 2015). This will result in shifts in host plant-pathogen dynamics, for instance over southern Africa, as prevailing average temperatures increase 2 - 4^{o}C higher than those experienced between 1961 and 1990 with rainfall decreasing in the range of 10 - 20 % (Jiri and Mafongoya, 2015). Higher temperatures promote proliferation of diseases which favour temperatures higher than the optimum growing temperatures for most crop species (Evans *et al.*, 2008).

For instance, as a direct response to the fluctuating weather conditions prevailing in Zimbabwe, pests such as the diamond back moth (*Plutella xylostella*) are becoming a challenge in vegetable production (Tibugari *et al*., 2012). Thus, alternative climate-smart, environmentally sustainable and resilient crop protection methods have to be identified and implemented in already existing mainstream disease management strategies. Commonly grown horticultural crops in most parts of Zimbabwe at subsistence and small scale commercial systems include cabbage (*Brassica olearacea,* L.*)*, covo (*Brassica oleracea var. sabellica*), rape (*Brassica napus,* L.*)* and tomatoes (*Solanum lycopersicum,* L.) (Pahla *et al*., 2014). Whilst commercial horticultural farmers mainly produce high value crops such as green, red and yellow pepper (*Capsicum species,* L.), cucumbers (*Cucumis sativus,* L.), Chinese cabbage (*Brassica rapa subsp. pekinensis,* L.), lettuce (*Lactuca sativa,* L.), carrots (*Daucus carota,* L.), beet root (*Beta vulgaris,* L.) and string beans (*Phaseolus vulgaris,* L.) (Siziba *et al.*, 2003). In the rural setting in Zimbabwe, vegetable gardens are located in one section of the village usually close to a river or water source and are mainly grown as a food source, with only surplus being sold to neighbours within the community (Pahla *et al*. 2014). The

greatest challenges to productivity and high yields faced by farmers in the vegetables are susceptible to fungal and bacterial diseases, which they are not equipped to efficiently manage (Nyamupingidza and Machakaire, 2003). Disease management problems are now being compounded by the variable climateic conditions (Brown *et al*., 2012).

The objective of this study was to: i) determine and infer vegetable farmers' perceptions on disease incidence and ii) identify the most common disease control methods implemented by vegetable farmers in Domboshava District. It also evaluated vegetable farmers' perceptions on the impact of fluctuations in weather patterns on disease incidence based on their observations for the past 10 years.

3.2 Materials and methods

3.2.1 Description of the study area

The study was conducted in Domboshava District of Harare, Mashonaland East Province, Zimbabwe, which lies between 17° 36' 40" S and 31° 10' 28" E. Domboshava District was chosen since it falls within the major vegetable growing regions and high rainfall areas. It lies largely to the north-eastern part of Harare. Its climate is characteristic of agro-ecological region IIB which receives good rains averaging between 650-800mm but is subject to frequent droughts, dry summer spells and short rainy seasons (Ministry of Agriculture, Mechanism and Irrigation Development, 2014). Temperatures are always quite high in summer (day temperatures often over 39ºC in summer) causing evaporation losses of 10–13 mm/day. The annual mean, maximum and minimum mean monthly temperatures in the district are 24.8°C, 27.4°C (November) and 22.3°C (July), respectively**.** The soils are sandy loams with a few patches of red clay soils in some areas (Nyamapfene, 1992).

3.2.2 Data collection and analysis

A questionnaire consisting of semi-structured questions was used to capture farmers' responses. Focus group discussions and key informant interviews were also carried out to triangulate questionnaire responses. The questionnaire was pre-tested by administering it on to a small sample

of farmers in a small area before using it in this study. The data collected included demographic matters and general farming systems and practices; pesticides use or non-pesticide practices, disease symptoms observed based on seasonality, and disease control methods and frequency of disease control.

The sample size comprised of 250 vegetable farmers. For purposes of this study, the farmers were categorized into the following groups: (i) market gardeners were those whose production target was to meet immediate market demands and grew vegetables for commercial purposes. Only a very small portion of their produce was consumed by the family. (ii) Subsistence farmers referred to those farmers who grew their produce to meet household consumption and demands. However, these farmers would sell small portions or surplus of their produce to meet social needs. (iii) Farmers who practiced mechanical farming methods were those who employed and practiced improved farm power processes to carry out their farming activities. These farming activities included use of appropriate labor-saving machinery such as rotary cultivators, conservation tillage equipment and disc plows. Activities on such farms included construction and adoption of contours, field strips, crop rotations and terrace farming and lastly; (iv) farmers classified as practicing cultural farming practices was compromised of those farmers who were still using labor-intensive methods with the aid of traditional tools (e.g. hand hoes, garden forks, shovels etc.) and used limited draft power. These farmers relied heavily on the mold board plow to till and prepare their lands for cropping.

The quantitative data which was collected was entered into Microsoft Excel and it was analyzed using the Statistical Package for Social Sciences (SPSS). Frequency tables and graphs were generated to indicate the trends occurring across study parameters for this study (SPSS, 2009). Significance differences between data were calculated at $P < 0.05$ level.

3.3 Results

3.3.1 Farmers Demographics and Crop Preferences

Male vegetable farmers made up of 65.4% of the respondents whilst 34.6% were female. The majority of the vegetable farmers in Domboshava practice small scale commercial farming

(53.8%), followed by market gardeners (30.8%) and the lowest percentage was made up of subsistence farmers (15.4%). 60% the farmers are between the ages of 45 – 60, 15% are above 60, 15% are between the ages of 19 – 25 and the remaining 10% are made up of the elderly above 61 years. The majority of the respondents have attained education levels of post secondary/high school level and primary school (70%), with the remaining 30% went up to primary education.

The respondents' most commonly grown crop preferences are in the following order: covo, which is a member of the kale family belonging to the *Brassica species*, tomatoes (*Solanum lycopersicum*, L.), rape (*Brassica napus*, L.), mustard greens (*Brassica juncea*, L.), onion (*Allium cepa*, L.), pumpkins (*Cucurbita maxima*, Duchesne.), cabbage (*Brassica oleracea*, L.), spinach (*Spinacia oleracea*, L.), common beans (*Phaseolus vulgaris*, L.) and soft gourd (*Cucurbita maxima*, Duchesne.) (Fig 3.1).

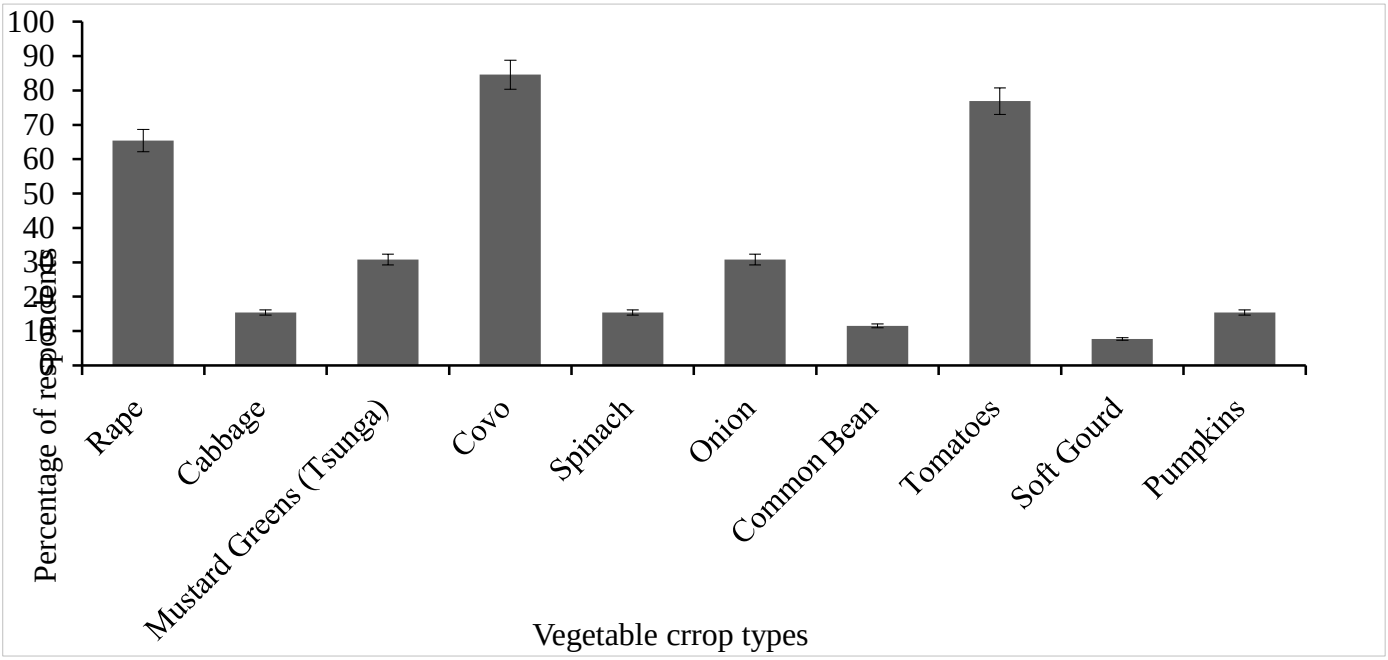

Figure 3.1: Preferences in crop types grown as indicated by Domboshava farmers.

According to respondents, the most commonly grown crops are rape, covo and mustard greens. The other crop which is grown in significantly high proportions are tomatoes, whilst the lowest preferred crops were common beans and soft gourd ($P < 0.05$, Fig 3.1).

3.3.2 Frequently observed disease symptoms of the

The most commonly observed vegetable disease symptoms were plant chlorosis, wilting leaves, powdery patches, root rots, dark spots and yellow halos, yellow V-shaped lesions, drying leaves, whitish blisters, stunting and water-soaked lesions (Fig 3.2). The most significant symptoms observed were chlorosis and wilting leaves ($P \leq 0.05$, Fig 3.2).

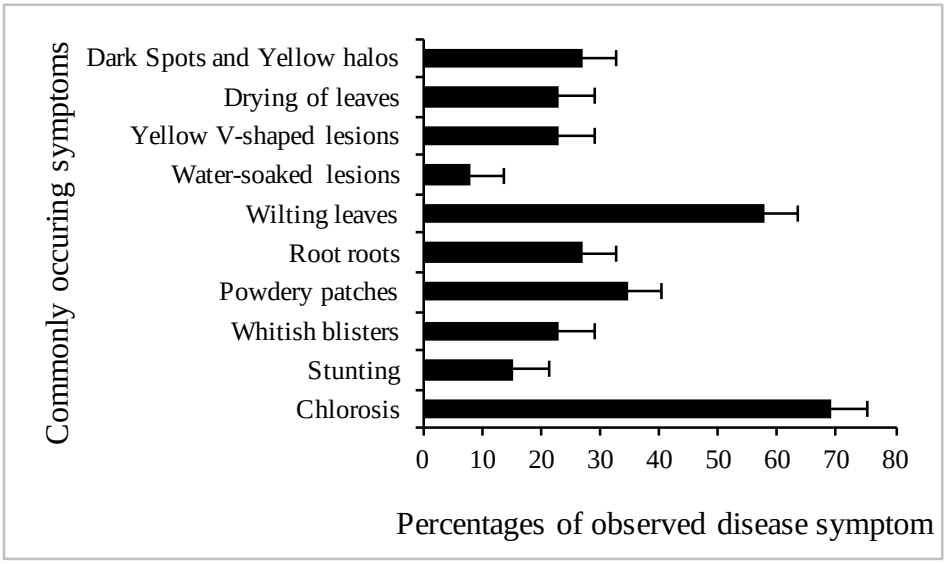

Figure 3.2: Commonly observed deviation from normal plant growth (disease symptoms) according to farmers' responses in Domboshava.

3.3.3 Farmer perceptions on seasonal disease incidence

The disease incidence and type in relation to seasonality based on farmers' response to observed symptoms are presented in Fig 3.3. Fungal and bacterial disease incidence in the area are high at 84.6% and 73.1% respectively within a particular growing season. Most of these diseases occur during the winter season [May – July] (30.8%) and rainy season [Nov – Feb] (23.1%). The least diseases occur during the spring season [March – April] (3.8%). Fungal diseases were the biggest disease problem [84.6%], while the least were bacterial diseases [15.4%] experienced in Domboshava (Fig 3.3 – Fig 3.4).

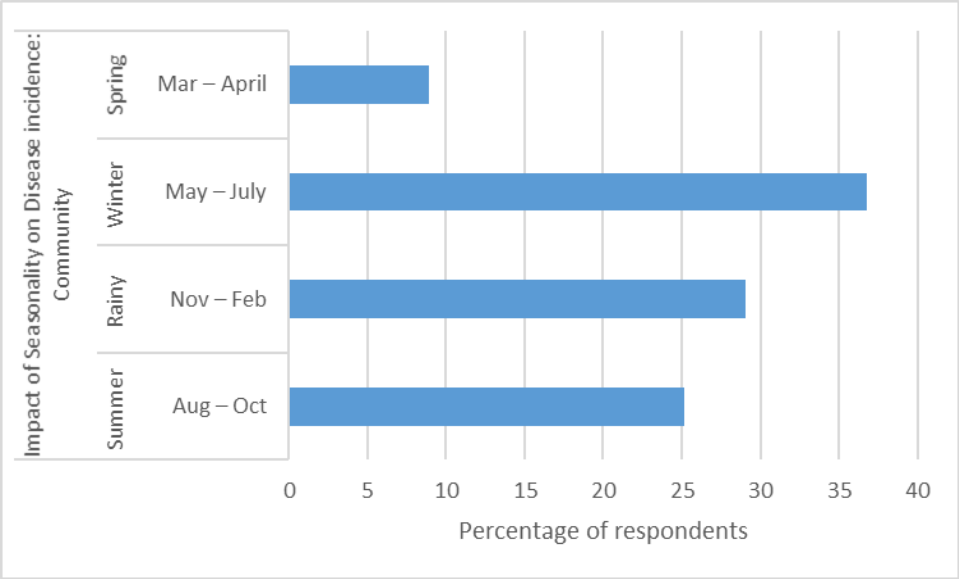

Figure 3.3: Farmers response disease incidence in relation to season type

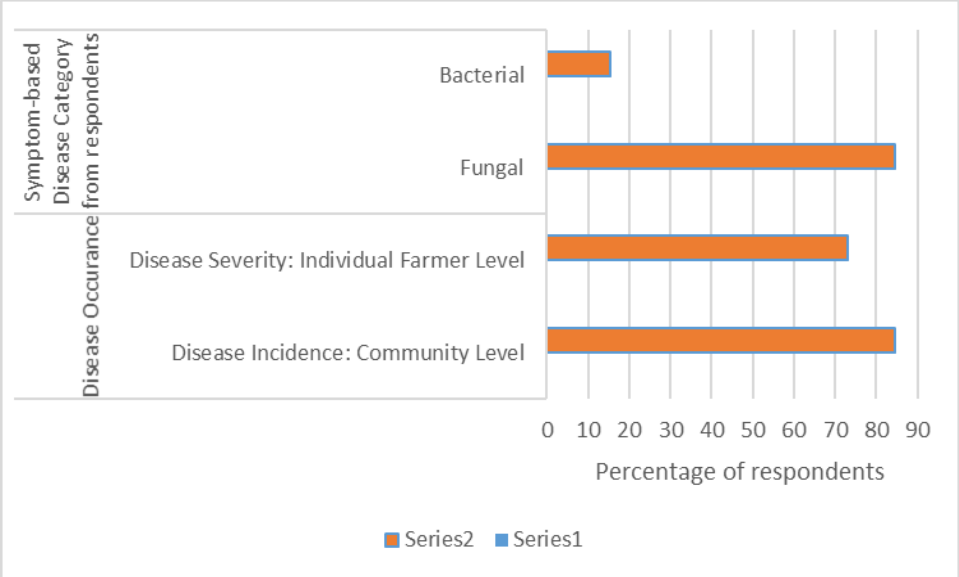

Figure 3.4: Farmers response on experienced disease type and their occurrence

3.3.4 Rate of disease incidence and crop losses experienced by farmers

The varying percentage losses and disease infection rates respondents are experiencing in their cropping enterprises are shown in Fig 3.5. Our study revealed that 53.8% of the farmers, which is the largest number of respondents, indicated that they experience extreme to severe disease infection rates. The other 46.2% of the respondents' indicated that the disease infection rates they

incur are moderate to mild in nature. The study revealed that 30.8% of the respondents experienced major crop losses due to disease outbreaks (Fig 3.5). Whilst the remaining 34.6% of the respondents' indicated that they incur high to very high losses as a result of disease incidence in their vegetable plots.

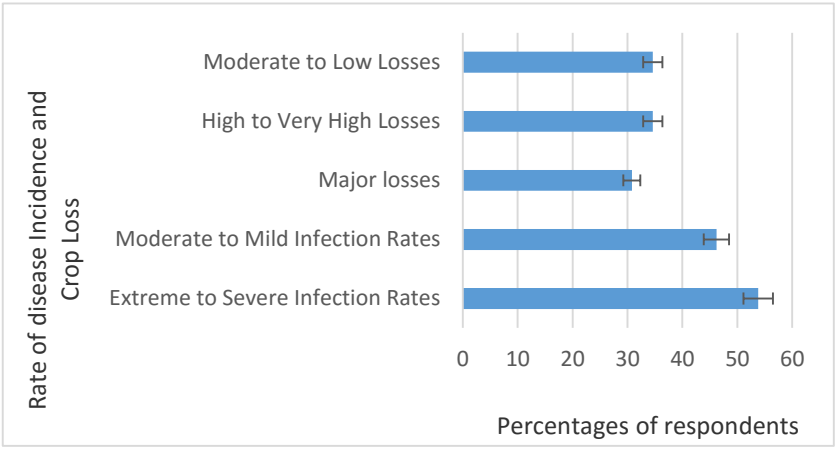

Figure 3.5: Crop losses due disease experienced by farmers in Domboshava.

The largest population of respondents indicated that they experience 'moderate to low', and those experiencing 'high to very high' crop losses due to diseases in the area are not significantly different ($P < 0.05$, Fig 3.5).

3.3.5 Most common plant disease control methods practised by Domboshava farmers

Figure 3.6 is an indication of the most commonly adopted disease control methods as practised by the respondents in Domboshava. The results indicated that 96.2% of the respondents' use chemical disease control methods in managing disease outbreaks in their vegetable plots. About 53.8% of the respondents' rely on cultural methods to manage vegetable diseases, whilst 11.5% of the respondents' rely on natural control methods involving living fences and distance between cropping fields. The minority 3.8% of the respondents rely on mechanical control methods. However, none of the respondents rely on biologocal control methods involving botanical

formulations or bio-pesticides. The differences in disease control methods existing among Domboshava farmers were significant ($P < 0.05$, Fig 3.6).

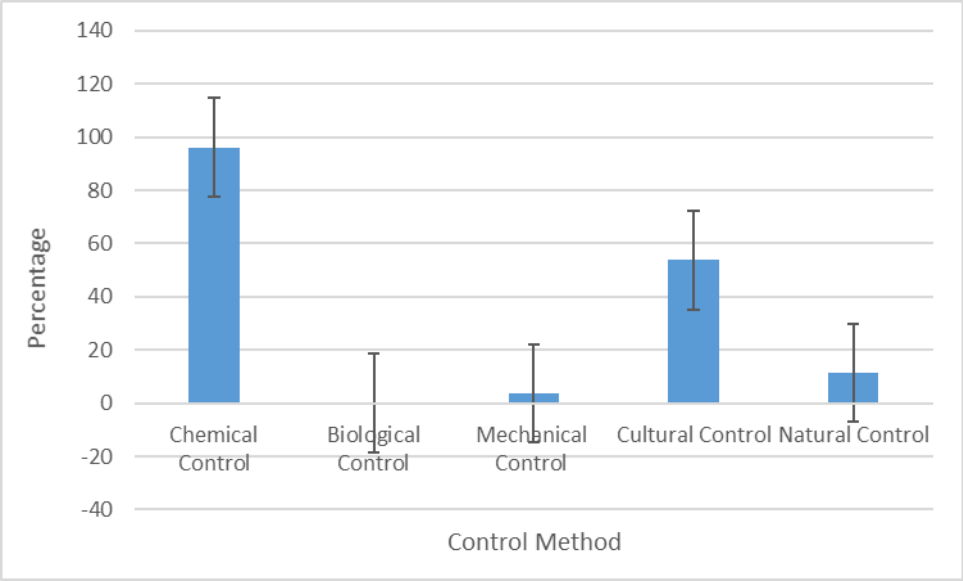

Figure 3.6: The most common disease control methods being practiced by farmers in Domboshava.

Cultural disease management strategies were also being implemented and these were significantly different from the natural control strategies being used by the farmers ($P < 0.05$). The results of this study also indicate that the adoption rate for using mechanical disease control strategies was significantly different from the other strategies ($P < 0.05$). However, none of the farmers used biological or bio-pesticide disease control strategies (Fig. 3.6).

3.3.6 Farmer perceptions on efficacy of chemical use and application frequency

About 65.4% of the respondents stated there has been an increase in chemical application frequency when managing vegetable diseases, with 34.6% stating there was no change in spraying frequency over the past 10 years (Fig 3.7). Furthermore, 76.9% of the respondents' indicate that there is an increase in amount of chemicals being rotated in dealing with vegetable diseases whilst 23.1% indicate that there is no change in the number of chemicals being rotated. About 53.8% of the respondents indicated that chemical disease control methods are effective while the remaining 46.2% of the respondents indicated that chemicals are ineffective in achieving vegetable disease control. About 38.5% of the respondents indicated that they sprayed once/week, while 19.2% they

sprayed twice/week, with the remaining 7.7% carring out disease control methods once/month. However, 34.6% of the respondents were uncertain as to how frequent they carried out disease spraying programs in their vegetable plots (Fig 3.7)

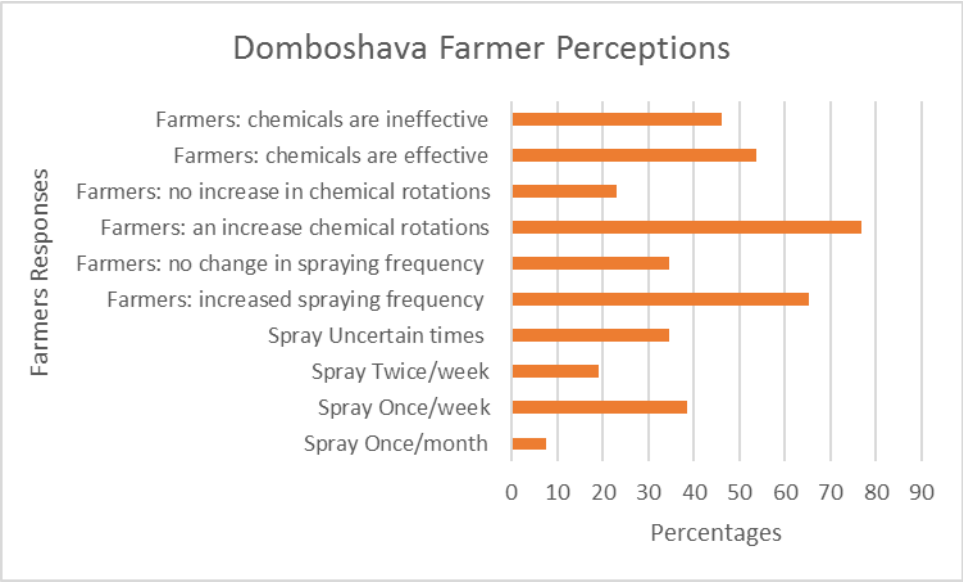

Figure 3.7: Farmers' Perceptions on chemical control efficacy on disease control and spraying frequencies

3.4 Discussion

The survey revealed that 65.4% of the respondents' were male with the remaining 34.6% being female. The greater percentage of vegetable growers is male mainly as a result of urban cities downsizing their workforce, causing the main bread winners to relocate to their rural homes. The farmer category data indicated that 30.8% and 53.8% of respondents' were market gardeners and small scale commercial farmers respectively. The least percentage was comprised of subsistence farmers (15.4%). The male dominated vegetable farmers are shifting the farming paradigms in this area from producing vegetables solely for home consumption, to practising farming as a business. The shift is necessitated by the high unemployment rates and prevailing economic challenges. These vegetable enterprises, therefore, must become income generating centres in order to meet the food and financial needs of the family. Furthermore, Domboshava is ideally situated in a Peri-urban area near the capital city, Harare. This makes marketing and transportation of horticulture produce easier and more viable given the existing road network passing through the area.

Respondents indicated that these crops are grown in response to market and consumer preferences. Leafy vegetables are supplied to hyper-supermarkets within the peri-urban areas surrounding Domboshava District, whilst the tomatoes are mainly grown for Mbare musika in Harare. The soft gourds and common beans are grown mainly for home consumption to supplement the family dietary requirements. However, respondents indicated that if a market arises, they were willing to sell these products. They however, also used such crops for barter trade and selling among themselves within the community.

The survey indicated that disease incidence is on the increase over the past 5 -10 year period; and that this observed phenomenon might be related to seasonality and prevailing changes in weather conditions. Zimbabwe has, over the past 10 years, been experiencing fluctuations in prevailing weather conditions, resulting in prolonged alternations between wet and dry spells (Jiri and Mafongoya, 2015). Prevailing weather variability significantly influences disease development, spread and distribution of most of the prevalent bacterial, fungal and viral crop plant diseases within a given crop production site (Deuter, 2008). This observed increase in disease incidence might therefore, indicate a possible correlation between the prevailing weather conditions and disease incidence within this sub-humid area of Zimbabwe. This assertion implies the need for further studies to validate this assumption. At the same time, variations in weather patterns in these vegetable growing sites is creating conducive conditions such as maximum and minimum temperatures and the moisture levels, which are ideal for pathogen survival (Mina and Dubey, 2010). The variable weather conditions within particular sites, play a major role in the migration of insect pests which could be vectors of viral diseases, which also influence the rate of secondary pathogen dispersal (Elad and Pertot, 2016). Disease causing micro-organisms have specific conditions that favour their growth, spread and promote spore formation (Larkin, 2016). Changes in temperature and rainfall patterns have resulted in creation of new invasion niches and expansion of the range of disease causing microorganisms (Chakraborty, 2005). This may result in new disease incursions since conditions that were normally preventing pathogen growth would have changed. Changes in the pattern of disease epidemics is likely to increase the losses that farmers will incur, whilst at the same time complicate disease control methods and strategies. In addition to complicated disease control, climate variability especially in terms of low rainfall will result in difficulties in irrigation planning since the replenishment of the underground storage is reduced,

resulting in increased costs and possibility of water-deficient crops (Deuter 2008). This will increase the vulnerability and susceptibility of crop plants to a wide host range of disease causing organisms.

The risk of disease infestation in crop production is a function of the prevailing environmental conditions and local climate (Chakraborty, 2005). The relationship between host plant ecology and disease causing pathogens is therefore, likely to change as weather conditions alternate between extremeties (Chakraborty 2005). High temperatures, low rainfall and other extreme events have the capacity to alter host plant physiology in ways that alter the way host plants and pathogens relate to each other. Yáñez-López (2012) states that the variable weather effects have resulted in development of pathogens with short life spans, higher reproduction rates and effective dispersal mechanisms, which makes them more virulent in nature. Such changes can either result in high disease incidence or failure of disease development based on farmers adaptive coping strategies to these pandemics. Management practices also have a bearing in the host-pathogen relationship as the climate is changing, with an increase in atmospheric CO_2 resulting in an increase in plant biomass, thus increasing the infective area for pathogenic attack (Elad and Pertot, 2016). During the dry spell periods, when lower rainfall is being received in Zimbabwe (Jiri and Mafongoya, 2015), farmers resort to applying supplementary irrigation, this coupled with high temperatures, creates conducive humid and warm environments for certain fungal and bacterial pathogens to proliferate. Disease pathogen development and multiplication is rapid when the climatic conditions are conducive for pathogen growth (Elad and Pertot, 2014). The shift in climatic conditions may provide ideal conditions for various diseases to invade certain areas where conditions were previously unfavourable, thus it is assumed that climate change impact will increase incidence and severity of diseases and pests (Mtisi and Prowse, 2012).

The majority of farmers in Domboshava are mainly relying on chemical disease control strategies to ensure enhanced yields. Fluctuations in weather conditions, a major component that has been affecting crop production over several years, has introduced so many uncertain variables in the cropping dynamics (Sarkar and Padaria, 2010). Temperature and rainfall are some of the aspects which have been used by farmers to predict climate since time immemorial. Thus, the changing, unpredictable occurrences in these two natural phenomenon, adds an element of marked

uncertainty to decisions farmers have to make regards their farming activities (Brown *et al*. 2012; Sarkar and Padaria, 2010). This uncertainty has the potential to influence the decisions made regarding which crop protection measures to implement. This becomes even more important since many farmers have observed that disease incidence and prevalence is also increasing in relation to fluctuations in weather conditions (Schlenker and Lobell, 2010). Fungal diseases are the main problem in the vegetable value chain from production, storage to processing (Salau and Shehu, 2015). Many developing countries, including Zimbabwe, are likely to be affected by variations in weather conditions, increasing their vulnerability to loss of food security through an inability to effectively cope with increased disease and pest pressures. According to Sarkar and Padaria (2010), India is vulnerable to climate change impacts, which has brought about increases in crop diseases, insect and pest attacks. Climate change in India has also decreased the land area being utilised for agricultural purposes and declines in soil fertility (Deuter, 2008). These impacts of climate change have consequently resulted in increased production costs for many smallholder farmers resulting in low incomes and deprived livelihoods.

The survey also revealed that frequency of diseases is correlated to the seasonality. This implies that prevailing temperatures and moisture conditions influence crop disease type and progression. Weather variability does therefore, alter the dynamics of disease development and spread in field crops, fruits and vegetables as it influences disease prevalence, incidence, and severity (Mina and Dubey, 2010). There are a wide range of vegetable fungal and bacterial diseases of importance, such as angular leaf spot in cucurbits, bacterial wilt in cucurbits, black rot in brassicas, bacterial canker in solanaceous crops, bacterial speck in solanaceous crops and bacterial spot (Douglas, 2003). Mildews are the other common diseases which occur in vegetables either as powdery mildew or downy mildew (Davis *et al*. 2008). These diseases result in quality and yield reduction of horticultural produce (Kader *et al*, 2003). Many vegetable producers aim for high yields but presence of diseases results in lower than the expected yields due to inadequate resources to purchase required chemicals (Siziba *et al*, 2003) and lack of knowledge and skills concerning disease management practices (Nyamupingidza and Machakaire, 2003).

Horticultural crops are known to be sensitive to growing conditions; and any changes in climatic attributes such as temperature and rainfall have the capacity to alter the growing patterns. Such

changes also affect flowering, time to maturity, crop quality as well as nutritional quality, which consequently influences their susceptibility to diseases (Deuter 2008). Vegetables exhibit environmental specificity whereby certain environmental and climatic conditions favour optimum growth with some vegetables needing cool weather conditions for example spinach (Kader *et al*, 2003). However, production of cool weather crops is becoming difficult under the current prevailing high temperatures in Zimbabwe. Abiotic stresses such as drought, heat, and moisture can increase a plants susceptibility to disease and produce symptoms such as wilting, leaf burn/scorching, leaf folding and chlorosis (Garret *et al*., 2006). Therefore, not all the symptoms observed by the respondents were a result of fungal or bacterial diseases.

Ngowi *et al.*, (2007) states that more and more farmers have adopted chemical control as the most effective method of dealing with crop diseases as an assurance to enhanced yields. This supports the results of this survey which indicated that 96.2% of the respondents rely on chemical disease control. Most cultural methods that were previously used to control diseases are no longer as effective (Ogle, 2016). As a result, there has been an increase in the use of fungicides, insecticides, and pesticides, a development which, however, has resulted in many detrimental environmental consequences. Chemicals have also caused disruption and contamination of the natural ecosystems (Ntow *et al*. 2006). The increased indiscriminate and over reliance on chemical disease control methods has resulted in loss of important indigenous knowledge systems on utilization of natural bio-pesticides in disease management. There is an urgent need to integrate indigenous knowledge systems with scientific research findings. This move would emphasize the important role played by traditional culture, natural resource management and sustainable cropping practices to farmers. The majority of farmers have shifted from a sustainable, constructive farming approach to an exploitative and destructive approach which relies mainly on synthetic pesticides and soil additives (Mhaka, 2015).

The alternative use of natural bio-pesticides in disease control as part of their pest management strategies, is increasingly being neglected by the majority of farmers in Domboshava. They are resorting to quick farming solutions in relation to the intensified agricultural activities as a response to increased population and food security issues (Potts *et al.*, 2015). This supports the results of this survey which indicated that 0% of the respondents used any form of biological

disease control. Although studies have shown that 90% of people rely on traditional medicinal plants, the majority are utilizing them to treat common human ailments, with the limited indigenous knowledge of their botanical applications as crop protectants being further neglected (Smith-Hall, 2012).

3.5 Conclusion

The study indicates that farmers are aware of the changing disease patterns occurring in response to a number of factors, among which might be weather or climate variability. It was also revealed that farmers have resorted solely to chemical disease control strategies with very few practising cultural, mechanical or natural disease management strategies to manage disease problems in Domboshava area, Zimbabwe. It has also been brought to light that none of the farmers in this area are utilizing bio-pesticides or botanical disease management strategies. The implications here are that farmers might not be aware of the negative human health and environmental impacts associated with indiscriminate or over-use of chemicals. They may also be unaware of alternative bio-pesticide or botanical disease control strategies. This implies an urgent need to raise awareness among farmers on the concerns and issues surrounding indiscriminate chemical use, and also on the potential of bio-pesticide disease control alternatives. These can be done through a series of trainings, workshops and setting up of demonstration plots working within the communities through the Ministry of Agricultural Research and Extension Services (AGRITEX) and other extension services.

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CHAPTER 4

EFFICACY OF *Moringa oleifera* LEAF AND SEED AQUEOUS EXTRACTS AGAINST SELECTED PLANT PATHOGENS.

Abstract

Fungal and bacterial pathogens are among the group of crop pathogens which cause serious diseases and losses in most vegetable crops. However, effective chemical control methods are yet to be developed to manage these pathogens. An *in-vitro* study to determine the efficacy of Moringa leaf and seed aqueous extracts in controlling the growth of three fungal pathogens (*Pythium ultimum, Rhizoctonia solani and Phytophthora infestans*) and three bacterial pathogens (*Pectobacterium carotovorum subspp. brasiliensis*, *Pectobacterium atrosepticum* and *Dickeya dadantii*) was conducted. The concentrations levels of 0%, 5%, 10% and 15% aqueous were used for the *Moringa* leaf or seed aqueous extracts. Distilled water only was used as the negative control at the 0% Moringa aqueous concentration. The experiment was a 2 x 4 factorial laid out in a Randomized Block Design, with three replications. A copper oxychloride positive control was laid out as a separate experiment to prevent cross-contamination of the chemical into the bio-pesticide treatments during the duration of the experiment. The results showed marked significant growth suppressive effects being exerted by both plant aqueous extracts on fungal and bacterial growth ($P < 0.05$), on all the pathogens under study except for *D. dadanti.* However, the antimicrobial effect of the extracts was more effective in suppressing fungal pathogenic growth compared to the bacterial growth. There was no significant growth suppression exerted on *D. dadantii* bacterial strain from both leaf and seed aqueous extracts at all the concentration levels. The Moringa seed aqueous had greater inhibitory ability on the growth of the fungal (*P. ultimum, R. solani and P. infestans)* pathogens as compared to the growth suppressive ability of the leaf aqueous extracts on the same fungal pathogens. There was significant interaction occurring between the sources of aqueous extract and type of pathogen ($P < 0.05$). There is need for further studies on efficacy of these aqueous extracts on disease suppression in the field on crops of economic importance.

Keywords: bacterial pathogens, *D. dadantii,* efficacy, fungal pathogens

4.1. Introduction

Control of pests and diseases is critical for achieving improved crop yields and harvesting quality of farm produce (Nedunchezhiyan *et al*., 2010). The use of synthetic pesticides is a well-known method of controlling pests and diseases for improved yields and quality. This enables farmers to meet increasing food demands in response to the increasing world population (Aktar *et al*., 2009). It, therefore, becomes difficult and almost impossible for farmers not to use synthetic chemicals for improved crop productivity. Despite the effectiveness of some chemicals in controlling diseases, the toxicity of these chemicals endangers the environment and reduces natural biodiversity which impacts negatively on important pollinator species diversity (Garibaldi *et al*., 2013). Pollinators play a vital role in quality and yield of most horticultural crops worldwide. The use of chemicals has also negatively impacted human health (Soto *et al*., 1994). This is due to the prolonged residual effect in the soil (Aktar *et al*., 2009) from where they eventually get into the food chain leading to build up in bodies of animals and humans causing health problems (Soto *et al*., 1994; Choursis *et al*., 2012). Based on the documented negative residual impacts of pesticides on the environment, human and animal health, the world has become more 'health-conscious' and hence the market demand for organically grown products has been rapidly increasing (Rex, 2004; Borgen, 2004; Ramesh *et al*, 2010).

Organic products tend to attract specific markets which respond to demand for high quality and nutritious products for humans (Nedunchezhiyan *et al*., 2010). Organically produced products are said to taste better than inorganically grown and have a longer shelf life (Nedunchezhiyan *et al*., 2010). The introduction of organic farming practices in controlling pests, diseases or weeds enables farmers to use resources that are readily available to them on their farms. This results in significant reduction in expenses related to use of insecticides and fungicides (Doughari, 2012). This shift, therefore, calls for non-synthetic disease control strategies. Plant aqueous extracts from different plant and tree species are being widely used in organic farming in controlling pests, weeds and diseases. An example being *Moringa*, which is indigenous to the sub-Himalayan regions of northern India (Abalaka *et al*., 2012) and some parts of Africa, (Choursis *et al*., 2012; Foidl *et al*., 2001). Recent studies on the use of plant aqueous extracts have proven them to be an alternative to synthetic pesticides and herbicides. These aqueous extracts have also proven to be safe and

environmentally friendly (Preethi *et al*., 2010). *Rhizoctonia solani, Pythium ultimum* and *Fusarium solani* are some of the important pathogens that have been successfully controlled using various other tree aqueous extracts (Koleva *et al*., 2002, Das *et al*., 2010).

4.2 Importance of selected pathogens

Pythium ultimum, is a fungal pathon which produces oospores that survive in soil and plant debris for long periods of time, which then initiate disease infection and development in the following crop (Filonow, 1999). *Pythium ultimum* species can cause serious diseases in greenhouse vegetable crops resulting in significant crop losses (Punja and Yip, 2003). Several *Pythium ultimum* species, including *Pythium aphanidermatum,* Edson., *Pythium irregulare* and *Pythium ultimum*, are known to cause damping-off and crown and root rot in greenhouse cucumber, pepper and tomato crops (Bardin *et al*., 2004). *Pythium ultimum* species are commonly known to cause seed rot, damping off, root rot and soft rot of fleshy fruits (Filonow, 1999; Agrios, 2005). According to Paulitz and Baker (1987), *P. ultimum* infection results in poor seedling establishment, damping-off, uneven growth, leaf chlorosis, premature defoliation, death of severely infected plants and lowered yields in bean crop. In other studies, *Phytophthora infestans*, has been identified as a serious pathogen of potatoes and tomatoes (Seebold *et al*., 2008) which is not easily managed by chemical applications as it spreads rapidly once infection has set in a field. In Zimbabwe, *Pectobacterium atrosepticum,* a bacterial pathogen, has been listed as one of the major causes of blackleg and tuber soft rot (Ngadze and Icishahayo, 2014) in tuber crops. *P. atrosepticum* is also known to cause soft rot disease which results in losses of up to 100% to horticultural crops, whilst en-route to distant markets and during storage (Wijekoon *et al*., 2008; Krauthausen *et al.*, 2011). According to Panda *et al*., (2012), *Pectobacterium carotovorium subspp. brasiliensis* bacterium, has caused severe economic impacts in Brazil and South Africa among its wide range of hosts which include banana (*Musa paradisiacal, L.),* beans (*Phaseolus vulgaris,* L.*),* cabbage, carrot, cassava (*Manihot esculenta,* Crantz.) coffee (*Coffea arabica,* L.), corn (*Zea mays,* L.*),* cotton (*Gossypium hirsutum,* L.*),* onion, other crucifers, pepper, potato (*Solanum tuberosum,* L.*),* sweet potato (*Ipomoea batatas,* L.*)* and tomato.

Most literature indicates the antimicrobial activity of Moringa leaf and seed aqueous extracts without specifying the pathogens they are able to suppress (Price, 1985; Foidl *et al*., 2001; Nepolean *et al*., 2009). Studies with more specific focus determining the efficacy of Moringa aqueous extracts on specific, economicaaly important pathogens in crop production, therefore are necessary.

This study was conducted to determine which of the two Moringa aqueous extracts (leaf versus seed) would be more effective in suppressing the growth and development of bacterial (*P. atrosepticum (Pba), Pectobacterium carotovorum subspp. brasiliensis (Pcb), D. dadantii)* and fungal (*P. ultimum, R. solani, P. infestans*) pathogens. The pathogens were selected as study parameters based on their economic importance from the damage they cause to agricultural crops.

4.3. Materials and methods

4.3.1 Experimental site and sources of pathogens and plant aqueous extracts

The experiment was carried out at the University of Zimbabwe, Crop Science Department pathology laboratory as an *in-vitro* study. Pure cultures of fungal strains, *R. solani, P. ultimum, P. infestans*, and bacterial strains of *P. atrosepticum, P. carotovorum subsp. brasiliensis and D. dadantii* were obtained from the pathology laboratory. Fresh disease free Moringa leaves and the disease-free seeds were obtained from the Forestry Commission Head Office in Harare, Zimbabwe.

The Moringa leaf, bark and seed aqueous extracts were analyzed using the GC/MS method to identify the bioactive and phytochemical compounds of the Zimbabwean Moringa accessions as described by Dhen et al. (2014). The analysis was carried out at the Central Analytical Facilities, Stellenbosch University in the Mass spectrometry Unit, at Matieland, South Africa. Three different solvents were used for the plant extract phytochemical analysis: Dichloromethane (DCM), Methanol (MeOH) and Solid Phase Micro Extraction (SPME) (Appendix A).

4.3.2 Experimental design and treatments

The experiment was a 2 x 4 factorial experiment laid out in Randomized Block Design with 3 replications. Factor A was *Moringa* plant aqueous extracts at two levels: a) leaf (MLE) and b) seed (MSE); Factor B were the *Moringa* aqueous concentration levels at four levels: a) 0%, b) 5%, c) 10%, and d) 15%. The test subjects were the pathogens at six levels: fungi with three levels namely a) *P. ultimum,* b) *R. solani* and c) *P. infestans,* and bacterial also at three levels namely a) *Pectobacterium carotovorum subsp. brasiliensis, b*) *P. atrosepticum* and c) *D. dadantii*. At 0% aqueous concentration level, distilled water only was used. The positive control factor of copper oxychloride was set up separately to avoid and prevent any chance of cross contamination of the Moringa treatments during the duration of trial. Contamination of any kind would have introduced biases into the study.

4.3.3 Media preparation and sub-culturing

Media was prepared by mixing 39 g nutrient agar (NA) and 54 g Potato dextrose agar (PDA) to 1000 ml distilled water, separately in sterilized conical flasks. *P. infestans* is hard to culture on the general standard media, hence the need to modify the quantities of media used in this study by slightly increasing from the standardized levels (Sopee *et al.,* 2012). The modification to the quantities of distilled water used was to improve spread, growth and sporulation of the test organism and the dissolution of the Moringa aqueous extracts (Sabat and Gupta, 2009). The media was later autoclaved at 121^{o}C and 15psi for 20 minutes and was left to cool before pouring into 9 cm petri dishes. Pouring was done under aseptic conditions using the lamina flow cabinet and 10 ml of media was poured in each petri-dish and stored for future use. The pathogen culture were sourced from the Crop Science Pathology laboratory, the department provided pure pathogens cultures in quantities as per calculations in consultation with the department.

Bacterial strains were sub-cultured by streaking in nutrient agar (NA) and fungal strains by cutting a 5 mm square disc from pure culture of the strain using a new, sterilized stainless steel surgical blade and placed upside down in PDA and incubated at 35^{o}C and 25^{o}C, respectively.

4.3.4 Preparation of the Moringa aqueous extracts

The Moringa leaves and seeds were both first thoroughly washed with distilled water to remove any dust, dirt and debris from their surfaces and allowed to air dry at room temperature under dark conditions for seven days. After drying, both the leaves and seeds were ground to a fine powder using mortar and pestle as described by Suleiman and Emua (2009). The aqueous extracts were then prepared by separately mixing ground seed and leaf powders with distilled water. Leaf and seed powder weighing 5, 10 and 15 grams respectively for each aqueous extract were placed in sterilized beakers and 100 ml of distilled water was added to these beakers to make 5%, 10% and 15% aqueous extracts respectively as described by (Hussain *et al*., 2011; Hossain *et al*., 2012). The mixtures were then stirred continuously for 30 minutes using a sterile glass rod and allowed to stand at room temperature for 24 hours. After 24 hours, the aqueous extracts were separately filtered through a 3 layered cheesecloth and finally, through a sterile Whatman number 1 filter paper placed in a funnel as described by Zahir *et al*., (2009); Preethi *et al*., (2010); Chollom, (2012). The aqueous extracts were further filtered through 45 micrometer Nitrocellulose micro-filters for further purification to avoid contamination and to achieve a more purified aqueous solution.

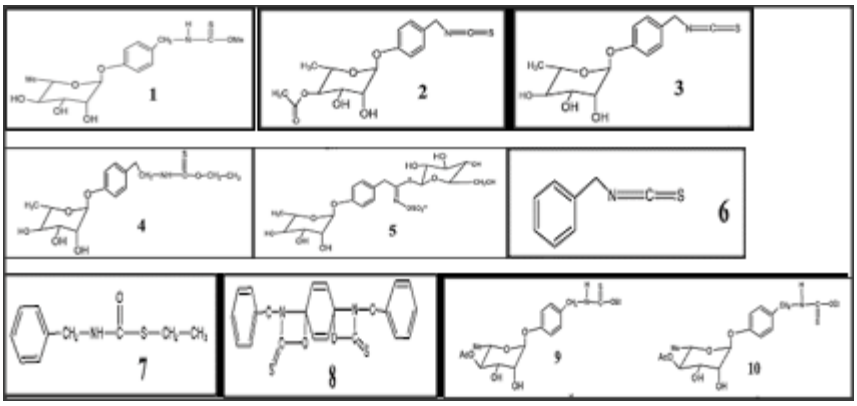
The three concentrations for each plant part and one control (distilled water) were then mixed with prepared PDA and NA in separate sterile petri dishes and later inoculated with fungal and bacterial pathogens respectively. 1ml of Moringa leaf and seed aqueous extract for each concentration level (5%, 10% and 15%), were added into each petri-dish containing molten potato dextrose agar (PDA) and nutrient agar (NA). Bacterial pathogen inoculation was done by streaking each of the different strains of bacteria individually onto the NA media where a 10mm line had been drawn at the center base of the petri dish thus streaking was along the drawn line. Fungal strains were inoculated by cutting a square piece from a pure culture and placing it upside down in the PDA. The plates were later incubated at 25^{o}C and 35^{o}C for fungi and bacteria respectively after which they were examined daily for the presence or absence of the fungal or bacterial growth for 7 days.

4.3.5 Pathogen growth measurements and data analysis

The data required to ascertain and monitor pathogen growth were obtained by measuring the width of the colony growth upon the streaked bacteria line in the petri-dish for the bacteria pathogens, and the diameter of the growth of fungal pathogens. Data was collected daily for seven days. The measurements for the fungal growth on diameter were inclusive of five-millimeter disc used during plating. The growth data for the fungal and bacterial pathogens was then analyzed using Genstat Version 14 statistical analysis package and the means were separated using the Fishers' Protected least significant difference (LSD) at 5% significant level.

4.4. Results

The results of the plant extract phytochemical analysis carried out on the Moringa plant extracts revealed the presence of flavonoids, serpenoids, terpenines and other active bio-active components (Plate 4.1, Appendix A).



Plates 4.1 – 4.10 Chemical structures of some of the bioactive compounds found in Zimbabwean *Moringa oleifera* accessions used in the study: 1. 4-(4′-O-acetyl-α-L-rhamnopyranosyloxy) benzyl isothiocyanate **2**. 4-(-L-rhamnopyranosyloxy) benzyl isothiocyanate **3.** Niazimicin **4.** 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate **5.** Benzyl isothiocyanate **6.** Aglycon of deoxy-niazimicine (N-benzyl, S-ethylthioformate) **7.** Pterygospermin **8.** Niaziminin **9&10.** O-ethyl-4-(α-L-rhamnosyloxy) and benzyl carbamate.

4.4.1 Antimicrobial action of *Moringa oleifera* aqueous extracts on fungal and bacterial growth

The Moringa seed aqueous extracts showed very strong antimicrobial activity, with the lowest growth being exhibited by *P. carotovorum subsp. brasiliensis* (Fig 4.5), *P. atrosepticum* (Fig 4.4),

P. infestans (Fig 4.3), and *P. ultimum* (Fig 4.1). The Moringa aqueous extracts' growth inhibitory action against the pathogens was lowest for *R. solani* (Fig 4.2), but showed almost no activity against *D. dadantii* ($P < 0.05$). The poorest antimicrobial activity of supernatant was also found against *D. dadantii* as the bacterium exhibited the highest pathogenic growth (80 mm for MSE and 85 mm for MLE at 15% concentration levels for both aqueous extracts) across both treatments ($P < 0.05$) (Fig 4.6).

However, there was evidence of within treatment effects on the antimicrobial action exhibited. There were significant differences in the antifungal and antibacterial activities existing between the MSE and MLE on the test organisms (Fig 4.1 – Fig 4.6) ($P < 0.05$). The antifungal action on *P. ultimum* was more pronounced for the aqueous MSE in comparison to the aqueous MLE (Fig 4.1). *P. ultimum* growth was lowest at 15% MSE concentration, and highest at 0% aqueous concentration. However, pathogen growth inhibitory effect for *P. ultimum*, was not significantly different at 5 and 10% MSE concentration levels ($P < 0.05$). The antifungal action exhibited against *P. ultimum* at 5%, 10% and 15% was not significantly different (Fig 4.1). The antifungal activity against *R. solani* was strongest with the MSE at 15% concentration level (50 mm growth) and highest pathogen growth was observed at 0% aqueous level (90 mm) (Fig 4.2). The antifungal action of the MSE decreased as the aqueous concentration levels decreased. The antifungal action against *R. solani* were significantly different from each other at all concentration levels (Fig 4.2). For the MLE however, the lowest pathogen growth rate for *R. solani* was recorded at 0% aqueous level (70 mm), whilst there was no significant difference among all the other concentration levels on growth (85 mm) of *R. solani*. The inhibitory effect of MLE on *P. infestans* growth was lower than that of MSE ($P < 0.05$) (Fig 4.3). *P. infestans* growth suppression was most pronounced at 15% MSE (25 mm) and at 5% MLE (35 mm) concentration levels respectively. However, MLE at 15% concentration had the lowest growth inhibiting effect on *P. infestans* (48 mm).

Moringa seed and leaf aqueous extracts had the greatest and most significant inhibitive impact on the growth of *P. atrosepticum* and *P. carotovorum subspp. brasiliensis* bacterial pathogens ($P < 0.05$) (Fig 4.4 and Fig 4.5). Compared with the growth rate of fungal pathogens, with that of bacterial pathogens, *P. atrosepticum* and *P. carotovorum subspp. brasiliensis*, the Moringa aqueous extracts suppressed growth of the bacterium more effectively than that of fungal pathogens. The highest growth attained by *P. atrosepticum* and *P. carotovorum subspp.*

brasiliensis bacterial pathogens was 21 mm (5% MLE); 21 mm (0% MSE); and 28 mm (5% MLE); 27 mm (5% MSE) respectively. The 15% MLE concentration produced the highest growth inhibition of *P. atrosepticum* pathogen (17 mm) which was significantly different from all the other concentration levels ($P < 0.05$); followed by 15% MSE (19 mm) which was significantly different from 0% (21 mm) (Fig 4.4). On the other hand, the highest growth rate achieved by all study fungal pathogens was in excess of 40 mm across all the concentration levels. The higher MLE levels (10 and 15%) resulted in lowered *P. carotovorum subspp. brasiliensis* growth rates across the treatments. However, 10% MSE concentration had greater inhibitory effect on *P. carotovorum subspp. brasiliensis* compared to 5 and 15% MSE concentration (Fig 4.5). Interestingly, it was different for *D. dadantii,* as the increase in MLE and MSE concentration levels resulted in increased pathogenic growth up to a certain point, after which it reached a constant for both MSE and MLE (Fig 4.6).

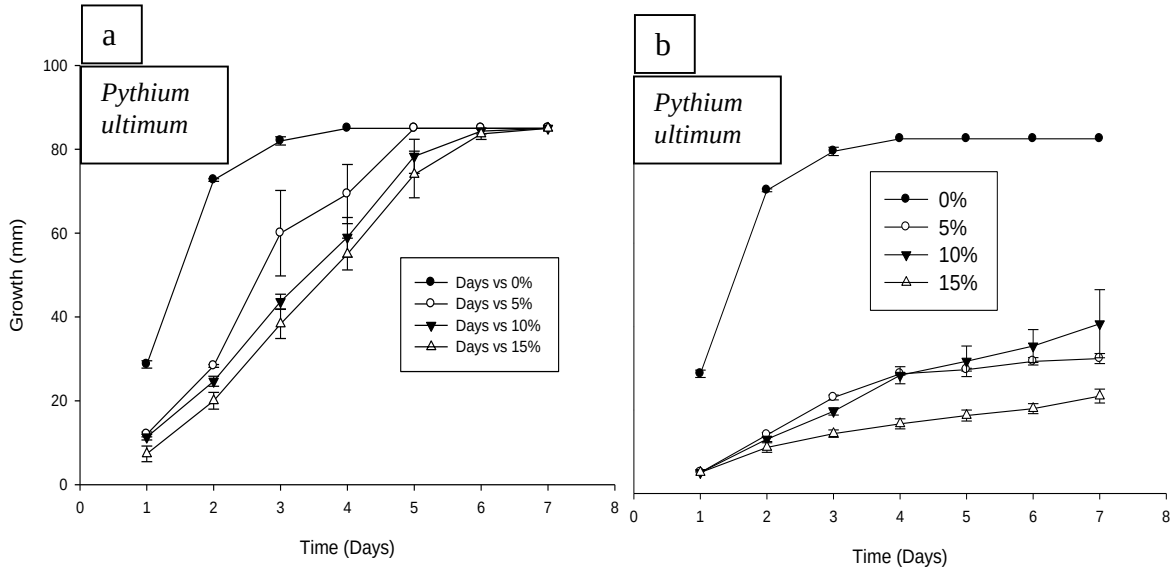


Figure 4.1 Antifungal activity of Moringa leaf (a) and Moringa seed (b) aqueous extracts on the growth *of Pythium ultimum*

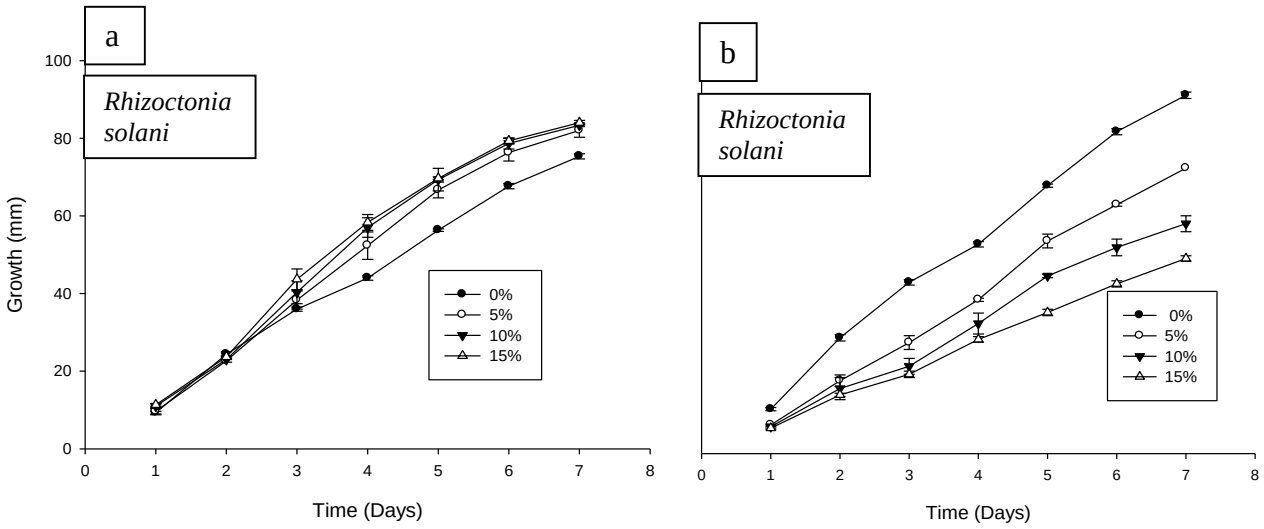


Figure 4.2. Antifungal activity of Moringa leaf (a) and Moringa seed (b) aqueous extracts on the growth *of R. solani*

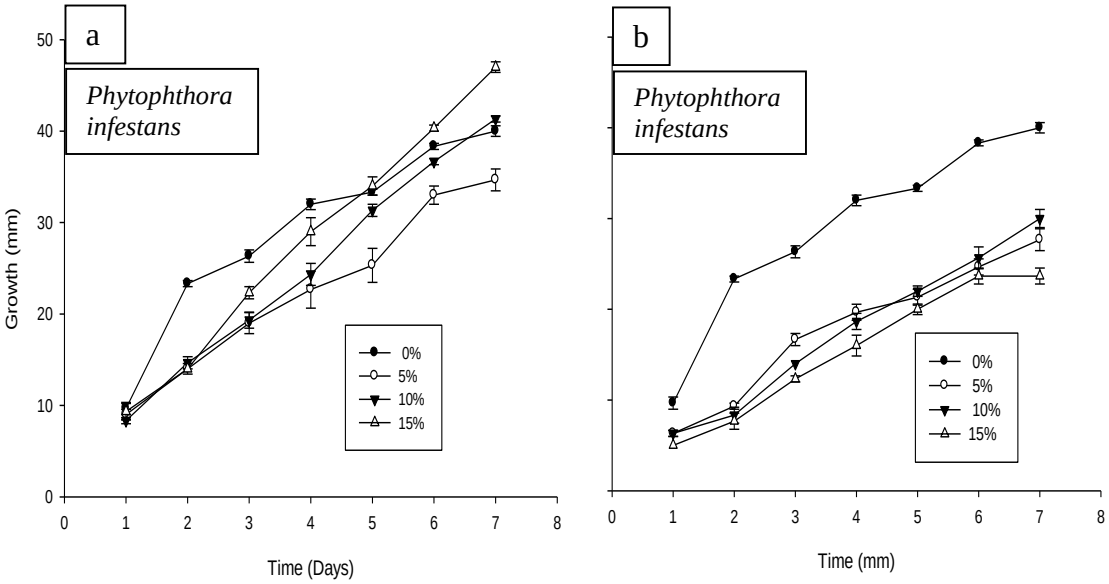


Figure 4.3. Antifungal effects of Moringa leaf (a) and Moringa seed aqueous extracts (b) on *P. infestans* growth

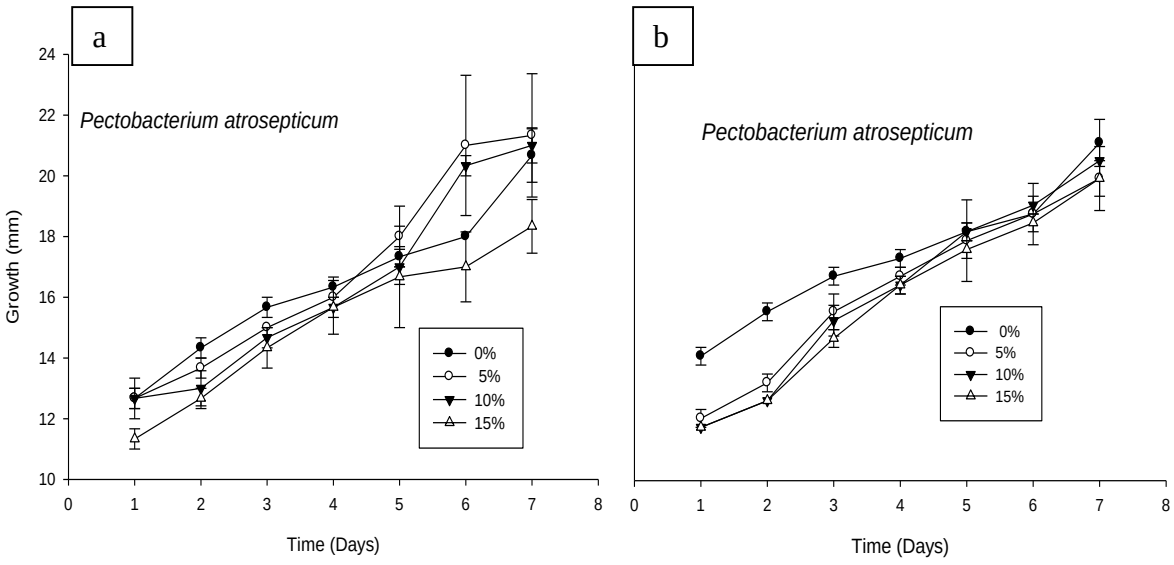


Figure 4.4. Antibacterial action of Moringa leaf (a) and Moringa seed (b) aqueous extracts on *P. atrosepticum* growth

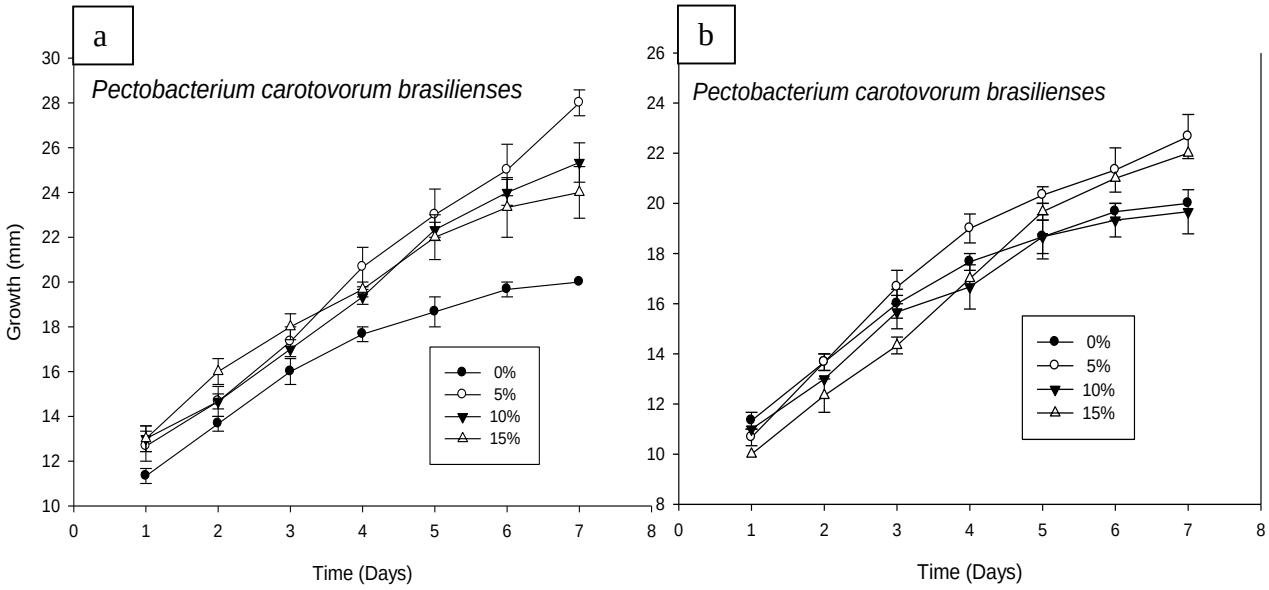


Figure 4.5. Antibacterial Effect of Moringa leaf (a) and Moringa seed aqueous extracts (b) on *P. c. brasiliensis* growth

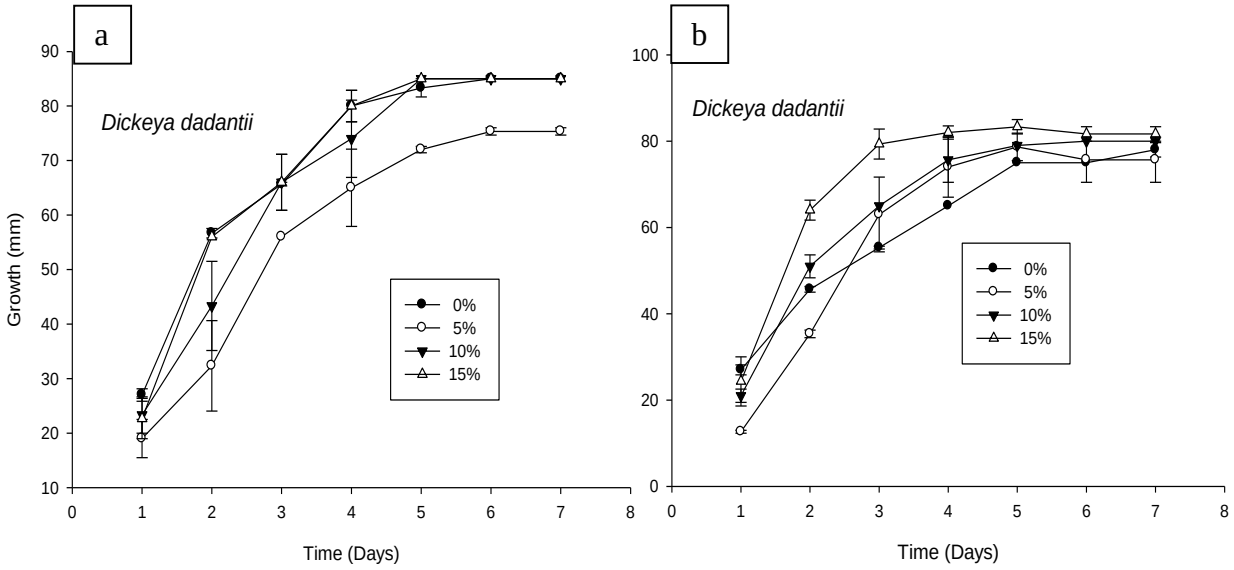


Figure 4.6. Antibacterial effects of Moringa leaf (a) and Moringa seed (b) aqueous extracts on *D. Dadantii* growth

4.5 Discussion

4.5.1 Antimicrobial action of phytochemicals in local Moringa accessions

The preliminary analysis of the leaf, bark and seed aqueous extracts of Moringa aqueous extracts used in the study revealed the presence of alkaloids, flavonoids, saponins and tannin (Appendix A). The presence of alkaloids and flavonoids reveal the efficacy of the plant against the pathogenic bacteria as Moringa seed and leaf aqueous extracts are highly bactericidal (Abalaka *et al*., 2012); containing active bactericidal substances such as *pterygospermin, moringine and glycosides*.

The Moringa leaf and seed aqueous extracts have varying degrees of antibacterial and antifungal activities against the test organisms, with the seed extract exhibiting stronger antifungal and antibacterial activity compared to the leaf extract. This could be as a result of the Moringa seed aqueous extract containing more of the *Pterylisgospermin* which is a bactericidal and fungicidal compound (Ogbunugafor *et al*., 2011; Caceres *et al*., 1991). The *Pterylisgospermin* compounds are found in higher concentrations in the seed compared to the leaf, causing the Moringa seed aqueous extracts to have a higher concentration of this bioactive compound, as the main bioactive ingredient. This resulted in stronger anti- fungal and -bacterial action being exhibited in the

Moringa seed compared to the leaf extracts. However, given the fact that the precise functioning, mode of action, and effect of these various metabolites present in Moringa is yet to be determined, there exists need for more studies to explain this observed phenomena (Prost, 2005).

The Moringa aqueous extracts used in this current study seem to have exhibited antibacterial and antifungal action against test pathogens due to the presence of 4-(4′-O-acetyl-α-L-rhamnopyranosyloxy) benzyl isothiocyanate; 4-(-L-rhamnopyranosyloxy) benzyl isothiocyanate; 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate and Benzyl isothiocyanate bioactive compounds. These phytochemicals infer strong pathogen growth inbiting properties (Akinbode and Ikotun, 2008; Al-Malki and Rabey, 2015).

The Moringa seed aqueous extracts showed strong antifungal activity against *P. ultimum, R. solani* and *P. infestans* fungal pathogens at all concentration levels compared to the leaf aqueous. This might not only because of the high levels of the *Pterylisgospermin* present, but also the presence of approximately 30% oil which is found in Moringa seed (Abalaka *et al*, 2012). When the seed was ground into a powder during extract preparation and the whole extraction process, oil was pressed out and released into the Moringa aqueous extract solution. This resulted in the Moringa seed extract having a frothy and saponificaed appearance which might have enhanced its antifungal efficacy, and also increased the contact time between test pathogens and extract. This improved contact is due to the Moringa oil being a good absorbent and moisturizer, which further created a conducive wet environment which enhanced the antifungal activity (Anwar *et al*., 2007; Bukar *et al*., 2010).

4.5.2 Antibacterial action of Moringa leaf aqueous extracts

The strong antibacterial activities of the aqueous leaf extract against *P. atrosepticum* might suggest that the extracts may be part of an integrated crop disease management strategy to suppress damage caused by these species. The stronger antibacterial activity of *Moringa* leaf extract compared to seed extract, might be linked to the presence of specific phytochemicals or bioactive compounds in the Zimbabwean Moringa accessions (Plates 4.1 – 4.10). For instance, the short poly-peptide, 4 (ά– L – rhamnosyloxy) benzyl- isothiocyanate is an important pathogen growth inhibitor. This bioactive peptide acts directly on microorganisms, resulting in growth inhibition by disrupting cell

membrane synthesis or the synthesis of essential enzymes (Abalaka *et al.,* 2012). The effectiveness of the Moringa aqueous extracts in suppressing *P. atrosepticum* pathogens' growth might also be attributed to the ability of *Moringa* leaf aqueous extracts to secrete phenolic compounds which inhibit the growth of micro-organisms including pathogens (Luqman *et al.,* 2012; Guest and Brown, 1997).

4.5.3 Effect of Moringa seed and leaf aqueous extracts on *Dickeya dadantii*

However, both Moringa seed and leaf aqueous extracts could not suppress the growth of *D. dadantii* bacterium. Application of both Moringa aqueous extracts to *D. dadantii* resulted in accelerated or rapid growth of pathogen. This observed phenomenon implies the need for further studies using higher extract concentration rates and longer duration of such studies. The rapid growth of *D. dadantii* pathogens in response to Moringa extract applications, can be attributed to its capacity to utlize both aqueous extracts as a food source for its own growth and proliferation (Zimmerli *et al*., 2000). In the absence of adequate research to provide an answer to the observed response of *D. dadantii* to Moringa extracts, there is great emphasis on the need for further studies at bio-molecular level. This approach would provide more valuable data and information given the fact that some pathogens are known to trigger potentiation of pathogen-specific cell reactions by the production of non-protein amino acids such as g-aminobutyric acid (GABA) and b-aminobutyric acid (BABA). These non-protein amino acids affect the biological functioning within the plant cells, which might trigger rapid cell multiplication (Zimmerli *et al*., 2000). However, although the mode of action of these non-protein amino acids are well documented regarding their effects for a number of animal pathogens, their mode of action has remained very unclear in plant pathogens (Zimmerli *et al*., 2000). This indicates an existing knowledge gap which needs further study.

It is not clear, however, why both *Moringa* aqueous extracts were more effective in suppressing the growth of the bacterial pathogens, compared to fungal pathogens. There are a lot of biotic and abiotic factors which influence reaction rate and intensity of pathogens to external stimuli such as tempearature and exposure time (Agrios, 2005). More research needs to be conducted to study the impact of the *Moringa* aqueous extracts on pathogenicity development at molecular level using

molecular markers. Although this study was repeated, there still exists a number of questions which were raised by the observations, and this calls for further research.

4.6 Conclusions

Moringa leaf and seed aqueous extracts might contain antimicrobial compounds which inhibited the growth of *P. ultimum, R. solani and P. infestans* (fungi) and *P. carotovorum subspp. brasiliensis*, and *P. atrosepticum* (bacteria). Additionally, both aqueous extracts were more effective in inhibiting bacterial pathogens compared to fungal pathogens. The inhibitory effect of the seed aqueous extracts was, however, more pronounced compared to the leaf aqueous extracts. There is need for further studies into understanding the mode of action of the antimicrobial compounds found in *Moringa* and the response of crop plants to the application of these compounds. There is also need to identify the specific mechanisms of the bioactive compounds present in the Moringa aqueous extracts in their antimicrobial activity. This will lay a firm foundation on which to base the application of *Moringa* aqueous extracts in controlling plants pathogens as part of an integrated approach to managing plant diseases of economic importance.

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CHAPTER 5[1]

MORINGA OLEIFERA EXTRACTS EFFECT ON FUSARIUM SOLANI AND RHIZOCTONIA SOLANI GROWTH

Abstract

An invitro study was conducted to test the effect of concentration levels of *Moringa oleifera* leaf and seed extracts in controlling the growth of *Rhizoctonia solani* and *Fusarium solani* pathogens The experimental design was a 2*7 factorial laid out in a Completely Randomized Design. Potato dextrose agar was amended with Moringa leaf extract and seed extract and mycelial growth of *R. solani* and *F. solani* were measured. This study was carried out at the University of Zimbabwe pathology laboratory during 2014/ 2015 season. **C**oncentrations levels of 10%, 15%, 20%, 25% and 30% from each extract were used. Distilled water (0%) was used as negative control, whilst 10% copper oxychloride was the positive control. Potato Dextrose Agar was amended with Moringa leaf extract and seed extract and mycelial growth of *R. solani* and *F. solani* were measured. All extracts showed a significant effect on reducing fungal growth (P=0.05). The higher the extract concentration level, the less the mycelial growth and no mycelial growth occurred on the positive control (10% copper oxychloride). Maximum percentage inhibition of 45 and 50 was recorded against *R. solani* using Moringa seed extract at 25 and 30% concentration respectively. Both Moringa extracts gave 50% inhibition growth of *F. solani* at 30% concentration levels. Moringa leaf and seed extracts contain antifungal properties which inhibited growth of *R. solani* and *F. solani*. Moringa extract concentration levels influenced the antifungal efficacy of the extracts, with higher concentration levels exhibiting an increased antifungal ability against the test pathogens. The phytochemical analysis of Moringa leaves and seed solvent extracts showed presence of Alkaloid, Flavonoid, Glycosides, Tanin and Phenolic compounds, Terpenoid etc.

[1] This chapter is based on a paper published as follows: M. Goss, P. Mafongoya and A. Gubba. 2017. *Moringa oleifera* Extracts Effect on *Fusarium solani* and *Rhizoctonia solani* Growth..*Asian Research Journal of Agriculture. 6(1): 1-10, ISSN: 2456-561X*

Keywords: concentration, growth inhibition, antifungal

5.1 Introduction

Fusarium solani and *Rhizoctonia solani* are important pathogens that infect many different genera of plants; they mainly cause serious root rot and stem rot on vegetables and ornamentals (Latiffah *et al.,* 2011). Root-rot caused by *Fusarium solani* is considered among the most deleterious diseases, resulting in major losses in brassicas and cruciferous vegetables in many parts of the world (Celar *et al.,* 2005). *Fusarium* species play an important role as plant pathogens, causing a wide range of disease such as vascular wilts and stem rots in a diversity of hosts (Carter, 1979). The fungus *Rhizoctonia solani* causes damping-off and wire stem of cabbage, cauliflower, other crucifer seedlings in the seedbed; and bottom rot and head rot of older plants in the field (El-Nagdi and Abd-El-Khair, 2008). Further studies have indicated *F. solani* and *R. solani* as being the most important soil borne fungal pathogens which cause the majority of the devastating diseases which exhibit symptoms of bottom rot and root rot diseases to a wide range of vegetables and other cruciferous crop plants including lettuce (Nawar, 2007). *Fusarium solani* and *Rhizoctonia solani* are listed among the major problematic soil-borne plant pathogens causing drastic reductions in yield and quality of crops in agricultural production world-wide and are also responsible for causing damping off, root-rots and wilts of vegetables (El-Mougy *et al*., 2012). The deleterious effects of these harmful parasites on crop stand, quantity and the quality of crops produced has resulted in an intensive usage of fungicides as a way of managing them. However, these management strategies are not only ineffective in achieving satisfactory control of the root diseases, but the intensity of their usage has raised concerns over their negative impact on the environment, fauna and flora, and human health (El-Mougy *et al*., 2012). This has also impacted negatively on overall productivity of cropping enterprises as a result of the increased input costs acquiring fungicides. There arises an urgent need therefore, to identify alternative methods and strategies to manage these soil-borne pathogens which are environmentally friendly and cost-effective in the long run. In order to identify such alternative strategies, medicinal plants such as the *Moringa oleifera* need to be researched upon and validations made regards their antimicrobial properties (Ali *et al*., 2004). This aim of this study was to evaluate the efficacy of Moringa leaf

and seed extracts against *Fusarium solani* and *Rhizoctonia solani* as an invitro experiment. There was also the need to determine at which concentration level the extract was most effective in inhibiting pathogenicity of the test pathogens. Validation of the antimicrobial action of the Moringa leaf and seed extracts would then enable further research into improving on extraction, identification and proper isolation of the bioactive compounds to enhance their effectiveness in field applications in agriculture.

Moringa is a multi-purpose tree which is being utilized for various applications in industry, traditionally and recently, a lot of research has gone into its medicinal use against human pathogens (Anwar *et al.,* 2007). However, not much research has been carried out regards the efficacy of Moringa as a Natural Bio-Agent against devastating crop pathogens of economic importance (Adline and Devi, 2016). Research on Moringa medicinal properties and its effectiveness against human diseases, its benefits to human and animal health and on human pathogens has been accumulating over the years as researchers are more fascinated with its anti-carcinogenic, antiulcer properties, improved human life (Adedapo *et al*., 2009; Aora *et al*., 2013) and reduction of human obesity (Bais *et al*., 2014). Further studies have revealed Moringa as an ideal supplementation in livestock feed formulations as a result of its high nutritional constituent (Banjo, 2012) and other yield benefits such as increased milk production dairy animals (Foidl *et al.,* 2001). The focus therefore has been, and continues to be, on improving human livelihoods and health using natural plant sources and this has overshadowed its importance and potential application as a bio-pesticide. Besides being packed with antioxidants, minerals, vitamins and having proven medicinal value for both human and animal benefit (Choudhury *et al.,* 2013), Moringa can be effectively utilized as a natural bio-pesticide and should therefore, be included in integrated pest management strategies once more focused research in its efficacy, extraction methods and extract preparations are carried out extensively and validated. Moringa can be used as the alternative crop disease management strategy due to the various bioactive ingredients it contains, that act in different ways against pathogenic infection within the plant (Holetz *et al*., 2002) which might even prove more effective compared to intensive and continued fungicide usage which results in problems such as; development of resistance by fungi to systemic fungicides and the specificity of fungicide formulations which affect only one pathway in the biosynthesis of fungi pathogens (Ogle, 2016), processes which reduce the efficacy of fungicide action. Hence, use of biological agents such as Moringa bioactive compound derivatives in control of soil-borne fungi pathogens notorious for

root-rot diseases, as a management strategy might prove to be more effective compared to fungicides (El-Mougy *et al.,* 2012). Not only are these bio-agents environmentally friendly compared to chemical methods, they have been used in various invitro studies to effectively inhibit pathogen growth (Najar *et al.,* 2011).

Moringa has been used in combination with other medicinal plants in invivo studies in the control of some pathogens. Moringa has been evaluated in an invitro study, for the control of *Rhizopus* pathogen which is a problem organism causing food spoilage and huge losses (Bukar *et al.,* 2010). Although many studies have been carried out to evaluate and assess Moringa efficacy against disease pathogens, the majority of these have been on human pathogens where it has been proven effective in invitro studies against *Salmonella typhi, Citrobactor spp, Shigella dysenteriae, Escherichia coli, Salmonella paratyphi* and *Pseudomonas aeruginosa* (Abalaka *et al*., 2012). Due to increased awareness about the risks involved in use of pesticides, much attention is being focused on alternative methods of pathogen control. There is a need to examine possible non-synthetic chemical approaches for disease management (Adandonon *et al.,* 2006) in agricultural applications. Research has demonstrated that biological disease control (Kuri *et al*., 2011) is a potentially feasible alternative to the use of pesticides. *Moringa oleifera* leaf extracts in combination with other bio-control agents have been successfully used as a seed treatment against *Scerotinia rolfsii* which causes damping off and stem rot in cowpea (Adandonon *et al.,* 2006). Based on these preliminary studies, it is imperative that Moringa bioactive agents efficacy be evaluated invitro against a wide range of crop pathogens which are deleterious in their impact on quantity and quality of produce and also which have proven to easily develop fungicide resistance (e.g. *Fusarium solani* and *Rhizoctonia solani,* the study organisms in this study). It is only after invitro studies have validated the effectiveness of Moringa bioactive agents against crop pathogens, can it then be effectively utilized by developing and adapting efficient extraction methods, correct formulations and determine correct application methods, rates and intervals. There are still so many areas of research regards *Moringa oleifera* based on its constituents, botany and phytochemical traits which need to be carried out to improve utilization of the bioactive phytochemicals present in Moringa as a bio-pesticide and a natural bio-agent in agricultural applications. The Moringa plant provides a rich and rare combination of zeatin, quercetin, kaempferom and many other phytochemicals that can inhibit the development of these pathogenic

fungi (Farooq *et al*., 2007) once adequate studies into its ideal extraction and application procedures have been exhausted in field research.

5.2. Materials and methods

5.2.1 Experimental Design

The experimental design which was used to carry out this study was a 2*7 factorial laid out in Completely Randomized Design replicated three times. Factor A was *Moringa oleifera* leaf and seed extracts at two levels; factor B was the concentration levels of the extracts (10, 15, 20, 25, and 30%). Negative control 0% concentration level and positive control 10% copper oxychloride (this is the main fungicide used locally for fungal disease control by most farmers in Zimbabwe). The Moringa leaf and seed plant extracts were analyzed using the GM/GC technique using three different solvents to identify the bioactive compounds and phytochemical constituents.

5.2.2 Preparation of medium and sub-culturing

Medium was prepared by mixing 54 grams Potato Dextrose Agar (PDA) with 1000ml distilled water, in sterilised conical flasks. The medium was later autoclaved at 121°C and 15psi for 20 minutes and left to cool before pouring into 9 cm petri dishes. Pouring was done under aseptic conditions using the lamina flow cabinet and 10ml of medium were poured in each petri-dish and stored for future use.

5.2.2.1 Isolation of *Fusarium solani*

The pathogen was isolated from cabbage which had yellows symptoms. The infected leaves were surface sterilized in 10 percent hypochlorite for 5 minutes and rinsed three times in sterile water and blot dried with sterile filter paper. The pieces were plated onto Potato Dextrose Agar and incubated at 25ºC for 7 days. The *F. solani* was then purified by sub-culturing the whitish cottony mycelium with some tinge of pink (Barnett and Hunter, 1972) and canoe shaped conidia which are slightly curved and septate or aseptate.

5.2.2.2 Inoculum preparation

One week old culture of *F. solani* was used. The culture was carefully teased with a sterile inoculating loop to dislodge the spores. A concentration of approximately 10^8 cfu per ml sterile distilled water was used. An Improved Neuber Haemocytometer was used to count the spores. The fungal strains were then introduced into the medium by cutting a 5 mm square disc from pure culture of the strain and placing them upside down in PDA and incubated at 25°C.

5.2.3 Preparation of Moringa oleifera seed and leaf extracts

The leaves and seeds for the Moringa extracts were obtained from Herbal Health Centre, an organic grower of Moringa and herbal plants in Harare. Both leaves and seeds were first thoroughly washed with distilled water to remove any dust debris and later air dried at room temperature under dark conditions for seven days. After drying, both the leaves and seeds were ground to a fine powder using mortar and pestle as described by Suleiman and Emua (2009). Aqueous extracts were prepared by separately mixing grounded seed and leaf with distilled water. Leaf and seed powder weighing 10, 15, 20, 25 and 30 grams were placed in sterilised beakers and 100 ml of distilled water was added to make 10, 15, 20, 25 and 30% extracts as described by (Hassan *et al.*, 2012). The mixtures were then stirred using a sterile glass rod and allowed to stand at room temperature for 24 hours. The extracts were separately filtered through a sterile Whatman number 1 filter paper placed in a funnel as described by (Okoi *et al.*, 2016). The extracts were further filtered through the microfilter for further purification to avoid contamination.

The five concentrations for each plant part and one control (distilled water) were mixed with prepared PDA in separate sterile petri dishes and later inoculated with fungal pathogens. 1 ml extract of each concentration (10, 15, 20, 25 and 30%) of seed and leaf *M. oleifera* were added in each petri-dish containing potato dextrose agar. Fungal strains were inoculated by cutting a square piece from a pure culture and placing it centrally upside down into the PDA. The plates were later incubated at 25°C after which they were examined daily for the presence or absence of fungal growth.

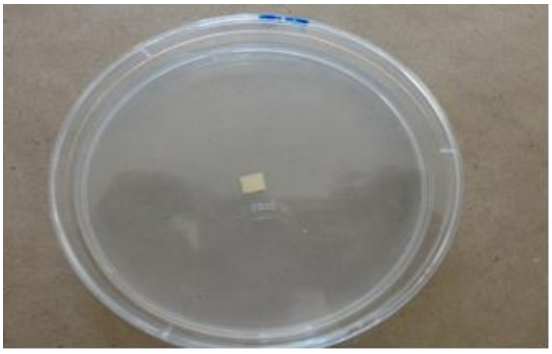

Plate 5.1: Fungal plating

The Moringa leaf and seed extract were analyzed using the GC/MS method to identify the bioactive and phytochemical compounds of the Zimbabwean Moringa accessions as described by (Dhen *et al*., 2014). The analysis was carried out at the Central Analytical Facilities, Stellenbosch University in the Mass spectrometry Unit, at Matieland, South Africa. Three different solvents were used for the plant extraction process namely, Dichloromethane (DCM), Methanol (Me OH) and Solid Phase Micro extraction (SPME).

5.2.4 Mycelia growth of the fungal pathogen

Mycelia growth of the fungal pathogens was measured in millimetres once a day for 7 days. Observations on colony diameter for the fungal pathogen were recorded and percent growth inhibition was worked out by using the following formula suggested by Whipps (1987).

$$\text{Percentage inhibition} = \frac{R_{1-R_2}}{R_1} \times \frac{100}{1} \qquad (1)$$

Where,

R_1 = Furthest radial distance of fungus in control plates (PDA only)

R_2= Furthest radial distance of fungus in treatment plates.

5.2.5 Data Analysis

The effectiveness of *Moringa oleifera* leaf and seed extracts was analysed using (Genstat version 14) statistical package and significant results were subjected to a LSD test at 5% significance level.

5.3. Results

5.3.1. Effect of concentration levels of Moringa extracts on radial growth of *R. solani* and *F. solani*

The effect of concentration levels was significant ($P<0.05$) on pathogen growth mean of *R. solani* and *F. solani* compared to the control (Table 5.1). The effect of extract was more pronounced at higher concentrations: the higher the extract concentration the less the mycelia growth and least colony mycelium growth was formed on PDA amended with copper oxychloride (10 ml). There was no significant difference for Moringa extracts of 25 and 30% on radial growth of *F. solani*.

Table 5.1: Effect of concentration levels on radial growth of R. solani and F. solani growth

Concentration levels (%)	**Radial growth (cm)**	
	R. Solani	***F. Solani***
Distilled water (0)	7.82^{f}	10.62^{f}
Copper oxychloride (10)	0.06^{a}	0.50^{a}
Moringa extracts (10)	6.36^{e}	7.91^{e}
Moringa extracts (15)	5.32^{d}	7.52^{d}
Moringa extracts (20)	4.70^{c}	6.77^{c}
Moringa extracts (25)	4.35^{bc}	6.35^{b}
Moringa extracts(30)	4.05^{b}	6.06^{b}
P-value	P<0.05	P<0.05
L.S.D	0.5	0.42
C.V%	11.2	7.3

NB: means with the same letter are not statistically different from each other at $P \leq 0.05$

5.3.2 Interaction of Moringa extracts and concentration levels on radial growth of *F. solani*

Results in Figure 5.1 indicated that there was significant interaction effect of Moringa extracts and concentration levels ($P\leq0.05$), with decrease in F. solani growth with increase in extract

concentration. Moringa seed extracts showed better inhibition of radial growth of *F. solani* at concentration levels of 20 and 25%. There were no significant effect on pathogen growth for both extracts at concentration levels of 10 and 15%.

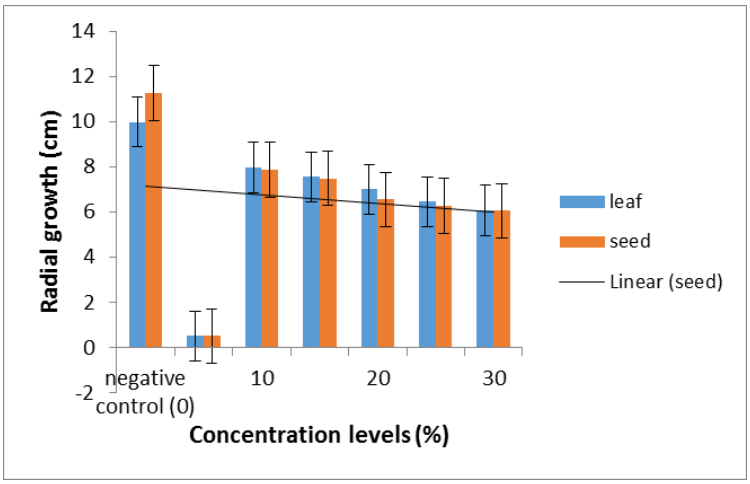

Figure 5.1: Interaction effects between Moringa extracts and radial growth of F. solani, June 2015. Bars represent least significant difference at $P \leq 0.05$.

5.3.3 Effect of concentration levels of Moringa extracts on percent inhibition of *R. solani*

The recorded percent growth inhibition for *R. solani* is presented in Table 5.2. There was significant ($P \leq 0.05$) response on the effect of concentration levels of Moringa extracts on pathogen growth. Moringa extracts at 30% concentrations showed stronger antifungal activity against *R. solani* than all other concentrations. Moringa extracts of 10% concentration levels had the least percent growth inhibition as compared to the positive control. The highest percent inhibition growth was recorded on the positive control with 10% copper oxychloride.

Table 5.2: Effect of concentration levels on percent inhibition of R. solani

Concentration (%)	Percent inhibition *R. solani*
Distilled water (0)	0[a]
Copper oxychloride (10)	93.97[g]
Moringa extracts (10)	19.33[b]
Moringa extracts (15)	33.26[c]
Moringa extracts (20)	40.44[d]
Moringa extracts (25)	45.96[c]
Moringa extracts(30)	50.33[f]
P-value	P<0.001
L.S.D	2.8
C.V%	14.4

NB: means with the same letter are not statistically different from each other at $P = 0.05$

5.3.4 Effect of Moringa extracts and concentration levels on percent inhibition *of F. solani*

The observations indicated that leaf and seed extracts significantly had an effect on the growth of *Fusarium solani* ($P \leq 0.05$) at different concentration levels (Figure 5.2). However, seed extract exhibited better growth inhibition compared to leaf extract at all concentration levels. Moringa extracts inhibitory effects on *F. solani* growth were more pronounced at their higher concentration levels, the higher the concentration the lower the percentage inhibition growth recorded.

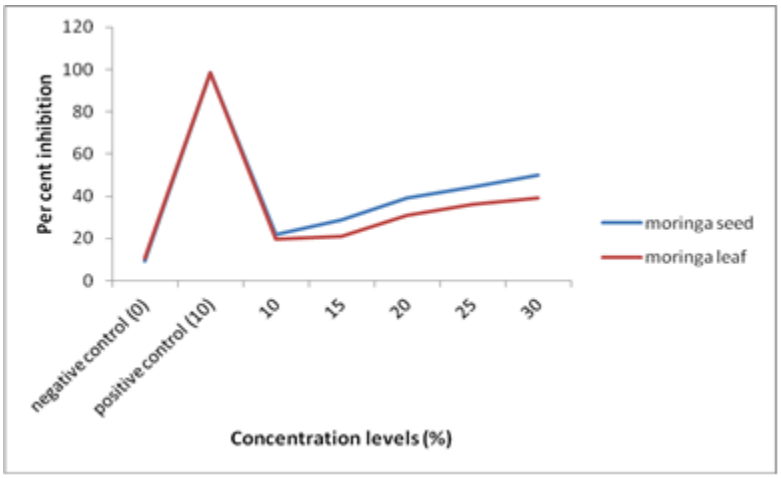

Figure 5.2: Effect of Moringa extracts and concentration levels on percentage inhibition of Fusarium solani, June 2015. Bars represent least significant difference at $P \leq 0.05$.

5.4. Discussion

Seed and leaf extracts both exhibited antifungal activity against both fungal pathogens, *Rhizoctonia solani* and *Fusarium solani* at all extract concentration levels. However, leaf extract was not as effective in its inhibitory properties compared to the seed extract, leading to the assumption that the seed contained more antifungal bioactive compounds which made it more effective. This could be because seed extract is said to contain more of *Pterygospermin* which is a fungicidal compound (Ali *et al*., 2004). Results further indicated a reduction in pathogen growth occurring gradually, with increase in the concentration levels of extracts in the growth medium. The increase of the concentration of the extract implied an increase in the active ingredients of the bioactive compounds which acted upon the fungus thereby affecting its physiological processes and consequently lowering the growth of the fungus. Studies done by Majali *et al* (2015), when they evaluated the antioxidant activity of Moringa extracts indicated the antioxidant and anti-inflammatory activities to influenced by the dosage rate used; with the higher dosage (300mg/kg) exhibiting enhanced activity over the lower dosage (100kg/mg) in the test subjects. These findings are also consistent with work done by Tijjani *et al* (2013) which revealed higher protection against castor-oil induced diarrhoea at dosages of 800mg/kg^{-1} compared with 400mg/kg^{-1} using *Detarium microcarpum* plant extracts as antidiarrheal agents. The highest records of reduction on radial growth of pathogen were recorded for Moringa seed and leaf extract at a concentration of 30%.

Moringa oleifera leaves contain some crystalline alkaloids, fatty acid, proteins, glycosides and niazirins which are believed to possess antimicrobial properties (Al Ameri *et al*., 2014). The phytochemical analysis which was carried out for the Moringa plant extracts used in this study revealed the presence of a wide range of bioactive compounds; among which were flavonoids/bioflavonoids such as 2-phenylchromen-4-one (2-phenyl-l,4-benzonpyrone), 3-phenylchromen-4-one (3-phenyl-l, 4-benzonpyrone), 4-phenylcoumarine (4-phenyl-l, 2-benzopyrone) and catechins (e.g. Flavan-3-ols) which possess antimicrobial properties (Ali *et al.,* 2004). As a result of these antimicrobial bioactive compounds present in the Moringa extracts, they were able to inhibit the growth of the test pathogens, albeit at different levels of efficacy. This difference might be explained by presence of higher amounts of Moringa oil in the Moringa seed extracts compared to the leaf extracts. Moringa oil is used extensively in the cosmetic industry for perfumery and skin products because it has a very good propensity to bind with other molecules (making it a good perfume base), and as such makes it a very good moisturizing agent as well (Chollom, 2012). These properties of Moringa oil are enhanced by their stability due to the presence of linoleic acid, its non-sticking ability, which are further enhanced by its ability to resist rancidity (Tesfay *et al.,* 2016). The higher ability of the Moringa seed extracts to inhibit pathogen growth, can therefore, be attributed to the seed extract being able to have enhanced and prolonged contact with the test pathogens within the PDA since it could easily be absorbed, enabling it to spread faster and more easily within the infected media due to its ability to bind readily with other molecules. This fact then improves its antifungal efficacy due to its more fluid viscosity, and longer more effective spread and contact time with the test pathogens. The presence of saponins in the Moringa extracts might also explain their ability to inhibit fungal growth. Studies done by Balamurugan and Balakrishnan (2013) revealed plant saponins to exhibit direct cytotoxic effects, a fact which might have been employed as an antifungal mechanism within the test pathogen cells. Furthermore, the presence of alkaloids such as the isoquinones, indoles, pyridines and benzyl-lisoquinolines present in both leaf and seed extracts, are known to infer inhibitory activity against several pathogens (Ncube *et al.,* 2008). In this current study, Moringa seed extract exhibited strong antifungal action against *R. solani* and *F. solani,* these are not entirely supported by work done by Zhao *et al* (2012) whose findings indicated very strong activity against *Botrytis cinerea,* yet very weak activity against *R. solani, C. orbiculare* and *S. sclerotiorum* using a bioactive secondary metabolite isolated from the Moringa roots.

Moringa oleifera is a multipurpose tree with a whole wide range of applications such as industrial, medicinal, nutritional, traditional and ayurverdic (Abdull *et al.,* 2014; Mishra *et al.,* 2011). However, despite all the research which is being focused on utilizations of Moringa, there are so many areas which are yet to be scientifically validated. For instance the greater percentage of Moringa research is focused on its nutritive, medicinal and health value to humans and livestock, with its phytochemical screening and analysis targeting these particular areas of interest, which then leaves a void in terms of how the nature and composition of Moringa can also be effectively applied to improving agricultural productivity, bearing in mind the need to use sustainable and environmentally friendly farming practices.

5.5. Conclusion

Moringa leaf and seed extracts can be effectively utilized as antifungal biological agents against R. solani and F. solani. However, these invitro studies need to be validated in field conditions to enable assessment of their efficacy within natural elements in the open fields. For Moringa to be effectively used as a natural biopesticide, many aspects of its phytochemistry, mode of action upon crop pathogens once it has been applied to crops, cost-effective extraction methods which take cogniscence of how easily Moringa denatures once exposed to harsh solvent and extraction processes, need to be taken into consideration. The antimicrobial action of Moringa can be and is attributed to a wide array of bioactive, phytochemicals which are present within it, thus implying that the modes of action upon bacterial and fungal organisms are different. In this fact, lies its efficacy and strength. The question which now needs to be addressed is how can this multi-array of phytochemicals within this tree be harnessed with as little interference of its natural genetic coding and antimicrobial natural modes of action? Only if scientist are able to achieve as little interference with the natural properties of this miracle tree will its full potential be harnessed for the benefit of our environment. Research has to shy away from isolating, characterizing and multiplying these phytochemicals aimed at reproducing them synthetically, thus altering the very code of these elements, and instead aim to study this plant in a bid to minimise modifying it and its properties to suit human need only.

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CHAPTER 6

IN-VITRO EVALUATION OF *Moringa oleifera* BARK EXTRACTS IN SUPPRESSING FOUR *Xanthomonas campestris* pathovars

Abstract

Xanthomonas campestris pathovars are listed among the top ten most devastating plant pathogens worldwide. Effective chemical control strategies have not yet been developed to manage the damage caused by *X. campestris* pathogens in crops of agricultural importance. In the current laboratory study, four *X. campestris* pathovars were used in evaluating the antibacterial effect of *Moringa* bark aqueous extracts. The experiment was conducted in the pathology laboratory, Crop Science Department at the University of Zimbabwe. The trial was carried out as a 4 x 4 factorial in Randomized Complete Design with 3 replicates. The Moringa bark aqueous extract concentrations used to evaluate the antibacterial effect for the suppression of the four *X. campestris* pathovars were 0, 5, 10, and 15%. Moringa antibacterial effect was evaluated by measuring the growth of bacterial colonies compared to the control. The results showed that all the Moringa bark aqueous levels had significant growth suppressing action on all 4 organisms ($P < 0.05$), with an increase in the bark aqueous concentration resulting in a decrease in colony length. There were no interaction effects. The highest bark aqueous concentration level (15%) resulted in slower establishment of all the pathovars. On the first day, 5% concentration level had already achieved bacterial colony length means of approximately 14mm, whilst that of 15% concentration had a mean colony length of approximately 11mm. The antibacterial action also indicated a degree of time dependency. Moringa bark aqueous extracts possesses antibacterial properties indicated by its ability in suppressing *X. campestris* pathovars growth at 5% significance level. In conclusion, Moringa bark aqueous extract can be utilized effectively as a natural bio-pesticide against *X. campestris* crop pathogens. However, there is need to validate these findings in field trials on brassica crops which are the major host plants for this pathogen.

Keywords: antibacterial, aqueous, bio-pesticide, pathovars

6.1 Introduction

Crop production losses incurred yearly are attributed to biotic and abiotic factors in the agro-ecosystem. Biotic factors include weeds (parasitic plants), insects and pathogens. According to Gilligan (2008), production losses due to pathogenic diseases can range from 30 – 100% annually. Xanthomonads (*Xanthomonas* species) cause numerous devastating diseases, especially the *Xanthomonas campestris* strains. They exhibit a very wide host range and are the causal agent of various plant diseases, many of which are economically important. The pathogenic pathovars of this bacterium have exceptional levels of specificity, causing them to be further classified on the basis of host plants they attack. Four of the approximate 125 pathovars of the species, have caused diseases of paramount importance in Zimbabwe (Berthier *et al.,* 1993). These are *X. campestris pv campestris, X. campestris pv. vesicatoria, X. campestris pv. malvacearum* and *X. campestris pv. phaseoli* which cause black rot of brassicas, bacterial spot of pepper (*Capsicum* group) and tomato *(Lycopersicon esculentum),* bacterial blight of cotton (*Gossypium hirsutum*) and common bacterial blight of beans (*Phaseolus vulgaris),* respectively.

Black rot is an intensively destructive disease of all crucifers which include cabbage, cauliflower, kale and broccoli, among others. It is a worldwide disease that is caused by the *X. campestris pv campestris* bacterium. Black rot is a seed borne, vascular disease which favors warm and humid conditions (Vicente and Holub, 2013). It is a major disease constraint for brassica vegetable production by smallholder farmers in many parts of Africa, including South Africa, Kenya and Zimbabwe. This disease can cause up to 100 percent losses as was encountered by smallholder farmers during the El Nino rains in 1998 in Tanzania (Massomo *et al.,* 2004). Initial symptoms of the disease are V-shaped lesions on the leaf edges, which may coalesce causing total wilting of the leaves, and veins of the infected tissue usually blacken and eventually rots occur as the disease progresses (Seebold *et al.,* 2008). Black rot renders a larger percentage of plants grown incapable of producing marketable produce, with the harvested produce also being unfit for storage or shipment (Seebold *et al.,* 2008). In Zimbabwe black rot is common in all of the five agro-ecological regions and the disease incidence ranges from 10% to 80% (Wulff *et al.,* 2002). Bacterial spot (*X. campestris pv. vesicatoria)* is a cosmopolitan disease of tomatoes. Although the disease occurs occasionally, it is a disease of great economic importance (Production Guidelines, 2013) because

it can cause huge losses and usually occurs simultaneously with another bacterial disease called bacterial speck (Delahaut and Stevenson, n.d.). The typical symptoms of bacterial spot are prominent water soaked lesions on both the fruits and the leaves. On the former, for bacterial speck disease, the lesions are more distinctive, corky, and black, whilst on bacterial spot, the lesions are surrounded by a yellow halo (Vincent and Holub, 2013; Fargier and Manceau, 2007). Most symptoms caused are superficial but they reduce produce marketability for both the fresh and the processing market (Extension, 1988).

The Bacterial blight disease of cotton is caused by *X. campestris pv. malvacearum.* The disease causes significant economic losses since it affects all the above-ground cotton plant parts, thus reducing the quality of the lint. Symptoms include seedling blights, leaf spots, boll blight and rot, and black arm on stem and petioles (Allen *et al.,* 2012). According to the Ministry of Agriculture, Mechanism and Irrigation Development (2014), this disease is still one of the five major diseases of cotton in Zimbabwe. Only two cultivars FQ902 and BC853 are said to be tolerant but not resistant to this disease. The disease can cause up to 100% losses since it attacks the base and the tip of the boll leading to the prevention of boll opening. Usually what causes the disease to take root is wrong diagnosis leading to wrong control measure as the disease symptoms mimic spray drift and Alternaria leaf spot (Ministry of Agriculture, Mechanism and Irrigation Development, 2014).

Common bacterial blight is a wide spread disease of the common bean that is caused by *X. campestris pv. phaseoli*. Symptoms of the disease occurs on the leaves, stems, flowers and even seed as leaf lesions, and sunken pod lesions which are dark red to brown in color (Oreille, 2009). Symptoms on the seed are hard to observe visually but infected plants become stunted and may even die. The disease can cause up to 40% yield loss and in Zimbabwe it poses a threat to both smallholder and commercial farmers in agro-ecological regions II, III and IV (Karavina, 2011). Feasible methods of control being implemented include cultural and preventative control methods which include use of certified seed, control of weeds and volunteer plants, and removal and destruction of diseased plants. Biological control using *Bacillus subtilis* has been used in Zimbabwe on *X. campestris pv campestris* and it has proven to be effective on different Brassica species during the dry and short rainy seasons in Zimbabwe (Wulff *et al.*, 2002). No satisfactory

chemical control however, has been established yet for all the four *Xanthomonas campestris* pathovars. This is mainly because chemical applications are done when crop has already been infected and symptoms have been observed. This then renders this method marginally ineffective in disease suppression, and in some cases, the chemicals may even cause pathogen resistance (Wulf *et al.*, 2002)

There is, therefore, a need to identify other easier, effective, environmentally and user friendly ways to not only prevent, but also control the bacterial agents for these economically devastating diseases. The main objectives of this study were to 1) assess the most effective Moringa bark aqueous extracts' concentration level which would result in increased efficacy in suppressing growth of the different *Xanthomonas campestris* pathovars; and 2) determine which *Xanthomonas campestris* pathovars can be significantly controlled by Moringa bark aqueous extracts. The bark aqueous was the only test Moringa aqueous extract due to seasonal variations in availability of Moringa plant products. The seasonality is as a result of the tree shedding most of its foliage during the cooler temperature periods of the year. The growth thus is slowed down which affects the biomass which can be harvested per tree.

6.2 Materials and methods

6.2.1 Preparation of the Moringa bark aqueous extracts

The experiment was an *in-vitro* study conducted in the Crop Science Department, plant pathology laboratory. *X. campestris* bacterium were isolated and cultured according to National Committee for Clinical Laboratory Standards and Watts (1999) procedure. Moringa bark powder was obtained from Forestry Commission Head Office, Harare, Zimbabwe.

The study was laid out as a 4 x 4 factorial experiment in a Randomized Complete Design. The only factor were the different Moringa bark aqueous concentrations (0, 5, 10, 15%), whilst the test organisms were the four *X. campestris pathovars:* a) *X. campestris pv. campestris,* b) *X. campestris pv. phaseoli,* c) *X. campestris pv. vesicatoria,* and d) *X. campestris pv. malvacearum* pathovars. The bark aqueous extracts were prepared by separately suspending 5, 10, and 15 grams of pulverized Moringa bark powder in 100 mls of sterile water respectively. The samples were shaken

for 30 minutes and allowed to sediment at room temperature for 24 hours. After 24 hours, each separate concentration aqueous extract was filtered firstly through triple layered cheesecloth, then through four sterile Whatman number 1 filter papers. Then last filtration was through syringe micro filters to obtain a pure bark aqueous extract solution. The micro filters were used to remove any microbes or microscopic materials that could cause contaminations after reacting with the agar.

6.2.2 Preparation of *Xanthomonas campestris pathovars*

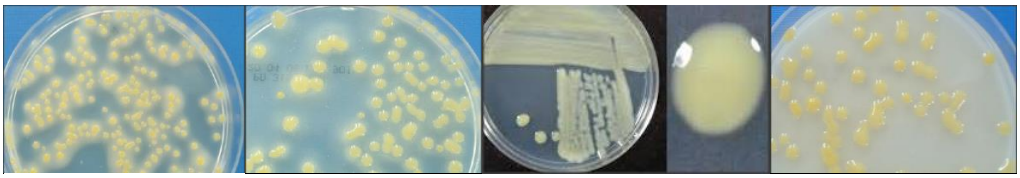
Two infected leaves/plant were collected from cabbage, green pepper, cotton and green beans growing in infected fields (Figure 6.1 a – d) early in the morning (07:00hours). These were placed and secured separately into polypropylene packets and taken to the laboratory for testing. The leaves were then shoursedded into small pieces (0.20 cm^2), placed into separate conical flasks containing 50 ml of distilled water. These were shaken for ten minutes and the resultant water was directly used for plating. 1 ml water from each separately prepared mixture, was placed into a sterile *petri dish* and 15 ml of melted medium was added to this, followed by rotary shaking for 20 minutes. After solidification of medium, the plates were incubated at 28±1°C.

A. **B.** **C.** **D.**

Figure 6.1: Plant leaves infected four different strains of *X. campestris*: a. *pv campestris* – cabbage *(Brassica oleracea var. capitata)* b. *pv vesicatoria* – pepper *(Capsicum group)* c. *pv malvacearum* – cotton *(Gossypium hirsutum)* and d. *pv phaseoli* – beans (*Phaseolus vulgaris).*

Three days after incubation bacterial colony appeared in each *petri dish.* These were collected and the bacteria were identified and isolated according to colony morphology which usually depict luxuriant yellow and mucoid colonies surrounded by zones of starch hydrolysis. The bacteria showed negative growth on MS media (Schaad *et al.,* 2011; Sijam *et al,* 1992; National Committee for Clinical Laboratory Standards and Watts, 1999). The depth of yellow differed according to the different strains of the *X. campestris* pathovors (Figure 6.2 a - d). To test for pathogenicity of the bacterial strains the PCR was used. To further valid pathogenicity, susceptible plant seedlings were

separately inoculated with test pathogens, grown and observed for 3 – 4 weeks before onset of the Moringa bark laboratory trials.



A. **B.** **C.** **D.**

Figure 6.2: Strains of *X. campestris* bacterial colonies: a. *pv vesicatoria* b. *pv campestris* c. *pv malvacearum* and d. *pv phaseoli*.

The growth medium used during isolation and identification of pathogens from infected plant material was modified by testing a series of different concentrations (Chang *et al.*, 1991) three weeks prior to the onset of the trials with the following nutrients: nitrogen source, sucrose, citrate and glutamate, in this sequence (Table 6.1). It was selected for each of the *X. campestris* pathovors to obtain conditions which would provide and maintain the best quality environment for their growth. *X.campenstris* pathovors were multiplied by streaking on nutrient agar and incubation at 37 degrees overnight. The bacteria was then diluted to approximately 10^4 CFU/ml with sterile distilled water using serial dilutions. Selective medium is essential to support growth and reduce contamination due to disintegration of targeted Xanthomonads as they are affected by the C/N ratio and the yeast levels influence its performance.

Table 6.1: Medium modifications

Medium[a]: Specific Pathogen	Yeast extract (%)	(NH4)2HPO4 (%)
1: *X. campestris pv campestris*	0.20	0
2: *X. campestris pv malvacearum*	0.20	0.20
3: *X. campestris pv phaseoli*	0.30	0
4: *X. campestris pv vesicatoria*	0.30	0.25

[a] Percentage (w/v)—all culture media including 2.5% sucrose, 0.01% $MgSO_4$_$7H_2O$ and 0.15% K_2HPO_4

Modified from Carignatto *et al*, 2011.

6.2.3 Media Preparation and Pathogen Inoculation

The following formulations were used to make the NA utilized for setting up the laboratory experiment for the four pathovors.

Yeast extract Dextrose-$CaCo_3$ Agar (YDC) ingredients

1. Yeast Extract……..10g/L
2. Dextrose…….20g/L
3. Calcium carbonate..20g/L
4. Agar …….15g/L

SM Agar ingredients PER LITRE

1. KH_2PO_4 1.0g
2. Na_2HPO_4 2.6g
3. NH_4Cl 1.0g
4. NaCl 2.0g
5. $MgSO_4.7H_2O$ 0.2G
6. $CaCl_2.2H_2O$ 67mg
7. Glucose 1.0g
8. L-methionine 0.2g
9. Starch 10.0g
10. Methyl violet 2B 1.0ml
11. Methyl green 2.0ml
12. Trace element solution 1.0 ml
13. Triphenyltetrazolium chloride 1.0ml
14. Cycloheximide 50mg
15. Agar 20g

Trace element ingredients in 100ml distilled water

1. EDTA 250mg
2. $FeSO_4.7H_2O$ 500mg
3. $ZnSO_4.H_2O$ 10mg
4. $NaMoO_4.2H_2O$ 25mg
5. $Na_2B_4O_7.10H_2O$ 18mg
6. $CoSO_4.7H_2O$ 10mg

The liquid media was then autoclaved at 121^oC at 15psi for approximately 15 minutes. The prepared media was left to cool for approximately 20 minutes. 12 ml of the Moringa bark aqueous

extracts for each concentration level together with the distilled water (control) were then mixed with the already prepared NA in separate conical flasks. After mixing, the mixtures were then poured into sterile petri-dishes to 15 mm thickness. The aqueous extracts and NA mixtures were then left to cool and solidify and stored for future use.

A loopful of 72 hours old bacterial culture for each *Xanthomonas* pathovar strain was added to 10 ml of sterile water and 1 ml of this 10^5 dilution bacterial suspension was plated by streaking a 10 mm line at the center of the petri dish. This was done in line with an identical marking drawn on the underside of the petri-dish with a visible marker. The molten agar was then poured into the petri dish to a 10mm thickness. The different Moringa bark extract concentrations were then further incorporated uniformly by thinly spreading them over the surface of the medium with a sterilized glass spreader to obtain an even spread. This procedure was repeated for all four concentration levels. The petri-dishes were then incubated at $37^o \pm 1^oC$ for 72 hourss, after which growth of each bacterial pathovar and its replicates was observed.

6.2.4 Data Collection and Analysis

Observations for bacterial growth were obtained by measuring bacterial growth in colony length (mm) and these were initiated 72hours after setting up experiment. Collection of data was done for seven consecutive days at 24 hour intervals. The efficacy of Moringa aqueous bark extract in suppressing bacterial growth was analyzed using Excel and Genstat version 14 statistical package. The significant means were separated using Least Significant Differences at 5%.

The Moringa bark aqueous extract was analyzed using the GC/MS method to identify the bioactive and phytochemical compounds of the Zimbabwean Moringa accessions as described by Dhen *et al*., (2014). The analysis was carried out at the Central Analytical Facilities, Stellenbosch University in the Mass spectrometry Unit, at Matieland, South Africa. Thee different solvents were used for the plant extract phytochemical analysis: Dichloromethane (DCM), Methanol (MeOH) and Solid Phase Micro Extraction (SPME).

6.3 Results

The phytochemical analysis revealed presence of the following bioactive compounds in the Moringa leaf and seed extracts used in this study: saponin, phenolics, phytate, flavanoids, terpenoids and lectins, N-Benzyl thiocarbamates, N-benzyl carbamates, benzyl nitriles and a benzyl ester methionine, cysteine; 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate, benzylglucosinolate, moringyne, mono-palmitic and di-oleic triglyceride. More detailed bioactive compounds for each extract type is provided in Appendix A.

6.3.1 Effect of Moringa bark extract concentration on bacterial colony growth

There was no interaction between the Moringa bark aqueous concentration and the *X. campestris* pathovors, the concentration level however, was significant in suppressing *X. campestris pv campestris* growth ($P < 0.05$). The observations indicated that an increase in extract concentration level significantly reduced the growth of the bacteria (Fig 6.3). As indicated in Fig. 6.3, an increase in concentration level from 0 – 15% caused a decrease in the growth of the bacterial colonies. However, there was no significant difference in bacterial colony length from day 6 – 7 at all the concentration levels. The highest bark aqueous concentration level (15%) resulted in slower establishment of all the pathovors, except at the 0% concentration (Fig 6.3). On the first day, 5% concentration level had already achieved bacterial colony lengths mean of approximately 14mm, whilst that of 15% had a mean of approximately 11mm. The overall colony length was lowest at highest concentration levels of 15% and highest at lower concentration levels of 5% bark aqueous respectively ($P < 0.05$) across all the treatments.

The overall study indicated that Moringa aqueous bark extract antibacterial efficiency was independent of the pathovar in question, and there was no interaction (Table 6.3). Indicating that each aqueous concentration level had a significant effect on each individual pathogenic pathovar. It was also observed that an increase in the aqueous concentration level was not directly proportional to an increase in suppression of the bacterial growth (colony length). Concentration levels from 5 – 10% indicated positive significant difference at all the levels (Fig 6.3).

6.3.2 Effect of time on antibacterial activity of the Moringa bark aqueous extracts

The variation gap of all the four pathovars ranged from 12.5 to 24 mm in 7 days. The observations revealed a trend indicating slow establishment of pathovars such as *X. campestris pv campestris* and *X. campestris pv malvacearum* in Day 1 (Fig 6.4). This resulted in a decrease in colony length and indicated the highest level of growth suppression was experienced during day 1 to day 4 (Fig 6.4). However, *X. campestris pv campestris* exhibited a quicker growth rate the next day [Day 2] than *X. campestris pv malvacearum* and by the 3rd Day, it had exceeded the growth of *X. campestris pv phaseoli* (Fig 6.4).

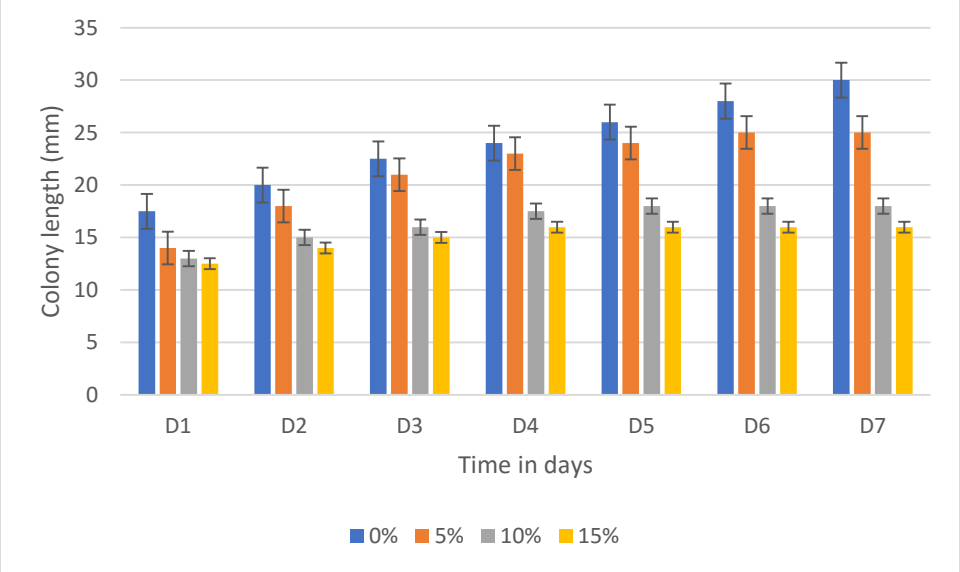


Figure 6.3: Effect of Moringa bark aqueous concentration level on growth of *Xanthomonas campestris* pathovars.

Table 6.2: P-values from Day 1 – Day 7

SV	DF	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Bacteria(B)	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Moringa(M)	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B*M	15	0.781	0.055	0.124	0.269	0.199	0.102	0.074
Error	25	-	-	-	-	-	-	-
Total	47	-	-	-	-	-	-	-

On the other hand the quick bacterial establishment by *X. campestris pv vesicatoria* ensured an increased overall colony length and the lowest level of suppression by the aqueous extract at all concentration levels among the four pathovars from Day 1 – 7. *X. campestris pv phaseoli* exhibited an intermediate growth among all the pathovars from Day 1, but by Day 7 it had an overall growth which was higher than *X. campestris pv campestris.* The longer the period in which the pathogens were exposed to the bark aqueous nutrient agar medium, the shorter the colony length, indicating the differential effect of time on colony length ($P < 0.05$) (Fig 6.4).

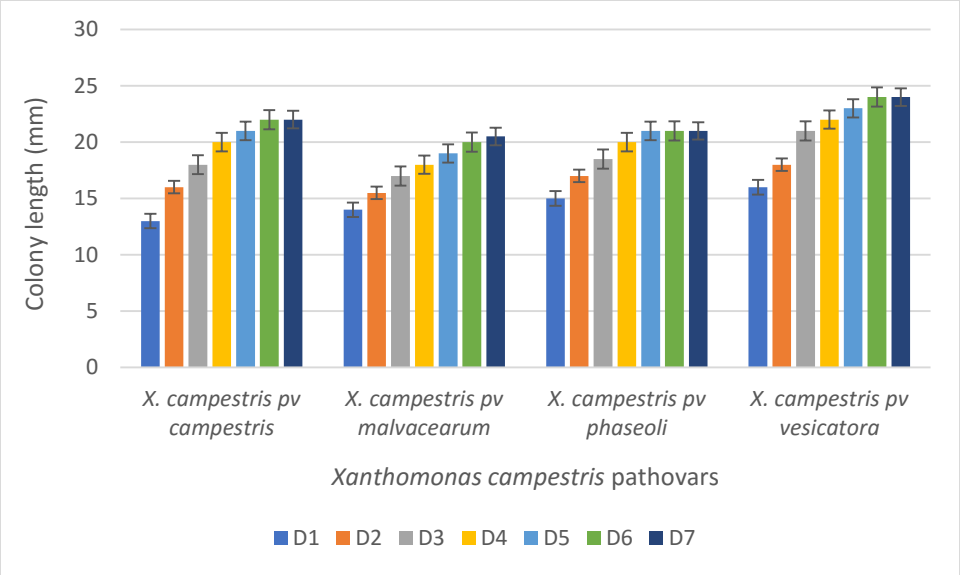


Figure 6.4: Effect of time on colony length of *Xanthomonas campestris* pathovars.

By day 4, colony growth had stabilised and any growth occurring thereafter was insignificant across all the four pathovars (Fig 6.4).

6.4 Discussion

6.4.1 Effect of Moringa aqueous extracts concentration of the on colony length

Moringa bark aqeous extract was able to effectively control all the test bacterial pathovars, irrespective of the difference in pathovar type. This could be due to the fact that all the *Xanthomona*s pathovars belong to the same *campestris* species *(*Berthier *et al.*, 1993). Implying that they could have been affected in almost similar fashion by the Moringa bark aqueous extracts at each level. In other studies, *Moringa* seemed to be able to control gram negative bacteria growth

at all levels of the Moringa aqueous bark extract used in these studies (Anwar, 2008). Jenifer (2015), highlighted that, *Moringa* possess strong antibacterial properties due to the presence of alkaloids, tannins, triterpenoids, and phenolic compounds (Appendix A) which exert an allelopathic effect on bacterial pathogens. In the current study, Moringa bark extracts exhibited antibacterial activity against Xanthomonas pathovars which was influenced by the concentration. Moringa bark is more readily available locally because it stores better under the tropical conditions of Zimbabwe and also because the few Moringa growers prune out their trees during the winter periods. This is the period when the Moringa tree is dormant and sheds off its leaves. Thus farmers process the pruned off branches into bark powders, implying that it is a resource which can be easily and locally available. Resource poor farmers might be able to therefore utilize this natural resource to improve the yield, quality and productivity of their cropping fields with no additionl expenses. Moringa bark is easy to process into powder using traditional, non-specialised tools such as the pestle and mortar at house hold level.

6.4.2 Effect of Moringa bark extract concentration on antibacterial activity

Overall colony length seemed to also be dependent on the concentration levels. As the bark aqueous concentration increased, the number of antibacterial compounds also increased, and thus increasing the antibacterial action of the Moringa bark aqueous. However, although the Moringa bark aqueous concentrations used were able to significantly suppress the growth of all the pathovars, they were not able to completely eradicate any of the four pathovars. This observation indicates the need for further studies using concentration levels that are higher than the 15% used in this current study. There is also need to lengthen the trial period to exceed the 7 days observed for this study. Higher suppression rate of the *Xcc* pathogen with the Moringa bark aqueous extract seemed to have resulted in greater antibacterial action. There exists the possibility that antibacterial activity of the Moringa bark aqueous extract might be further enhanced by longer exposure of test pathogens to the Moringa aqueous extracts. Satish *et al.,* (1999) reported that *Moringa* leaf aqueous extracts did not have any marked suppression on *X. campestris phasoeli, X. campestris malvacearum, and X. campestris vesicatoria.* This might be attributed to the effect of the heat sterilization extraction method they used in their study. The Moringa extraction method they used could have denatured the antibacterial compounds due to the heat since Moringa is said to be sensitive to heat processes (Foidl et *al.,* 2001) . The other reason could be that very little contact

time was given between the Moringa aqueous extracts and test pathogens in their study. This might have given the bark aqueous extracts less time to express their full antibacterial potential since results were obtained only after 24 hours. All these factors, therefore, might have caused the concentration levels of the bioactive ingredients to be too low to show any reaction with the study pathogens. Results of our current *in-vitro* study clearly exhibited the potential ability of Moringa bark aqueous extracts to significantly suppress the growth and development of the *Xanthomonas campestris* pathovors under study.

Moringa bark aqueous extracts might be effectively used to control diseases caused by the *X. campestris* pathogens because they were able to suppress and stop further development and growth of the study organisms in this study. From day 4, colony growth ceased for all the four pathovars. This ability might be harnessed and a natural bio-pesticide developed entirely from the Moringa bark. In the face of the devasting impacts of these test organisms, Moringa bark might provide a potentially viable, yet environmentally friendly alternative which might provide the much sought after answer to managing black rot, black speck, blights and other diseases caused by this pathogen. According to Adandonon *et al.,* (2006) different Moringa plant aqueous extracts can be utilized in the agricultural sector as bio-pesticides in pest and disease management, since it has been used to effectively control microbes such as *Sclerotium* that cause stem rot and sometimes damping off in cowpea.

Choudhary *et al*., (2011) and Atif *et al.,* (2014) reported that Moringa bark aqueous extracts had high inhibitory effect on gram negative bacteria, *P. aeruginosa, Escheria coli,* and *Staphylococcus auereus.* Besides being an effective, natural bio-pesticide, Moringa can improve crop productivity through enhanced yield and quality. Culver *et al.,* (2012), concluded that aqueous extracts from Moringa leaves can increase the yield and growth of tomatoes in both greenhouses and in the field. The seedcake that remains after the extraction of the oil from the seed can also be used as an environmentally friendly fertilizer and as livestock feed (Emmanuel and Zaku, 2011). In the same way, the solid phase which remains after bark extraction can be used as an organic soil amendment to improve crop productivity and soil structures. Whilst using it as a bio-pesticide, would enhance crop productivity and yield. Akinnibosun *et al.,* (2009), states that Moringa ethanol and water

based bark aqueous extracts, are able to inhibit the growth of bacteria such as *S. aureus* and *E. coli* among others in *in-vitro* trials. This is supportive of findings in this current study.

6.5 Conclusion

Moringa bark aqueous extracts exhibited antibacterial properties in these current studies, with prolonged contact with test pathogens resulting in enhanced antibacterial activity against the test pathogens. Moringa bark aqueous can therefore become part of an integrated disease control strategy. There is need to validate these findings under field conditions on host plants such as cruciferous and brassicas crops. These are among the majority of crops grown by small scale vegetable farmers who practice market gardening in Zimbabwe. However, further studies to validate whether contact time would influence the antibacterial activity against these same pathovars in this study is highly recommended.

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CHAPTER 7

Fusarium solani AND *Rhizoctonia solani* CONTROL USING Moringa *oleifera* PLANT EXTRACTS IN GREENHOUSE LETTUCE (*Lactuca sativa* L.)

Abstract

Greenhouse grown crops are very susceptible to fungal diseases. The greenhouse environment provides conducive conditions for the proliferation of pathogens due to the high plant populations, high temperatures and high humidity levels. These conditions cause rapid spread of the fungal spores, resulting in high disease outbreaks which reduce the yield and quality of produce. To manage these diseases requires integrated disease management strategies which include chemical and bio-pesticide applications. Greenhouse trials were conducted to test the efficacy of *Moringa* leaf and seed aqueous extracts in controlling bottom rot and stem rot diseases caused by *Rhizoctonia solani* and *Fusarium solani* during the 2014/2015 season in lettuce *(Lactuca sativa*) respectively. The experiment was a 2 x 5 x 3 factorial laid out in a Completely Randomised Block Design replicated three times. Negative control (0%) had no Moringa aqueous treatments nor chemical control, whilst 10% copper oxychloride was the positive control. Lettuce was inoculated with both pathogens at 2, 4 and 6 weeks after crop emergence and treated with Moringa leaf and seed aqueous extracts at 10, 15, 20, 25 and 30% concentration levels. Measurements on percentage disease incidence, severity, head weight and diameter were recorded. The results show that the highest disease control occurred in lettuce plants treated with 25 and 30% Moringa leaf and seed aqueous concentrations. Lettuce treated using 25 and 30% Moringa seed aqueous extract significantly suppressed disease at a higher rate on *F. solani*, recording a lower disease incidence of 33.3% ($P < 0.05$). Whilst Moringa leaf aqueous extract at 30% concentration, recorded higher *F. solani* disease incidence of 52.7% ($P < 0.05$). Moringa leaf and seed aqueous extracts at 30% concentration levels, both gave 50% disease incidence suppression on *R. solani*. The highest disease severity was observed at 10% Moringa leaf and seed aqueous concentrations for both pathogens, whilst least disease severity for both pathogens was exhibited by Moringa leaf and seed aqueous extracts at 30% concentration levels. Lettuce which was inoculated 2 weeks after crop emergence and treated with Moringa aqueous extracts at 30% concentration levels, exhibited highest head weight and diameter measurements. The results indicate that *Moringa* seed and leaf

aqueous extracts can be effectively utilized as fungicides since they inhibited disease progression in lettuce for both pathogens. The Moringa leaf and seed aqueous extracts can also be utilized to improve yield and quality of horticultural crops.

Keywords: Disease severity, Fungicides, Leaf and Seed aqueous extracts

7.1 Introduction

Lettuce is a leafy vegetable which is typically eaten fresh and raw, in salads, hamburgers, tacos, and many other dishes in many countries. As with most vegetables lettuces are marketed through the national fresh produce markets, restaurants and chain stores, and is mainly produced for commercial purposes (Kader, 2003). Therefore, it remains an important source of livelihood for many communities as it is regarded as a high value, quick turn over horticultural crop due to its short growing season (Adam, 2013).

Vegetable crops such as lettuce, are infected by a wide range of fungal diseases which reduce crop yield and quality (Barakat and Al-Masri, 2005). *R. solani* and *F. solani* are soil borne pathogens which cause serious diseases such as bottom rots and root rots in lettuce respectively, reducing crop yield and quality (Zhang *et al*., 2014). They are also responsible for major losses in a wide range of economically important agricultural and horticultural crops world-wide by the damage caused (Grosch *et al*., 2005). Although these two pathogens are listed among the most notorious pathogens, effective control strategies to manage them is still not available in horticulture or organic farming (Grosch *et al*., 2005). These pathogens exhibit characteristics which cause difficulty in effectively controlling them. *R. solani* and *F. solani* have the ability to survive in organic matter for many years as sclerotia or mycelium under adverse environmental conditions. They also exhibit a wide host range, giving them adequate support for their saprophytic life cycle by the organic matter present in soil (Grosch *et al*., 2005). Control of these pathogens is largely reliant on alternating fungicides, there are no registered fungicides specific for controlling these pathogens (Adesina *et al.*, 2009). For year round lettuce production in Zimbabwe, it becomes necessary to grow lettuce in the greenhouse during the winter months (May to early August months), which provides the most ideal environment for the spread and development of *R. solani*

and *F. solani* (Raaijmakers *et al.*, 2009). Farmers are resorting to excessive and intensive chemical use to control these pathogens. However, chemical control strategies have raised environmental and health related concerns due to their residual effects in the environment, negative effects on human/animal health and the negative impacts on beneficial insects such as bee pollinators and predatory insects (Pimentel, 2005; Horrigan *et al*., 2002).

Numerous studies have been done which indicate that *Moringa* possesses antimicrobial properties (Bansode and Chavan, 2014, Bukar *et al*., 2010) and it also contains compounds (plant growth regulators) which enhance plant growth (Mathur, 2006). However, the majority of these studies have been done as *in-vitro* studies (Abalaka *et al.*, 2012). The main objectives of this study were to: 1) determine the stage of lettuce growth at which *Moringa* aqueous extracts would be most effective in suppressing growth and development of *R. solani* and *F. solani;* and 2) determine the effect of *Moringa* aqueous extracts on yield (weight, size) of lettuce heads.

7.2 Materials and methods

7.2.1 Experimental site and trial management

The experiment was carried out in the greenhouse at the Department of Crop Science, University of Zimbabwe, which lies in natural region II with Latitude and longitude of 17° 55' S and 31° 08' E respectively. It is at an altitude of 1 479 meters above sea level, with an average annual rainfall range of 750-950 mm. The soil comprises mainly of red clay loam soils. The experiment was a 2 x 7 x 3 factorial laid out in completely randomized block design. Factor A was *Moringa* aqueous extracts at two levels that is leaf and seed; Factor B was the concentration levels of the aqueous extracts (10, 15, 20, 25, and 30%) plus the positive and negative controls; and factor C was the growth stages (2 weeks, 4 weeks and 6 weeks after crop emergence). Negative disease control (0%) did not have any Moringa aqueous nor fungicide disease control treatments, and the positive control was copper oxychloride 10%. The positive control copper oxychloride treatment, was set up separately to avoid and prevent any chance of cross contamination of the Moringa treatments during the duration of trial. Chemical contamination of any kind would have introduced biases into the study, resulting in flawed and inaccurate data being collected.

The two land areas used to raise the lettuce seedlings were pre-irrigated before land preparation was initiated to facilitate salt leaching (movement with water below the root zone) and increase soil moisture for microbial activity. The nursery seedbeds were then tilled until they both had a fine tilth and friable structure to a depth of 20 to 30 cm (Aalders *et al*., 2009). Transplanting of seedlings was done two weeks after crop emergence from the nursery into the pots. Lettuce seedlings were planted separately in 30cm diameter pots in heat-sterilized red clay loam soils obtained from crop science department fields. Crops were irrigated to soil saturation point after every 2 days. Frequent, light irrigation, was applied to maintain the moist, favourable conditions for lettuce development until head formation and final harvest-time.

Split applications of nitrogen (N) at a rate of 5g/plant were done starting 2 weeks after transplanting into individual pots. Subsequent applications of nitrogen were applied as supplemental dressings at 3week intervals thereafter. A second series of cultivation and fertilizer dressings was conducted approximately 14 days towards the end of early head formation. Phosphorus (P) fertilizer at a rate of 5g/plant was banded 6 to 9cm below the soil surface, 2cm away and, besides the seedling plant. Weeds were controlled manually by hand pulling the weeds or through light manual cultivation within the pot using a hand fork, depending on weed size. Chemical sprays for the control potted plants were done whenever scouting efforts revealed the presence of pests.

7.2.2 Fungal inoculum preparation and application

When preparing the fungal spores, 10 ml of sterile distilled water was added to each plate containing pathogen pure culture, and colonies were carefully scraped with a sterile needle. The resulting spore suspension from each isolate was adjusted to 1×10^8 spores/ml and used for the inoculation of lettuce plants. Two weeks, four weeks and six weeks after crop mergence, leaves were inoculated using the brushing method (Oliver and France, 2016). Data was collected every 72 hours. Lettuce plants under the negative control plot, were sprayed with distilled water and positive control was sprayed with 10% copper oxychloride.

7.2.3 Moringa leaf and seed aqueous preparation

Preparation of *Moringa* seed and leaf aqueous extracts was carried out using the method laid out by Okoi *et al*., (2016). Both leaves and seeds were first thoroughly washed with distilled water to remove any dust debris and later air dried at room temperature under dark conditions for seven days. After drying, both the leaves and seeds were separately ground to a fine powder using mortar and pestle. Moringa aqueous extracts were prepared by separately mixing ground seed and leaf powder with distilled water. Leaf and seed powder weighing 10, 15, 20, 25 and 30 grams were placed in sterilised beakers and 100 ml of distilled water was added to make 10, 15, 20, 25 and 30% concentration aqueous extracts as described by Hassan *et al*., (2012). The mixtures were then stirred using a sterile glass rod for 30 minutes and allowed to stand at room temperature for 24 hours. The Moringa aqueous extracts were separately filtered thoursough a triple layered cheese cloth initially, then through a sterile Whatman number 1 filter paper placed in a funnel as described by Okoi *et al*., (2016). The aqueous extracts were further filtered through the micro filter for further purification to avoid contamination. All five concentrations of the Moringa leaf and seed aqueous extracts were applied three times weekly, using a hand–held sprayer. The Moringa aqueous treatment applications were initially applied on lettuce plants at 2 weeks after crop emergence, 4 weeks after crop emergence and 6 weeks after crop emergence. After which the Moringa aqueous extracts were applied three times weekly for the duration of the study.

7.2.4 Data collection and analysis

Data which was collected was; disease incidence, disease severity and yield parameters of lettuce.

The disease incidence (%) was recorded once every week for 4 weeks using the following formula (Anjorin, 2016):

$$\text{Disease incidence (\%)} = \frac{\text{(Total number of infected plants)}}{\text{(Total number of plants assessed)}} \times 100 \quad (1)$$

Disease severity assessment was carried out once every week for 4 weeks by scoring diseased plants using a 1-5 scale as described by Mamza *et al*., (2008) where:

1= All leaves without symptoms.

2= 1- 25% total leaf number with symptoms.

3 = 26 - 50% total leaf number with symptoms.

4 = 51 - 75% total leaf number with symptoms.

5= 75% or more –total leaf number with symptoms.

The yield parameter measurements taken were head diameter, using a 30cm ruler across the horizontal section of the lettuce head, whilst head length was measured longitudinally from the top of lettuce to the bottom-end of the lettuce head, were it connected with the lettuce stem. Weight of lettuce heads was measured in grams using a balance scale.

Data analysis on the effectiveness of Moringa leaf and seed aqueous extracts was analysed using Genstat version 14 statistical analysis package. The means were separated using the least significant difference (L.S.D) at 5% significant level.

The Moringa leaf and seed aqueous extracts were analyzed using the GC/MS method to identify the bioactive and phytochemical compounds of the Zimbabwean Moringa accessions as described by Dhen *et al*., (2014) (Appendix A). The analysis was carried out at the Central Analytical Facilities, Stellenbosch University in the Mass spectrometry Unit, at Matieland, South Africa. Three different solvents were used for the plant extraction process namely, Dichloromethane (DCM), Methanol (MeOH) and Solid Phase Micro Extraction (SPME).

7.3 Results

The phytochemical analysis revealed presence of the following bioactive compounds in the Moringa leaf and seed extracts used in this study: saponin, phenolics, phytate, flavanoids, terpenoids and lectins, N-Benzyl thiocarbamates, N-benzyl carbamates, benzyl nitriles and a benzyl ester methionine, cysteine; 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate, benzylglucosinolate, moringyne, mono-palmitic and di-oleic triglyceride. More detailed bioactive compound information for each extract type is provided in Appendix A.

The interaction between concentration levels and disease severity of both *R. solani* and *F. solani* on lettuce was highly significant ($P < 0.05$) (Table 7.1). The observations indicated that plants treated with 25 and 30% Moringa aqueous concentrations, had lower disease severity indices compared to plants treated at lower Moringa aqueous concentration levels. Plants treated with 25 and 30% Moringa aqueous concentrations recorded lower fungal disease indices which were not

significantly different from each other for both test pathogens ($P < 0.05$). The antifungal activities at all the other Moringa aqueous concentration levels, were however, significantly different from the antifungal action exhibited at 25 and 30% concentration levels. The highest disease incidence occurred in plants treated with distilled water (0%), and the 10 and 15% Moringa aqueous concentration levels. However, the antifungal activity exhibited by Moringa aqueous extracts at 20% concentration level, was significantly different from the both the higher and lower Moringa aqueous concentration levels ($P < 0.05$). The lettuce plants which were under the negative control with no Moringa or chemical sprays, succumbed to disease and died off. The results also indicated that 10% copper oxychloride suppressed *R. solani* pathogen more effectively than it suppressed the *F. solani* pathogen.

Table 7.1: Effect of Moringa aqueous extract concentration on disease severity of *R. solani* and *F. solani.*

Concentration levels	Radial growth (mm)	
	R. solani	*F. solani*
0 %	7.82^{f}	10.62^{f}
Copper oxychloride	0.06^{a}	0.50^{a}
10 %	6.36^{e}	7.91^{e}
15 %	5.32^{d}	7.52^{d}
20 %	4.70^{c}	6.77^{c}
25 %	$4.35b^{c}$	6.35^{b}
30 %	4.05^{b}	6.06^{b}
P-value	P<0.001	P<0.001
L.S.D	0.5	0.42
C.V%	11.2	7.3

NB: Means with the same letter are not statistically different from each other.

There was evidence of significant interaction between Moringa leaf aqueous concentration levels and the stage of lettuce growth at which pathogen inoculation was done, on disease severity of *F. solani* in lettuce ($P < 0.05$). Lettuce plants which were inoculated with *F. solani* pathogen at 2

weeks after crop emergence, had lower disease severity indices across all the Moringa concentration levels when compared to inoculation stages of 4 and 6 weeks. However, there was no significant interaction effect on antifungal activity occurring on Moringa leaf aqueous extract at 10 and 15% concentration and disease severity on lettuce inoculated at stages 4 and 6 weeks after crop emergence (Fig 7.1). Lettuce plants treated with Moringa leaf aqueous extract at 25% and 30% concentration, and inoculated at 2 weeks after crop emergence, showed a lower incidence of *F. solani* disease than lettuce inoculated at the other growth stages. This observation was significantly different from positive control and the other growth stages (Fig 7.1)

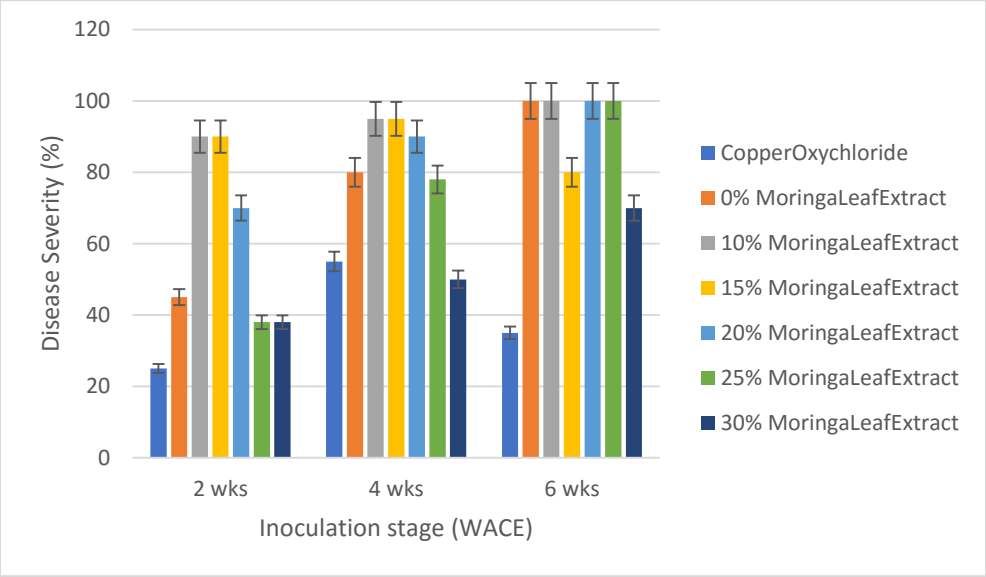

Figure 7.1: Interaction effects of Moringa leaf aqueous concentration level and stage at which lettuce was inoculated with *F. solani* on disease severity of *F. solani*.

Disease incidence was significantly influenced by Moringa aqueous seed extract concentration levels and inoculation stages of *R. solani* of the lettuce plants ($P < 0.05$) (Fig 7.2). Disease incidence was significantly lower in lettuce plants which were inoculated at 2 weeks after crop emergence at all Moringa seed aqueous concentration levels, in comparison to plants inoculated at 4 and 6 weeks after crop emergence. Lettuce plants inoculated at 2 weeks after crop emergence in combination with Moringa seed aqueous extract at 30% concentration, had significantly lower disease incidence compared to lettuce inoculated at 4 and 6 weeks after crop emergence, at the same Moringa seed aqueous concentration level (Fig 7.2). Moringa seed aqueous extract at 30% concentration recorded lowest disease incidence at all the 3 lettuce plant growth inoculation stages.

It exhibited the strongest antifungal action across all the Moringa treatments. There was no significant difference in disease incidence occurring between plants inoculated with *R. solani*, at 2 and 6 weeks after crop emergence for Moringa seed aqueous extract at 30% concentration and the positive control. It is important to note that there are significant differences occurring within all the Moringa seed aqueous treatments at all the Moringa seed extract concentration levels in response to the stage at which the lettuce plants were inoculated on disease incidence in lettuce ($P < 0.05$) (Fig 7.2). The disease incidence levels occurring when lettuce was inoculated with *R. solani* at 6 weeks after crop emergence, showed no significant differences for Moringa seed aqueous extract concentration levels of 10, 15, 20 and 25%.

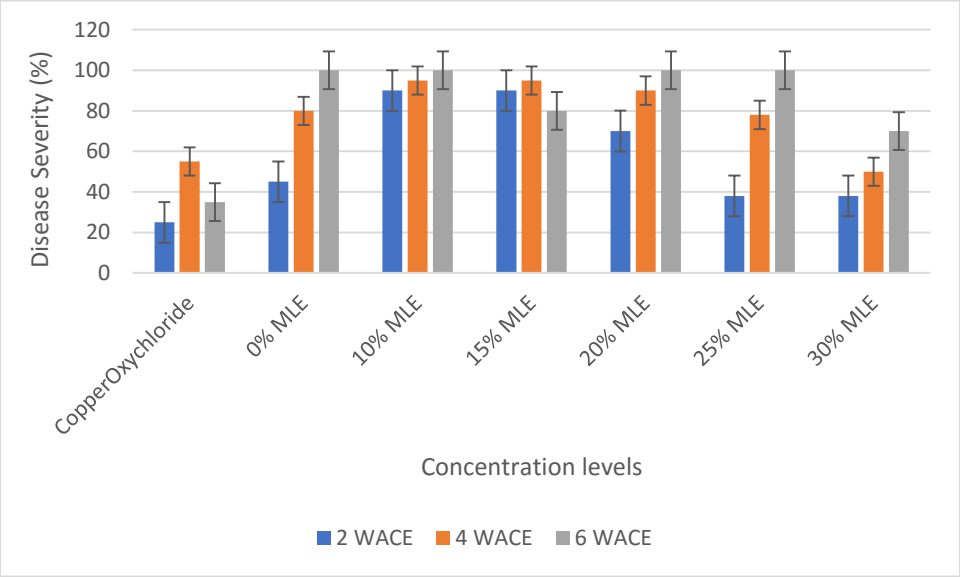

Figure 7.2: Interaction effect of Moringa seed aqueous extract concentration and lettuce inoculation stages on disease severity of *R. solani*

There was significant interaction influence on disease incidence between Moringa leaf aqueous extract concentration levels and growth stage at which lettuce was inoculated on disease incidence of *F. solani* ($P < 0.05$) (Fig 7. 3). Lettuce plants inoculated at 2 and 4 weeks after crop emergence, in combination with Moringa leaf aqueous extract at 25 and 30% concentrations significantly had the lowest disease incidence ($P < 0.05$). Disease incidence was very low when plants were inoculated at an early stage and also as the concentration of the Moringa leaf aqueous extract increased. Plants inoculated at 6 weeks after emergence and treated with Moringa leaf aqueous extract had the highest disease incidence across all the concentration levels (Fig 7.3).

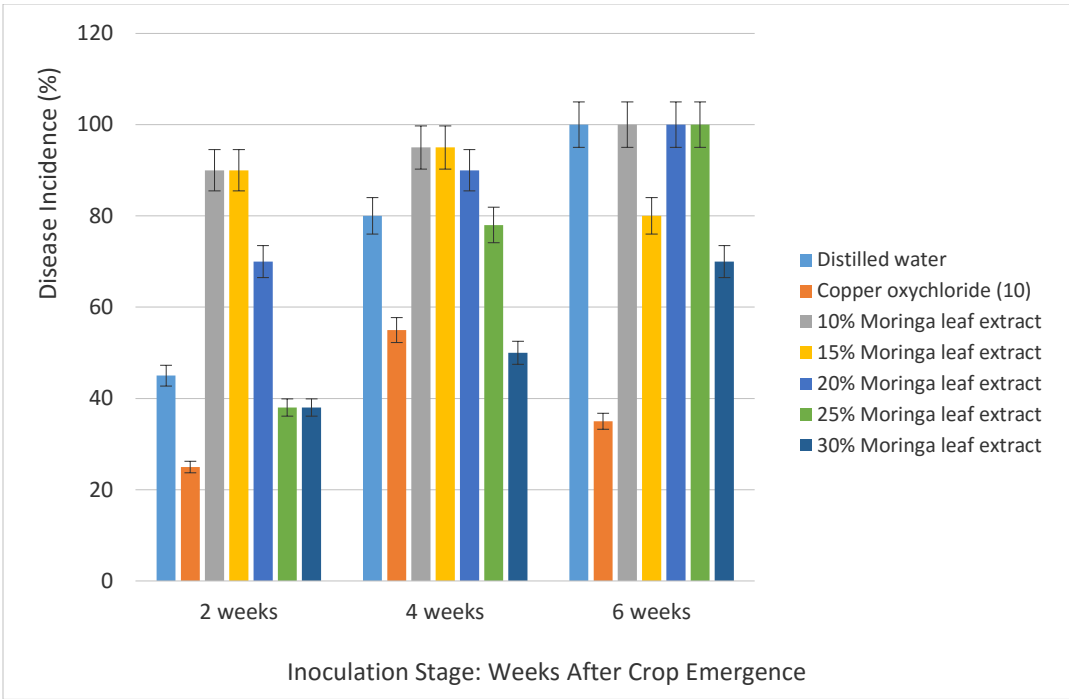

Figure 7.3: Interaction effect of Moringa leaf aqueous concentration and growth stage at which lettuce was inoculated with *F. solani* on disease incidence.

The observations indicated that the growth stage at which lettuce was inoculated with *F. solani* coupled with the Moringa seed aqueous concentration, significantly influenced the rate of disease incidence ($P < 0.05$). The lettuce plants which were inoculated at 2 weeks after crop emergence in combination with the higher Moringa seed aqueous concentration rates, significantly reduced *F. solani* disease incidence in lettuce (Fig 7.4). The rate of disease incidence occurring in lettuce inoculated at 2 weeks after crop emergence was significantly lower compared to all the other inoculation stages at 4 and 6 weeks after crop emergence ($P < 0.05$). Interestingly, disease incidence observed in lettuce which was inoculated at 2 weeks after crop emergence, at Moringa seed aqueous of 30% concentration had significantly higher antifungal activity against *F. solani* compared to the positive control (10% copper oxychloride) (Fig 7.4).

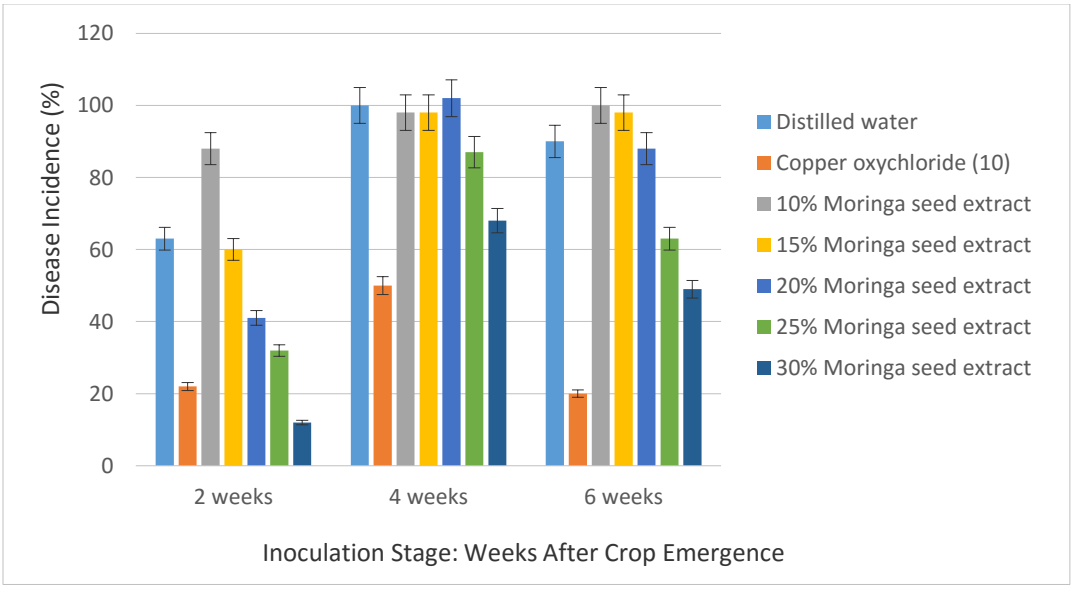

Figure 7.4: Effect of Moringa seed aqueous concentration level and stage at which lettuce was inoculated with *F. solani* on disease incidence.

The head weight of lettuce was significantly influenced by Moringa aqueous extract type and concentration and growth stage at which lettuce was inoculated ($P < 0.05$) (Fig 7.5). The results indicated that the negative control (no Moringa aqueous extracts nor fungicide application) had the lowest head weights ranging between 100 – 130g for all the three growth stages. The results show a similar trend for all the lettuce inoculation growth stages, were the lower Moringa aqueous concentrations produced lower head weights of lettuce. Lettuce plants which were inoculated with *R. solani* at 2 weeks after crop emergence and treated with Moringa seed aqueous extracts at all concentration levels, had bigger head diameters, which were not significantly different from each other ($P < 0.05$). However, Moringa seed aqueous extract at 10% concentration had the lowest head weight at 2 weeks after crop emergence inoculation stage. This was significantly different from the other head weights within this same inoculation stage ($P < 0.05$). There were no significant differences in head weight among lettuce plants which were inoculated at 4 and 6 weeks after emergence and treated with Moringa seed aqueous extract at 15, 20%; and at 25 and 30% concentration levels. The lowest lettuce head weights were obtained from Moringa seed aqueous extract at 10% concentration in for plants inoculated at 2 and 6 weeks after crop emergence (Fig 7.5).

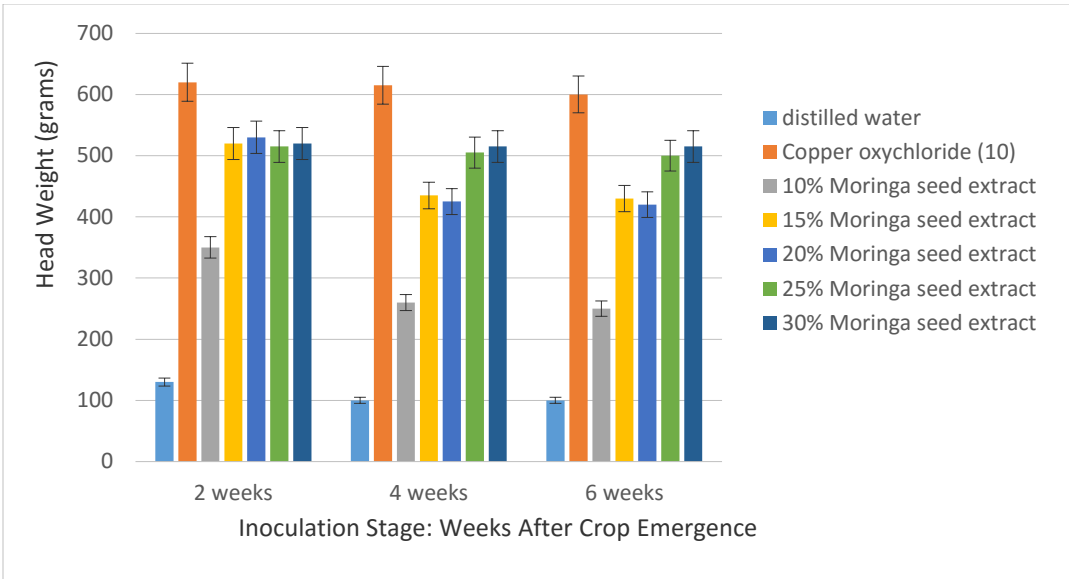

Figure 7.5: Interaction effects of Moringa seed aqueous concentration and lettuce inoculation stages on lettuce head weight inoculated with *R. solani*.

The observations indicated significant interaction effect of Moringa leaf aqueous concentration and stage of lettuce growth when inoculated with *F. solani* on head weight of lettuce ($P < 0.05$) (Fig 7.6). Lettuce plants which were inoculated with *F. solani* at 2 weeks after emergence and treated with Moringa leaf aqueous extract had the highest head weight at all concentration levels of Moringa as compared to the negative control, and there was no significance difference from the positive control ($P < 0.05$). The negative control had the lowest lettuce head weight, followed by Moringa leaf aqueous extract at 10% concentration. The results also show that plants inoculated at 4 and 6 weeks after emergence had no significant differences at all Moringa concentration levels on head weight of lettuce ($P < 0.05$).

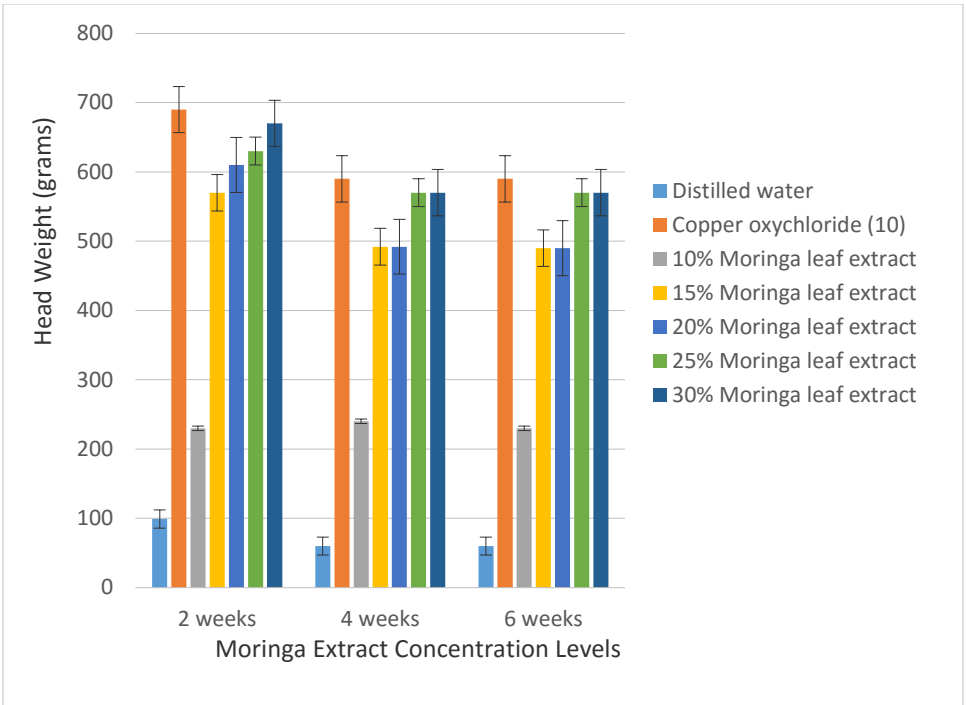

Figure 7.6: Interaction effects of Moringa leaf aqueous concentration and lettuce inoculation stages on lettuce head weight of *F. solani*.

The stage at which lettuce plants were inoculated and concentration level of the Moringa seed aqueous extract, significantly influenced the rate of increase in head weight of lettuce ($P < 0.05$) (Fig 7.7). The weight of lettuce heads inoculated at 2 weeks after crop emergence had the highest head weights compared to those inoculated at 4 and 6 weeks after crop emergence ($P < 0.05$). Lettuce head weights treated with Moringa seed aqueous extract at all concentration levels within each individual inoculation stage, were not significantly different from each other. The 10% Moringa seed aqueous extract concentration level, produced lowest head weights across all the treatments. The highest head weight of lettuce was recorded in lettuce treated with 10% copper oxychloride (Fig 7.7).

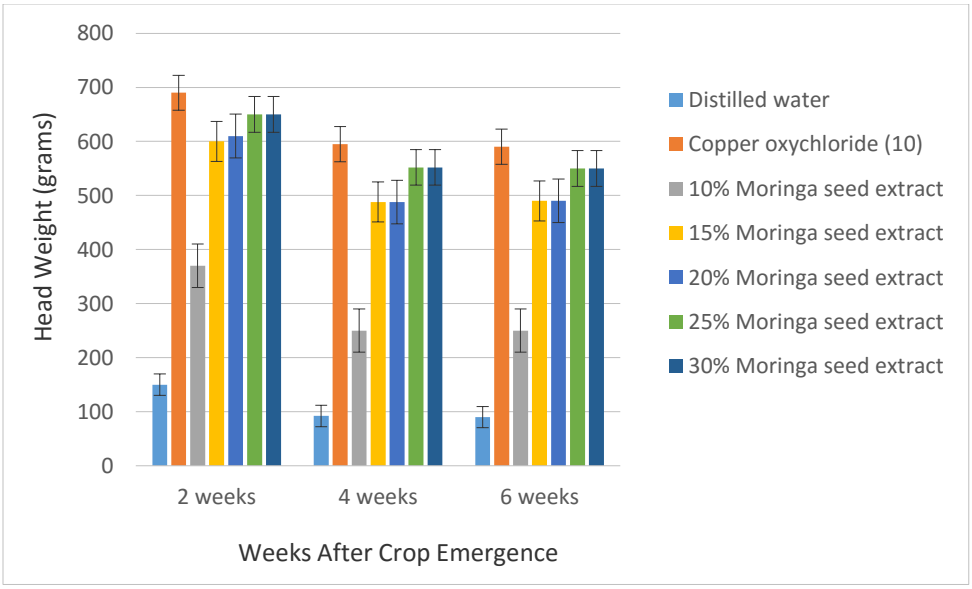

Figure 7.7: Interaction effects of Moringa seed aqueous concentration and lettuce inoculation stages on lettuce head weight of *F. solani*.

Effects of Moringa leaf aqueous extract concentration and stage of inoculation of lettuce were highly significant in influencing the head diameter of lettuce ($P < 0.05$) (Fig 7.8). All Moringa leaf aqueous concentrations performed better compared to the negative control. The lettuce head diameters were increasing in response to an increase in Moringa leaf aqueous extract concentration for the lettuce plants inoculated at 4 and 6 weeks after crop emergence. However, Moringa leaf aqueous extract at 10% concentration, produced the lowest head diameters which were significantly different from the 15, 20, 25 and 30% concentration levels for all the lettuce growth inoculation stages. Lettuce plants inoculated with *R. solani* at 2 weeks after emergence and treated with Moringa leaf aqueous extract had better performance at all concentrations levels. The highest head diameters recorded for Moringa leaf aqueous extract, were recorded at Moringa leaf aqueous extract of 30% concentration. Moringa leaf aqueous extract at 30% concentration produced larger lettuce head diameters in lettuce inoculated at 4 and 6 weeks after crop emergence compared to the lettuce inoculated at 2 weeks ($P < 0.05$). Positive control had the highest head diameter of lettuce.

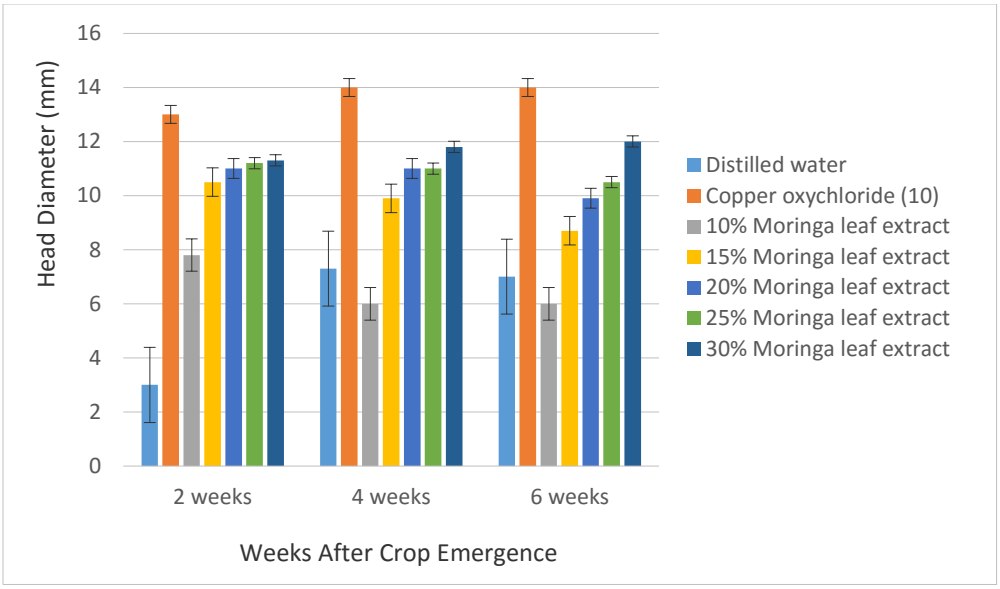

Figure 7.8: Effect of Moringa leaf aqueous concentration and stage of inoculation of lettuce with *R. solani* on lettuce head diameter.

The results showed that Moringa seed aqueous extract concentration and growth stage at which lettuce was inoculated had significant influence over the head diameter of the lettuce ($P < 0.05$) (Fig 7.9). The largest head diameters were observed in the lettuce plants treated with 10 % copper oxychloride, followed by those treated with Moringa seed aqueous extract at 20, 25 and 30% concentrations respectively. The smallest lettuce diameters were observed in the negative control and the Moringa seed aqueous extract at 10% concentration. The lettuce head diameters observed in Moringa seed aqueous extract concentrations of 20 and 25% were not significantly different from each other in the lettuce inoculated at 4 weeks after crop emergence ($P < 0.05$).

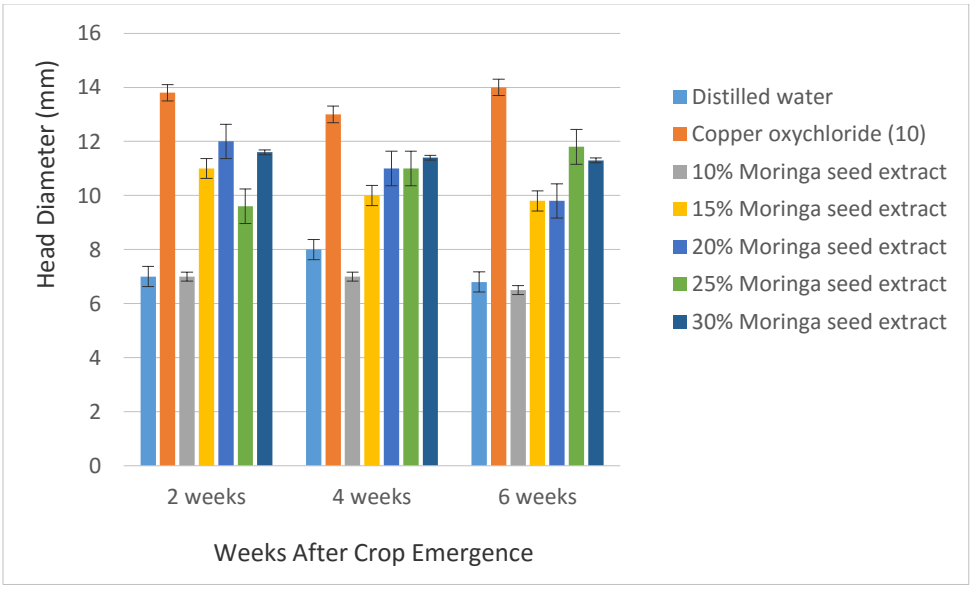

Figure 7.9: Effect of Moringa seed aqueous concentration and stage of inoculation of lettuce with *F. solani* on lettuce head diameter

Interaction effect of Moringa seed aqueous concentration levels and inoculation stages of pathogen was highly significant on head diameter of lettuce ($P < 0.05$), with the head diameter increasing as the aqueous concentration increased (Fig 7.10). All Moringa seed aqueous extract concentrations produced bigger lettuce head diameters compared to the negative control. Lettuce plants inoculated with *F. solani* at 2 weeks after emergence and treated with Moringa seed aqueous extract exhibited better performance at all concentration levels except for the 10% concentration. The lettuce head diameters were not significantly different from those of the positive control (10% copper oxychloride fungicide) at 2 weeks after crop emergence inoculation ($P < 0.05$). The negative control had the lowest head diameter of lettuce. The head diameters for lettuce inoculated at 2 and 4 weeks after crop emergence and treated with Moringa seed aqueous extract at 15 and 20% concentration, did not show any significant differences ($P < 0.05$). The lowest lettuce head diameters were observed in the negative control for the 2 weeks after crop emergence plants. The lettuce head diameters for the 4 and 6 weeks after inoculation growth stages, were significantly larger for the negative control compared to the Moringa seed aqueous extract at 10% concentration level ($P < 0.05$).

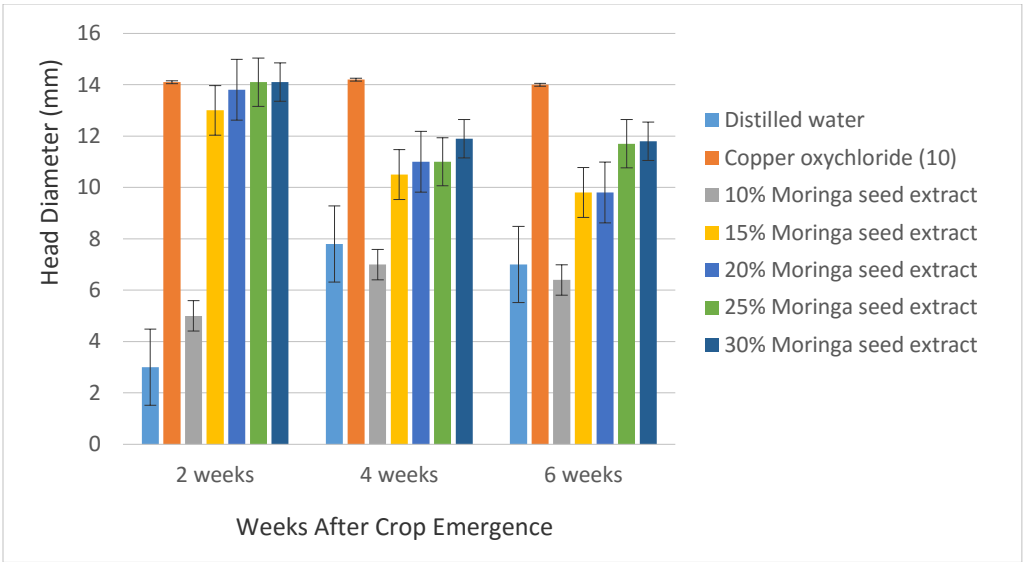

Figure 7.10: Effects of Moringa seed aqueous concentration levels and stage of lettuce inoculation on head diameter of lettuce.

The results show that the plant growth regulator effect of Moringa leaf aqueous extract on lettuce head diameter growth, was significantly influenced by the crop growth-stage at which the first Moringa leaf aqueous applications were initiated upon the test crop and concentration rate ($P < 0.05$) (Fig 7.11). Lettuce heads treated with Moringa leaf aqueous extract at 10% had the lowest head diameters across all the treatments. Lettuce head diameters were highest under the copper oxychloride treatment, which were however, not significantly different from all the Moringa leaf aqueous extract concentration levels for the 2 weeks after crop emergence inoculation stage ($P < 0.05$). The smallest lettuce head diameters were observed in lettuce plants inoculated at 6 weeks after crop emergence across all the treatments except for copper oxychloride treatment.

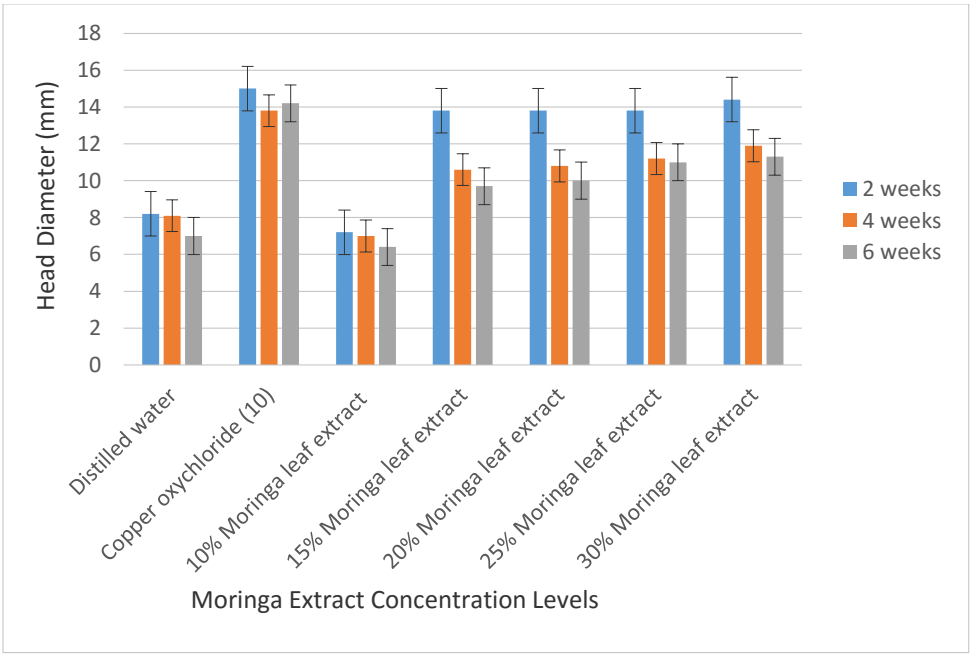

Figure 7.11: Interaction Effects of Moringa aqueous treatment concentration levels, lettuce inoculation stages and lettuce head diameter

7.4 Discussion

The effectiveness of plant aqueous extracts depend on the nature and amount of active ingredients it contains. The ability of Moringa aqueous extracts to suppress fungal disease development in greenhouse lettuce, confirms that Moringa contains active antimicrobial compounds which are released in varying amounts (Kumar *et al.*, 2010). The increased Moringa aqueous extract concentration probably resulted in an increase in the antifungal properties, which resulted in lowering the growth of the fungal pathogens (Tijjani *et al.*, 2014). Lettuce plants inoculated at an earlier growth stage (2 weeks after lettuce emergence) exhibited the lowest disease incidence and severity across all Moringa extract concentrations which might be due to several factors. One of which would be that the young, vigoriously growing lettuce leaves were able to absorb and utilize the foliarly applied Moringa aqueous extracts at a more efficient rate. Furthermore, young plants grow more vigorously compared to older plants which reduces pathogen infection rate due to the higher levels of meristematic cells (Anjorin *et al.*, 2016). The vigorous growth by young plants results in more efficient water utilisation from the soil, reducing moisture content in the ambient environment resulting in drier, unconducive conditions for pathogen growth (Oliver and France,

2016). Larger plants result in leaves being in closer contact, reducing aeration space between them and increasing plant density per m^{-1}. This scenario results in the creation of microclimates within the plant canopy increasing humidity and contact time between fungal inoculum and host plant in conditions which favour infection (Oliver and France, 2016). The lettuce plants were benefiting nutrients from the foliar applications of Moringa leaf aqueous extracts since these leaves are high in minerals and proteins (Ferreira *et al.*, 2008). Pathogens must first breach the natural barriers presented by healthy plants in order for them to cause harm and damage to the host cell. These barriers may be physical (the cuticle, cell wall, stomata aperture or lenticel) or chemical (Aubertot *et al*., 2006). Nutrients play an important role within the plant by reducing diseases to acceptable levels at which point further control through cultural practices, conventional, or organic biocides, might prove to be even more successful and less expensive (Abdel-Fattah *et al.*, 2011). Nitrates moderate the severity of black scurf (*R. solani*) in potatoes, root rots of beans and peas, and foot rot in wheat as a mobile regulator of enzyme activity, while K is involved in all cellular functions which influence disease severity (Yang *et al*., 2006; Gajda and Kurzawińska, 2004). Calcium is a cell-wall and plant membrane structural component and it plays a major role in the integrity and function of these structures. The activity of pectolytic enzymes which are released by fungi is inhibited by the calcium ion (Ngadze and Icishahayo, 2016). Copper acts to detoxify oxygen radicals and hydrogen peroxide, thus limiting damage to plant cells. Manganese plays a key role in the production of phenolic compounds and lignin formation, two of the major items in a plants arsenal against disease (Freeman and Beattie, 2008). The fact that zinc is an active ingredient in some fungicides is evidence that it is directly toxic to some pathogens, and some studies attributed tomato tolerance to *Fusarium* wilt to the presence of iron without affecting the development of the fungus (Balbi-Peña *et al*., 2014). The Moringa aqueous extracts used in this study contain all these elements and antifungal properties (Adline and Devi, 2016). Therefore the more of the foliarly applied Moringa aqueous extracts absorbed by the lettuce plants in this study, the more enhanced the antifungal and plant growth regulatory activities exhibited.

Moringa leaf aqueous extracts contain zeatin, a growth hormone which is effective in enhancing yields in a variety of crops. Makkar *et al.*, (2007) reported that juice from fresh Moringa leaves can be used to produce an effective plant growth hormone using zeatin. Zeatin promotes the growth of plants by enhancing more rapid growth and cell division to occur (Iqbal, 2014). This might

explain the better performance obtained from Moringa aqueous extracts on lettuce head diameter and weight growth parameters. Moringa plant aqueous extracts accelerated growth of young plants when applied as a foliar spray, resulting in development of heavier roots, stems, leaves, and bigger fruits with higher sugar content (Iqbal, 2014). It has been reported that foliar application of *Moringa* leaf aqueous accelerated growth of tomato, peanut, corn and wheat at early vegetative growth stage. These Moringa leaf aqueous extracts also improved resistance to pests and diseases, and enhanced more and larger fruits and generally increased yield by 20 to 35% (Ashfaq *et al*., 2012). These results further validate the observations exhibited in this current study were the Moringa aqueous extracts enhanced the weight and diameter of lettuce heads. This proves Moringa plant contains plant growth enhancing biochemical which can be efficiently utilized to improve horticultural crop yield and quality.

High levels of sugars in plant tissues enhances plant resistance in most fungal pathogen–plant systems (Morkunas and Ratajczak, 2014). Sugars are said to constitute the primary substrate which provide energy and structural material for defence responses in plants. They may also act as signal molecules interacting with the hormonal signalling network regulating the plant immune system (Morkunas and Ratajczak, 2014). These sugars are present in the bioactive compounds of Moringa aqueous extracts in a form which is produced in the Calvin cycle during photosynthesis (Morkunas and Ratajczak, 2014). Sugars enhance oxidative burst at early stages of infection, increasing lignification of cell walls, stimulating the synthesis of flavonoids and inducing certain pathogen resistant proteins (Balbi-Peña *et al.*, 2014). Secondary metabolites such as terpenoids, phenolic and alkaloids are the bioactive compounds directly involved in the plant defence mechanisms against pathogens (Freeman and Beattie, 2008). *Moringa* contains these phytochemical compounds in significant amounts (Kooltheat *et al.,* 2014). The presence of these bioactive compounds in the Moringa plant extracts used in this study (Appendix A) further conferred antifungal properties which suppressed bottom rot and root rot diseases in this current study.

The observations attained from this study are in agreement with other findings which state that roots, flowers, bark, stem, leaves and seeds of Moringa possess antimicrobial properties (Patel *et al.*, 2016; Adline and Devi, 2016). Sreelatha and Padma (2009) reported antioxidant activity from

the leaves of *Moringa* due to presence of kaempferol, which plays an important role in plant disease control. *Moringa* contains several compounds which constitute an important source of microbiocides, pesticides and many pharmaceutical drugs such as saponin, steroids, tannin, glycosides, alkaloids and flavonoids in the aqueous extracts (Zaffer *et al.*, 2012). The flavonoid present in Moringa, contain phy-toalexins present in some plants, which are toxic to fungal pathogens. Isoflavonoids reduce the development of fungi by inhibiting the growth of fungal mycelia, spore germination, and also limit fungal pathogenicity (Holetz *et al*., 2002). The fungicidal action of Moringa biocompounds, is related to the movement and disorganization of cell organelles by disturbing fungal respiration and nutrient uptake (Kooltheat *et al.*, 2014). Seed treatment with Moringa leaf not only improved the vegetative growth, but also enhanced the grain yield of maize even when applied in very small amounts (Phiri, 2010; Akinbode and Ikotun, 2008). Foliar application of Moringa leaf aqueous extracts also stimulates earlier cytokinin formation thus preventing premature leaf senescence, resulting in larger leaf area with higher photosynthetic pigments (Basra *et al*., 2011, Rahman *et al*., 2009). *Moringa* leaf is a rich source of ascorbate (Sreelatha and Padma, 2009). Exogenous application of ascorbate increase levels of ascorbic acid which exert a protective effect on growth, and improve photosynthetic capacity of plants against salt induced oxidative stress (Wahid and Jamil, 2009). This might be an explanation to the results obtained in the current study. As indicated by the biochemical analysis results in Appendix A, foliar application of the Moringa aqueous extracts in this current study, released the isoflavonoids, alkaloids, tanins and saponins which acted as antifungal bio-pesticides against the test organisms under study.

Although numerous studies have been carried out regarding Moringa antimicrobial properties against pathogens, the greatest limit to these studies is that the majority of these mainly target human pathogens and are mostly invitro experiments. In addition, Moringa aqueous extract efficacy, is not being studied on its own merit. The studies on Moringa antimicrobial activities, has focused on studying its mechanisms within the human cells and pathogens which cause human diseases. However, information on such mechanisms within plant cells is yet to be validated. Sharma *et al.*, (2007) has also indicated the need for further studies. *In-vitro* studies are done in controlled, artificially created environments which are different from the physical realities in field grown crops. Thus efficacy of Moringa observed *in-vitro*, might not necessary be the same when

observed in the field. Thus, there is need to validate such findings in crops of economic importance working with pathogens which are listed among those notoriously difficult to manage with conventional pesticides. The limited studies which have involved *Moringa* in field or potted experiments have to a large extent, used *Moringa* in combination with other biological control agents such as Neem or trichoderma, thus removing the emphasis on evaluation of the antimicrobial activity away from Moringa. This current study was carried out in an effort to add on to the existing body of knowledge regarding the efficacy of Moringa aqueous extracts. Moringa aqueous extracts have great potential as a viable bio-pesticide against soil borne crop pathogens in field applications.

7. 5 Conclusion

The results showed the antifungal activity of Moringa leaf and seed aqueous extracts against *R. solani* and *F. solani* in greenhouse lettuce. Moringa seed aqueous extract was the most effective at suppressing *R. solani* disease. These results indicate the great potential of developing natural bio-pesticides against fungal pathogens in crop production. Such developments are crucial in reducing the detrimental effects on the environment, flora, fauna, animal and human health. Moringa aqueous extracts were also shown to enhance the weight and diameter of the lettuce heads in this study. Thus, Moringa aqueous extracts can be used to enhance growth and quality of leafy vegetable crops.

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CHAPTER 8[2]

USE OF *Moringa oleifera* EXTRACTS TO CONTROL BLACK ROT (*Xanthomonas campestris pv. campestris*) IN FIELD GROWN CABBAGE (*Brassica oleracea*), BEATRICE, ZIMBABWE.

Abstract

Black rot is a devastating pathogen in cabbage production, which is proving difficult to eradicate from production plots. The disease persists in crop debris, residue and in alternate host plants. Current chemical strategies are not effective in managing this disease in intensive production systems. This study aimed to evaluate whether the antibacterial compounds present in Moringa plant aqueous extracts were effective in suppressing black rot disease (*Xanthomonas campestris pv campestris)* in open-field grown cabbages (*Brassica oleracea*). Field experiments were carried out and repeated over a period of 6 months during the October 2015 to April 2016 season at Victory Farm in Beatrice, Zimbabwe. The experimental design was a 3 x 3 factorial laid out in a split plot in two blocks with three replicates. Three Moringa aqueous extracts (leaf, bark, and seed) at 3 concentration levels of 60, 100 and 140% were foliar applied weekly. The applications started from 5 weeks after crop emergence to the cabbage crop for the duration of the study. The antibacterial activity for each of the different Moringa aqueous extracts was evaluated by recording number of totally defoliated plants once per week. The results indicated high antibacterial significance of all the three Moringa aqueous extracts as they were able to control black rot disease at varying levels in the cabbage plants ($P < 0.05$). The highest inhibition of black rot disease progression was recorded during the 8th week after crop emergence with the Moringa seed aqueous

[2] This chapter has been published modified as: M. Goss, P. Mafongoya, A. Gubba and Tesfay Sam. 2017. Black Rot (*Xanthomonas campestris pv. campestris*) Control in Field Grown Cabbage (*Brassica oleracea* var. Sugar Loaf) with *Moringa oleifera* extracts. *International Journal of Plant & Soil Science 18(2): 1-11, 2017; ISSN: 2320-7035.* This was presented as an International conference paper at: The 2nd Intenational Conference on Pesticidal Plants (ICPP). 6 -9 February 2017. Elephant Hills Resort Hotel. Victoria Falls. Zimbabwe. Black rot, *Xanthomonas campestris pv campestris* control in Cabbage using *Moringa oleifera* Lam. extracts

extract recording the least mean leaf defoliation of 2.965. This was followed by the bark aqueous extract, with a leaf defoliation mean of 3.312 and lastly leaf aqueous extract, with leaf defoliation mean of 3.486. Moringa seed aqueous extract had the highest antibacterial activity against the black rot disease in cabbages in this study. From this experiment, it can be concluded that **b**acterial black rot disease in cabbages can be effectively managed by using either seed, bark, or leaf Moringa aqueous extract sprays. The 100 and 140% concentration levels were most effective, compared to the 60% concentration level. Further studies need to be carried out to assess if the utilization of the Moringa seed aqueous extract as a seed dressing would not increase its antibacterial effects against the test pathogen since it is an important seed borne disease of brassicas and crucifers.

Keywords: antibacterial, aqueous extraction, leaf, seed, bark extracts

8.1. Introduction

Xanthomonas campestris bacterium can be transmitted either by being carried on the seed, through wounds caused by insects, through natural openings/hydathodes (significant port of entry), through fluid filled stomata openings or even via irrigation water [Singh *et al.*, 2016; Lancaster, 2006]. However, seed contamination by this bacterium is the main effective and most important vehicle of transmission for this pathogen. The bacterium attaches itself to the seed before plant establishment. The different pathovars of this bacteria thrive mostly in humid soils and temperatures ranging from 20 to 30^{o}C and a good example is the *Xanthomonas campestris pv. phaseoli*. This bacterium thrives at temperatures around 27^{o}C, causing economic losses in common beans (*Phaseolus vulgaris, L*) [Francisco *et al.*, 2013]. The bacteria, however, exhibits a much more reduced spread in dry weather and is also less active at temperatures above 50^{o}C [Francisco *et al.*, 2013]. This is because the wilting caused to the host plants will ultimately inhibit water transportation which disrupts conducive conditions for the bacterium to thrive. The bacteria's regeneration time is temperature dependent and one bacterium needs approximately 2 hours to produce 2 bacteria by fission, with the shortest generation time occurring at approximately 27^{o}C [Berthier *et al.*, 1993]. These characteristics aid in making black rot disease, a serious pathogen in

Zimbabwe. Tropical climates experience warm to hot climates most of the year. *Xanthomonas campestris* pathovars exhibit a wide host range, with each pathovar being specific and exceptionally distinctive to particular host plants. Xanthomonas pathovars are listed as number five among the top 10 plant bacterial pathogens of economic importance worldwide [Mansfield *et al.*, 2012]. This indicates their ability to cause devastating diseases of economic importance. The pathovars *campestris, vesicatoria, malvacearum,* and *phaseoli* are among the most detrimental pathogens in field crops. It also causes huge economic losses because of the damage inflicted on the cabbages, making them unmarketable [Vicente and Holub, 2013]. The cabbage produce will also be unfit for human consumption or processing. Black rot can cause up to 40% yield losses and in Zimbabwe, it poses a huge threat to both smallholder and commercial farmers in agro-ecological regions II, III and IV [Karavina, 2011] which are the hubs of vegetable production. The most common control strategies being implemented include cultural and preventative methods such as use of certified seed, control of weeds/volunteer plants, and removal or destruction of diseased plants. Biological control using *B. subtilis* has been effectively used in Zimbabwe against black rot diseases on different Brassica species in dry and short rainy seasons [Wulff *et al.*, 2002]. However, this strategy is not easily and readily available for resource constrained farmers.

Black rot is a major constraint for vegetable production by smallholder farmers in many parts of Africa including South Africa, Kenya, and Zimbabwe with devastating impacts on productivity. Crop losses of up to 100% to this disease have been reported by smallholder farmers during the rains in 1998 in Tanzania [Massomo *et al.*, 2004]. In Zimbabwe, black rot disease causes problems in all the five agro-ecological regions and disease incidence can be as low as 10% or as high as 80% [Wulff *et al.*, 2002]. Black rot disease affects above ground plant-parts and proceeds quickly in plants which have been weakened by poor crop protection practices. These create multi-focal inoculation sites for the pathogens. Plants are susceptible to black rot at any growth stage, and this is seen by the initial, characteristic V-shaped, small, wilted lesions which appear on the leaf margins, then progressively enlarge and coalesce. The lesion patch has small black veins, which in severe cases, results in vein discoloration of the stems. After reaching the stem, the bacteria moves systemically to other healthy uninfected plant parts [Jensen *et a.l*, 2010]. The infected areas will eventually enlarge, progress towards the leaf base, turn yellow to tan and then dry out.

Alternatively, dependent on prevailing weather conditions, the infected areas will after coalescing, rot into slimy masses as a result of secondary infections. Diseased leaves may also become stunted on one side and then drop prematurely [Seebold *et al.*, 2008]. One of the major challenges of this disease is that it inevitably paves way to soft rots which produce a very unwelcoming smell and cause quick rotting before harvesting the produce. In extreme cases, the crop might even fail to establish or produce anything worth harvesting [Kocks *et a.l,* 1998].

There is no satisfactory chemical control which has been established for all the four *Xanthomonas campestris* pathovars [Arias *et al.*, 2000]. This is mainly because most chemical applications are done when crop has already been infected and symptoms have been observed rendering this method marginally ineffective in disease suppression. In some cases, the chemicals may even cause pathogen resistance [Nadu, 2010; Wolf, nd.]. Alternative methods to control black rot using Black night shade have been reported to be effective [Chipurura, 2010]. However, these methods only reduced black rot virulence, but were not effective in achieving its control. Once the Xanthomonas pathogens infects plants, they cause a large percentage of the economic parts incapable of fetching any market prices, unfit for storage, or even processing [Schaad *et al.*, 1980]. There is need therefore, to identify other biological control agents to aid in combating black rot, without necessarily resorting to extensive and over use of chemicals. Moringa has great potential due to the antimicrobial properties it contains. If properly harnessed, these can be utilized effectively as alternative bio-pesticides to manage black rot disease.

The Moringa tree has naturalized in the northern parts of the country, which are drier (Binga, Zambezi valley, Victoria Falls) and more conducive to its establishment [Goss, 2012]. Based on its varied utilizations, the local communities can further benefit from it as a bio-pesticide and not only as a food source, livestock feed or hedge plant [Maroyi, 2006]. Moringa is a multipurpose tree whose potential needs to be harnessed and implemented in sustainable crop disease control strategies. Studies have indicated that plant aqueous extracts can be effectively used to control crop diseases. Moringa is one of the plants that have been utilized for its anti-microbial properties, and thus is a good choice for this current study [Al-askar and Rashad, 2010].

The main objectives of this study were to 1) evaluate the efficacy of Moringa leaf, seed, and bark aqueous extracts against black rot disease in open field cabbages and 2) determine which part of the Moringa plant aqueous extracts was most effective in suppressing black rot disease in cabbages grown under natural conditions.

8.2. Materials and Methods

8.2.1 Experimental Site and Methodology

The study was carried out at Victory Farm, Beatrice which lies in Mashonaland East Province, latitude 18 o 15'3.72" S, longitude 30^{o} 51'9.96E, approximately 1900msl and receives an average rainfall of 450 -600mm/annum. The soils in this area are predominantly sandy loam soils. This farm is under organic farming.

The experiment was laid out in a Split Plot, 3 x 3 factorial, in a Randomized Complete Block Design with 3 replicates. Moringa aqueous type was the main plot factor at 3 levels that is, leaf, bark, and seed, whilst the subplot factor were the Moringa aqueous concentration levels at 3 levels, 60%, 100% and 140%. The control was the use of Neem aqueous extracts in controlling black rot disease. The 140% Moringa leaf extract concentration was the only one which produced a runny paste. This challenge was overcome by running the extracts through a stainless steel laboratory sieve initially, before straining the extracts through a double-layered mutton cloth, cheesecloth and finally small sized guage stainless steel laboratory sieves.

8.2.2 *Xanthomonas campestris* Pathogen and Moringa aqueous extract preparation

The pathogen was prepared by collecting diseased leaves from an infected cabbage plant. The pathogens were isolated, purified, cultured and multiplied after Kochs postulate procedure [Rivers, 1937]. These cultures were then stored until they were needed for inoculation of the study plants.

To prepare the plant aqueous extracts, firstly the Moringa leaf and bark were air-dried in a well earated shade house for 7 days. Moringa seeds cannot air dry within 7 days, therefore the seed was dried in a laboratory oven set at 30^{o}C to avoid sample degradation for 5 days. After drying, the leaves, bark and seeds were ground to a fine powder using a laboratory grinder to get finely ground

powder. The Moringa aqueous extract solutions were prepared by suspending 60g, 100g, and 140g powders of each of the aqueous types separately in 1000 mls of sterile water. The amount of sterile water was increased to increase flowability and the viscosity of the resultant extract solutions. The modification to the quantities used also improve the spread and dissolution of the Moringa aqueous extract solutions in the field (Sabat and Gupta, 2009). The samples were shaken and stirred continuously for 30 minutes and allowed to sediment at room temperature for 24 hours. The extracts were firstly strained through a stainless steel laboratory sieve to remove the bulk of the organic residues from extractant solution. They were then strained with a double-layered muslin cloth, through a cheesecloth and finally run through small sized guage stainless steel laboratory sieve. This process was repeated for each extract type to obtain the 3 concentration levels needed for the study. The land was ploughed using a tractor and the beds were made using hand hoes. Two blocks A and B each with 9 beds were prepared. The beds measured 4m x 2m. The beds were pre-watered to field capacity before sowing. Liquid organic fertilizer was applied as fertigation through sprinkler irrigation 3 times per week to field capacity. The farm has a biodigestor hence the liquid manure is the waste obtained from cattle, sheep, goat, and pig droppings. The irrigation system is also run using biogas generated from the biodigestor. The farm is a model farm which trains farmers in sustainable permaculture and farming practices through efficient use of renewable energy. The cabbage seeds were sown at a rate of 3kg /Ha (actual sown were 5 seeds per planting station). The seedlings were then later thinned to leave one plant per station at four weeks after planting.

8.2.3 Cabbage bacterial inoculation and data collection

To inoculate cabbage plants with the black rot bacteria, the fully expanded top two leaves at the center of each cabbage seedling, were pricked and a third of a section of the leaf was cut using sterilized, stainless steel scissors, to improve the penetration of bacteria. The freshly prepared inoculum suspension from the cultured bacteria was sprayed onto each individual plant using 1 liter hand sprayers to run off point (until the whole plant was drenched with aqueous solution). The inoculation was done at 5 weeks after crop emergence.

The three Moringa aqueous extracts at concentrations of 60, 100 and 140% were foliar applied achieving full cover spray at each application, on cabbage plants once weekly basis from 7 weeks after crop emergence. The data collection exercise was initiated at 7 weeks after crop emergence. Recording of leaf defoliation was done once a week for the duration of the study. Totally defoliated plants were counted to evaluate the suppressive efficacy of the different Moringa plant aqueous extracts using the scoring method modified from [Anjorin *et al,* 2016] (Table 8**.**1). Assessment of totally defoliated leaves was part of the scoring process done. Plants showing symptoms exhibited based on Table 8.1 score of 1 – 9, were also counted and recorded.

Table 8.1. Disease Severity scoring table for black rot disease in this study

Scale	Disease severity
1	No symptoms
2	Very few symptoms, 1-3 small lesions on 1/2 leaves
3	3-5 leaves with more than 3 yellow lesions
4	Enlarged lesions on 3 or more leaves
5	Coalescing lesions forming wilted tissue.
6	Necrosis, with the veins turning black or brown
7	Plants completely defoliated and dying.

Modified from Anjorin, Jolaoso, and Golu. (2016).

The data was analyzed using Excel and GenStat 14th edition. The means were separated using LSD at 5% level where there were significant differences.

8.3. Results

A GC/MS phytochemical analysis which was carried out on the Moringa aqueous extract sources revealed the presence of bioactive compounds such as phenylpropanoids, alkaloids, choursomene neolignans, flavonoid and tannin (Appendix C) which is consistent with other findings [Holetz *et al*, 2002].

The three Moringa aqueous extracts (bark, leaf, and seed) showed great antibacterial activity by significantly controlling black rot disease ($P < 0.05$) in this field study. The highest disease control efficacy was recorded during the 10th week after crop emergence (Table 8.2). Moringa seed aqueous extract recorded the least mean leaf defoliation of 2.965 and 38% disease severity as the highest recorded at 140% concentration at 10 WAE (Fig 8.1). The Moringa seed aqueous extract managed to suppress black rot at a significantly higher rate across all the three concentration levels, compared to the other two extract types. Moringa bark aqueous extract also significantly suppressed black rot disease progression by recording a leaf defoliation mean of 3.312 (Table 8.2) compared to the Moringa leaf extract. Moringa bark extract exhibited higher antibacterial activity against black rot disease at 9 and 10 WAE, recording 32% as the highest severity (Fig 8.1). Moringa leaf aqueous extract had the highest leaf defoliation mean (3.486) and highest level of disease progression. The Moringa aqueous types were significantly different from each other in their antibacterial effects on the black rot disease as indicated by the least leaf defoliation occurring in cabbages under the Moringa seed treatment ($P < 0.05$). Cabbages which were under the Moringa bark aqueous treatments exhibited an intermediate defoliation rate into the 10th week after emergence, after having succumbed the greatest to the black rot bacterium initially. The highest rate of leaf defoliation was exhibited in cabbages under the Moringa leaf aqueous treatment. Interestingly there were no observed interactions occurring between aqueous concentration level and ability to suppress Black rot disease black rot disease among all the three Moringa aqueous types. However, cabbages under the 60% concentration level for all the three Moringa plant aqueous extracts were least effective in their antibacterial action against black rot disease. The Moringa aqueous at 100% and 140% concentration levels were not significantly different in their antibacterial action against the test pathogen from each other ($P < 0.05$) as indicated by the observations obtained in this study (Plates 8.1 – 8.4).

The results indicated an interesting trend in which the Moringa bark aqueous at 7 weeks after crop emergence (WAE), had the largest black rot disease mean defoliation level compared to both the Moringa leaf and the seed aqueous extracts (Fig. 8.1). However, by the 8th WAE, its efficacy at suppressing black rot disease severity had increased such that it was performing comparably well in relation to Moringa seed aqueous. At the end of the 10th WAE, Moringa bark aqueous had managed to surpass the antibacterial efficacy of Moringa leaf aqueous in suppressing black rot

disease in the cabbage plants. The results indicated significant differences in the antibacterial activities of each of the three Moringa aqueous extracts from each other in their antibacterial properties against Black rot disease ($P < 0.05$). Moringa seed aqueous extract had the lowest defoliation mean, followed by Moringa bark aqueous and the highest mean defoliation was recorded for Moringa leaf aqueous extract (Fig. 8.1). Moringa seed aqueous extract consistently maintained the lowest cabbage leaf defoliation rate throughout all the weeks. Moringa leaf aqueous extract had significantly higher cabbage leaf defoliation means at 8WAE, 9WAE and 10WAE ($P < 0.05$). At 8WAE, the antibacterial activity of Moringa bark and seed were not significantly different from each other shown by the same rate of cabbage leaf defoliation.

Table 8.2. Means showing effect of Moringa aqueous extracts on black rot disease severity in cabbages

Treatment	7WAE	8WAE	9WAE	10WAE
Bark aqueous	4.042	2.762	2.92	3.312$^{b\#}$
Leaf aqueous	2.014	2.969	3.148	3.486^{c}
Seed aqueous	1.912	2.694	2.875	2.965^{a}
P value	0.739	0.069	0.087	**0.009**
LSD5%	0.8791	0.5278	0.3617	0.09
CV %	8.2	2.5	2.1	1.1

#Means with different letters are significantly different at $P < 0.05$

Key: WAE = Weeks After crop Emergence

The Moringa aqueous type antibacterial effects were significantly different from each other ($P < 0.05$), affecting the level of defoliation and devastation of the crop not only statistically (Table 8.2 and Fig. 8.1), but physically as exhibited symptomatically in the field.

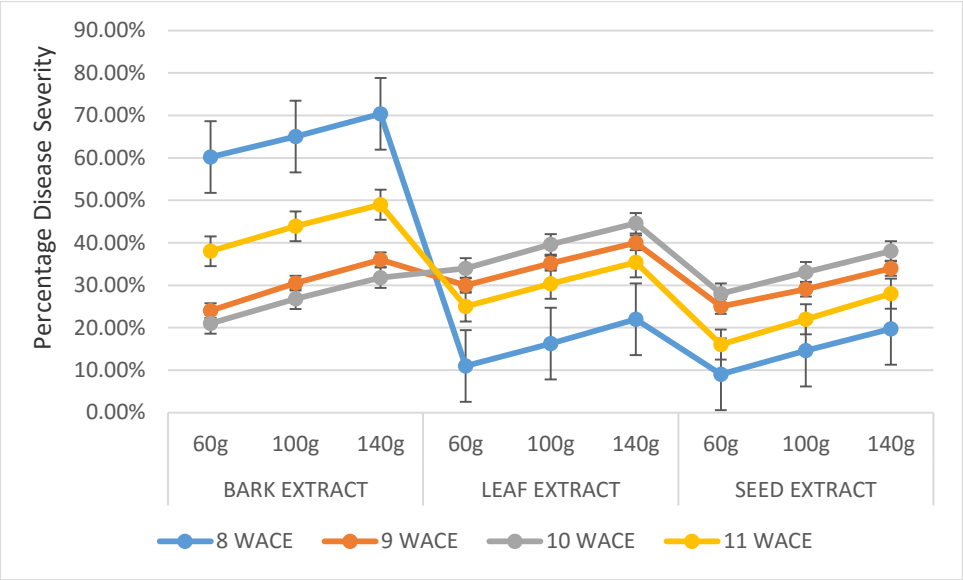

Figure 8.1. Effect of Moringa seed, leaf and bark aqueous extracts on mean cabbage leaf defoliation at 7 - 10 weeks after emergence. P = 0.009

Plates 8.1 – 8.4 are showing the physical manifestation of the effect of Moringa aqueous type on black rot disease severity and impact on cabbage growth, defoliation, and incidence of secondary physiological conditions such as rotting and plant death.

a. b.

Plates 8.1a and 8.1b. Antibacterial effect of Moringa seed aqueous in suppressing black rot disease in field grown Cabbages at 100% and 140% levels respectively


a. b.

Plates 8.2a and 8.2b. Antibacterial effect of Moringa bark aqueous in suppressing black rot disease in field grown Cabbages at 100% and 140% levels respectively


a. b.

Plates 8.3a and 8.3b. Antibacterial effect of Moringa leaf aqueous in suppressing black rot disease in field grown Cabbages at 100% and 140% levels respectively



Plates 8.4a - 8.4e: Antibacterial activity of all aqueous types at 60% concentration.

From top left to top right insets: (a) Moringa seed aqueous extract, (b) Moringa bark aqueous extract, (c) Moringa leaf aqueous extract: exhibiting physiological disorders arising from black rot disease;

From bottom left to right insets: (d) Moringa seed aqueous, (e) Moringa bark aqueous and (f) Moringa leaf aqueous. Showing the secondary infections and rotting effects of black rot disease in cabbage plants.

8.4. Discussion

The presence of bioactive compounds such as phenylpropanoids, alkaloids, choursomene neolignans, flavonoid, and tannin compounds to state a few, (Appendix C) are responsible for the antimicrobial activity found in the Moringa extracts used in this study. The flavonoid eugonol, is antibacterial in its activity against pathogens [Holetz *et al*, 2002]. The Moringa plants antibacterial properties were able to suppress black rot progression in this study. Moringa has been utilized for decades as part of an important medicinal plant traditionally, mainly as a remedy for all sorts of human conditions and illnesses [Dubey *et al*, 2013]. This points to its potential utilization as a bio-pesticide and the fact that it would be easily adopted. This is because over 80% of rural populations rely on traditional medicines or remedies which they can afford financially and these are readily available [Duraipandiyan *et al*, 2006]. It would be important to engage local farmers in a wider study involving larger farming communities located in various agricultural zones to validate its efficacy under a wide range of climates and natural environments. The current study was carried out and repeated from September 2015 until April 2016, this period overlapped with two contrasting seasons in Zimbabwe. During the September to November period, Zimbabwe experiences its hottest months as a country, which is then followed by its rainy season from the

months of November/December until March when the rains tail off and eventually come to an end [Davis, 2011]. It is possible that these conditions might have influenced the antibacterial efficacy of the Moringa aqueous extracts against black rot disease since this was an open field experiment. The cabbage plants were grown under natural, open-field conditions. Moringa aqueous extract efficacy might have been affected by contrasting extremities in weather which occurred during this study period. There was a period of intense heat followed by the prolonged wet and rainy periods. These conditions might have influenced the ability of one aqueous to express its antibacterial properties stronger compared to the other type. The phytochemical and bioactive compounds present in Moringa are affected by varying degrees of temperature [Tesfay *et al,* 2016]. The amounts of phenol compounds within the Moringa seed increase with raise in temperatures, hence the improved efficacy of seed aqueous as an antibacterial agent in comparison to leaf and bark aqueous extracts. However other *in-vitro* studies have shown no significant negative influence of temperature on the antibacterial activity of Moringa leaf aqueous against *S. aureus* and *Salmonella typhi* pathogens [Ju and Sally, 2014]. However, this was an *in-vitro* study, therefore many other factors were controlled and held constant.

Moringa seed aqueous extracts in this study exhibited high antibacterial properties by suppressing deleterious progression of the black rot disease in the cabbage plants. *In-vitro* screening studies on antibacterial properties of Moringa aqueous extracts against enteric pathogenic bacteria such as *S. aureus, P. aeruginosa, Salmonella typhi, E. coli* and *Vibrio cholera*, revealed strong antibacterial potency of Moringa against several gram negative and gram positive bacteria [Elangovan, 2014]. This antibacterial action of Moringa on the *Xanthomonas campestris* pathogen in this study, can be attributed to the presence of phenols (hydroxybenzoic acids, 2-hydroxy benzoate) and flavonoids (kaemperferol, quercetin, isorhamnetin, L-rhamnose) (Appendix C) which infer antimicrobial properties to the plant [Aja *et al*, 2014]. Tannin, present in the current Moringa plant extracts used in this study, imparted antimicrobial activity to the plant. Naturally occurring phytochemical compounds within plants such as p-hydroxybenzoic, reduced the growth of *Clostridium botulinum* types A and B, whilst ethyl p - methoxycinnamate, reduced mold growth in other similar studies [Chipurura, 2010]. The enhanced antibacterial efficacy of Moringa seed aqueous compared to leaf and bark aqueous extracts might be linked to the higher concentrations

of these bioactive phytochemicals within the seed aqueous compared to the bark and leaf aqueous extracts (Appendix C). Moringa leaf has the lowest concentration of tannins, which might explain the low antibacterial action the leaf aqueous exhibited compared to the performance of the bark and seed aqueous [Ferreira *et al,* 2008]. The presence of tannins at cellular level, not only result in a firmer, drier and thermally more stable structure, but they also contain high levels of antimicrobial activity due to the presence of trihydroxy benzoic acid (Appendix C) [Chipurura, 2010]. Thus, the low concentration of this bioactive compound in the leaves might have reduced its antibacterial action against black rot disease in this study. Moringa seed also contains approximately 49.8 – 57.25% oil with undetectable levels of linolenic acid which makes Moringa oil one of the most stable plant oil [Tesfay *et al,* 2016]. This stability might have enabled the phytochemical compounds present in Moringa seed aqueous not to break down due to the contrasting hot and wet conditions. This might have kept the antibacterial action of the seed aqueous stable and efficient against black rot disease.

Moringa showed marked bacteriolytic effect of Moringa on viable myxococci enterobacteria in an *in-vitro* study [Luqman *et al,* 2012], and the pterygospermin compound was the antibacterial principle which acted against a range of bacterial pathogens [Oxford and Singh, 1946]. The presence of the pterygospermin compound in the Moringa extracts used in this study inhibited and suppressed the black rot disease in cabbages grown in this study (Appendix C). Furthermore, Moringa leaf and seed aqueous extracts have inhibited bacterial growth effectively with their anti-Quorum-Sensing (anti QS) ability, which causes them to exhibit bacteriocidal properties in other studies [Singh, 2009]. All these further validate the antibacterial properties observed in this current study. Further studies have indicated the Moringa leaf aqueous extracts' effectiveness in inhibiting growth of the *P. aeruginosa* pathogen being significantly low. The *P. aeruginosa* pathogen exhibited resistance to the antibacterial action of the Moringa leaf aqueous extract [Abalaka *et al*., 2012]. These results are consistent with the findings in this current study whereby Moringa leaf aqueous extract exhibited very low antibacterial action against the test pathogen. Factors such as part of the plant tissue of Moringa aqueous extract being used, and seasonal changes in its growing environment, influence levels of the phytochemical and bioactive compounds present at the time of utilization. Summer samples of Moringa leaf, stem and bark aqueous extracts had higher ash,

calcium and phenolic compounds and stronger antioxidant activity compared to the winter aqueous extracts [Shih *et al*, 2011]. The antioxidant activity of the Moringa plant was a function of the part of the Moringa plant used, and it was exhibited thus: leaf > stem > bark. [Shih *et al*, 2011]. These indicate the potential differences which exist in the percentages of bioactive compounds present in the Moringa plant aqueous extracts as influenced by seasonality and plant part positioning on the Moringa plant. This would explain the differences observed in the rate of antibacterial action efficacy shown by the bark, seed, and leaf aqueous extracts against the test pathogen in this study. It might also explain why the Moringa leaf aqueous in this study showed the highest level of disease severity among the cabbages being sprayed with leaf aqueous extract. The low amounts of phenolic compounds might have reduced the antibacterial efficacy of the leaf aqueous extracts in this current study (Appendix C). Moringa seed aqueous extracts contain antibacterial properties which compete favorably with modern day antibiotics in human health pathogens. Moringa seed aqueous extracts contains a niaziridin-rich aqueous fraction, which enhances the bioactivity of several antibiotics (rifampicin, tetra cycline and ampicillin) that were effective against bacteria [Karim and Azlan. 2012]. Hence the ability of Moringa seed aqueous extract to exhibit the highest antibacterial action against black rot disease by achieving the least mean and percentage of defoliated cabbage leaves in this current study.

There is need for further studies to determine the plant growth stage at which Moringa aqueous extracts can be more efficient as bio-pesticides against crop pathogens. Application methods need to be studied for enhanced efficacy of the bio-pesticide in crop disease management strategies. Level of Moringa aqueous pulverization to enhance extraction process needs to be investigated. Many questions concerning improving efficacy and sustainable, climate smart utilization of Moringa still remain unanswered.

8.5. Conclusion

Black rot disease in field-grown cabbages can be controlled using Moringa leaf, seed and bark aqueous extracts. Moringa seed aqueous extracts exhibited higher antibacterial action against black rot compared to Moringa bark and leaf aqueous extracts, it also brings to light areas which need

further studies. This current study has validated that Moringa does possess antibacterial properties which are effective against black rot in cabbages produced under natural, open-field conditions. There is need to validate how these same Moringa aqueous extracts would perform in other host plants grown under different agro-ecological zones and natural conditions in Zimbabwe. Although a lot of research has been done to validate the antimicrobial properties of Moringa, not enough open-field studies have been documented. Hence there is still that need. Black rot is a devastating disease of many crops of economic importance and locally in Zimbabwe, organic farmers are the most affected by it as they grapple with the realities of failing to salvage any marketable produce. Hence the need to identify alternative biologically based, non-chemical disease control strategies in Zimbabwe. This work is a step towards that need.

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CHAPTER 9

CONCLUSIONS AND RECOMMENDATIONS

9.1 Major findings regarding antifungal and antibacterial activity of Moringa aqueous extracts

This study investigated the efficacy of *Moringa* leaf, seed and bark aqueous extracts when utilized as bio-pesticides against bacterial and fungal crop pathogens of economic importance infecting horticultural crops in Zimbabwe. The choice to use *Moringa* in the study were based on: 1) the tree has high antifungal and bacterial properties and it is has already naturalized in the drier parts of the country. These are the areas where the majority of resource-poor and marginalised farming communities are located in Zimbabwe. 2) Moringa aqueous extracts contain flavonoid and isothiocyanates compounds which are said to infer antimicrobial properties to the tree. 3) The tree is drought tolerant and thrives well under poor-nutrition soils and is a multi-purpose tree. The tree therefore has the potential to improve community livelihoods as a direct source of food and through sustainable food security by enhancing crop disease management strategies and crop productivity. These studies carried out over 3 growing seasons validate that Moringa aqueous extracts contain significant amounts of antifungal and antibacterial compounds which effectively controlled four fungal pathogens - *P. ultimum, Rhizoctonia solani, Fusarium solani* and *Phytophthora infestans;* and three bacterial pathogens - *Pectobacterium carotovorium subspp. brasiliensis, P. atrosepticum* and *Xanthomonas campetris* pathogens.

The Moringa plant aqueous extracts were effective in suppressing fungal and bacterial pathogens under *in-vitro* and open field conditions. The Moringa plant aqueous extracts achieved effective control of bottom/root rots (*R. solani* and *F. solani* respectively) and black rot (*Xanthomonas campetris*) diseases, after having being applied as foliar sprays in lettuce and cabbage respectively. The Moringa plant aqueous extracts enhanced lettuce head weight and diameter.

The survey which was carried out to identify prevalent diseases and study sites within the sub-humid areas in Zimbabwe, revealed the following aspects on diseases and their control methods: 1) Farmers are aware of the changing disease patterns occurring in response to the variations in

weather patterns. During the November – July months, the farmers experience higher levels of fungal and bacterial disease outbreaks. In comparison, lower disease incidence are being experienced during the months of August – October, and March – April. 2) Horticulture farmers face major challenges with fungal disease outbreaks, whilst bacterial diseases are not as prevalent. 3) The majority of farmers have resorted solely to using chemical disease control strategies to manage these outbreaks, with very few of the farmers' practising cultural or mechanical disease management strategies. 4) None of the farmers are utilizing bio-pesticides or botanical disease management strategies as an alternative method.

9. 2 Implications of findings for commercial development of Moringa bio-pesticides

The implications of these studies concerning the efficacy of Moringa aqueous extracts against crop pathogens is that these aqueous extracts can be developed into effective fungicides and bactericides. These natural bio-pesticides can be implemented into mainstream integrated crop disease management strategies. Moringa leaf and seed aqueous extracts can be effectively used in disease management strategies to control diseases such as damping-off, root rots, and stem rots and black rot in brassica crops. This indicates great potential for developing commercially viable natural bio-pesticides against disease pathogens in crop production. Moringa aqueous extracts can also be used to enhance growth and quality of horticultural crops as natural plant growth regulators. This also indicates a potentially viable commercially product. The antifungal and antibacterial activities shown by the Moringa aqueous extracts, imply a need for more intensified and diversified study to enable increased uptake of this technology in all sectors of the farming community.

The survey results imply that farmers might not be aware of the negative human health and environmental impacts associated with indiscriminate or over-use of chemicals. Farmers were also not aware of the possibility of the utilization of alternative bio-pesticide or botanical disease control strategies. There is thus an urgent need to raise awareness among farmers on the concerns and issues surrounding indiscriminate chemical use, and also on the potential of bio-pesticide disease control alternatives. These can be done through a series of trainings, workshops and setting up of demonstration plots working within the communities through the Ministry of Agriculture, extension services and other stake-holders in the agriculture industry.

9.3 Recommendations for improved validation and efficacy as a Moringa bio-pesticide

Extensive field experiments involving vegetable disease management using various parts of the Moringa plant aqueous extracts must be carried out in various locations and across different agro-ecological zones. Such studies would determine the effect of different agro-ecological conditions on the efficacy of these antimicrobial compounds.

Crop pathogens of economic importance in local agricultural production sites have to be identified and the correct Moringa botanical mixes prepared to suit particular areas, taking into consideration the geospatial nature of each site. Such field trials must be carried out using horticultural crops of economic and social significance within their particular environments.

Collaborative research between agronomists, pathologists, chemists, plant breeders, pharmacists, animal scientists, forestry specialists and crop protection specialists to scientifically validate information regarding Moringa antimicrobial properties, is vital.

Identification and development of effective, yet low cost plant extraction methods which will not alter, denature, dilute or mutate the natural antimicrobial bioactive compounds within *Moringa* is critical.

Verification of extraction and application methods and stage of plant growth which would improve efficacy of these antifungal and antibacterial properties of the Moringa aqueous extracts.

Determination of how the level of Moringa aqueous pulverization would influence the antifungal and antibacterial action of the aqueous solution is needed.

9.4 Areas for future research to stabilize Moringa bio-pesticide formulations

1. There exists need for further study to provide clarity on the mechanisms involved in the antifungal and antibacterial action of individual Moringa plant part aqueous extracts (seed, leaf, and bark). Such studies need to be carried out using molecular markers to track the various processes and reactions triggered in and occurring within the study plant.

2. Mechanistic and systems approaches are necessary which takes cognizance of the components of the biocontrol processes needs to be adopted to investigate the network of interactions which exist.
3. Use of molecular markers to track how Moringa aqueous extracts break down fungal and bacterial pathogen cells whilst effecting their antimicrobial action in plant tissues is important.
4. Devising ways and processes of stabilizing the Moringa aqueous formulations aimed at producing low-cost commercial bio-pesticides, which can be stored and used when needed on a large scale.
5. Combining Moringa bio-pesticide with salts, organic acids, glucose or other additives for increased performance, and evaluating how incidental micro-organisms or mixtures affect bio-pesticide efficacy. Carrying out studies to evaluate any improved or reduced efficacy in commercial, large scale or open field applications is necessary.
6. Development of specific protocols for each specific host plant-pathogen system based on pathogen epidemiology and the unique features pertaining to each pathogen is important.
7. There is need to enhance uptake of bio-pesticide technologies and practices among farming communities.

APPENDIX A

RESULTS OF THE GC/MS ANALYSIS CARRIED OUT ON LOCAL ZIMBABWEAN *Moringa oleifera* ACCESSIONS USED IN THE STUDY

Moringa plant part	**Main Phytochemicals Present**			
	Flavonoids	**Phenolic**	**Tepernoids**	**Others**
Leaves	kaempferol, rhamnetin, isoquercitrin and kaempferitrin beta-sitosterol, glycerol-1-(9-octadecanoate), 3-O-(6'-O-oleoyl-beta-D-glucopyranosyl), beta-sitosterol and betasitosterol-3-O-beta-D-glucopyranoside, tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones, alkaloids, glycoside compounds, glucosinolates and isothiocyanates glucosinolates, isothiocyanates, glycoside compounds and glycerol-1-9-octadecanoate sterols, saponins, trepenoids, phenolics, flavanoids like quercitin, isoquercitin, kaemfericitin, isothiocyanates and glycoside compounds Glycoside niazirin, niazirinin and three mustard oil glycosides, 4-[4'-O-acetyl- α -L-rhamnosyloxy) benzyl] isothiocyanate, niaziminin A and B			
Seed	palmitic, linolenic, linoleic and oleic acids tannins, saponin, phenolics, phytate, flavanoids, terpenoids and lectins N-Benzyl thiocarbamates, N-benzyl carbamates, benzyl nitriles and a benzyl ester methionine, cysteine, 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate, benzylglucosinolate, moringyne, mono-palmitic and di-oleic triglyceride Nitriles, isothiocyanate, thiocarbanates, 0-[2'-hydroxy-3'-(2''-heptenyloxy)]-propylundecanoate, 0-ethyl-4-[(α -1-rhamnosyloxy)-benzyl] carbamate, methyl-p-hydroxybenzoate and β-sitosterol			
Bark	alkaloids 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate 10 L-arabinose, D-galactose, D-glucuronic acid, L-rhamnose, D-mannose, D-xylose and leucoanthocyanin			

APPENDIX B

SURVEY QUESTIONNAIRE

My name is …….. an undergraduate student at the University of Zimbabwe, working on a research topic entitled **"*The evaluation of Moringa oleifera as a bio-control agent for vegetable diseases in Zimbabwe*".** I am kindly asking for your ideas, opinions and information pertaining to the different symptoms and types of diseases which are major problems in your vegetable gardens in Domboshava. I am appealing for your assistance by freely supplying your views and comments. All the information collected here will be treated with strict confidentiality as it is only for the purpose of academic research.

Instructions:

Please tick ✓ or circle the response which best describes your situation where applicable and fill in where there are blank spaces

1. Gender of the respondent: **1**. Male **2**.Female
2. Age of respondent: 1. 45 – 60 2. 19 – 25 3. Above 61 years

3. Which type of vegetable grower group do you belong to?
    1. for food for the family only
    2. subsistence (i.e. planned for food and surplus sales)
    3. market gardening
    4. small scale commercial
    5. large scale commercial

4. What types of vegetables do you grow?

Code	**1.Leafy**	**2.Bulbs**	**3.Legumes**	**4.Fruits**	**5.Cucurbits**
1	Rape	onions	Beans	Tomatoes	Cucumbers
2	Cabbage	Garlic	Peas	Green pepper	Pumpkins
3	Tsunga	Leeks		Red pepper	Watermelons
4	Covo			Chillies	Kiwino
5	Spinach				Mapodzi
6.	Other specify …… …… ……	Other specify …….. ……..	Other specify …… …….	Other specify …….. …… ……	Other specify …….. …… ……

		…….	…….	…….	…….

5. What are the indicators of the presence of disease in your vegetables?

Disease	**Symptoms**		
1. Fungal	Chlorosis and/ necrosis	Whitish blisters	Powdery patches
	Stunted growth	Clubbed roots	Cottony fungal growth
	Water-soaked rotting	Wilting of the leaves	Greying or reddening of the leaves
2. Bacterial	Water- soaked lesions	Greasy looking leaves	Yellowing V-shaped lesions
	Cankers	Wet, slimmy, soft rots	Dying of the leaves
	Small dark spots surrounded by yellow halos	Reddish or tan spots	Disagreeable odours
3. Other specify			

6. Which disease/s based on symptoms in table in question 4 above is most problematic in your area?
   1. Fungal
   2. Bacterial
   3. Other.....Specify.....

7. How do you view the problem of diseases in your area for the past two seasons/ years?
   1. It has increased
   2. It has decreased
   3. There is no change

8. Which crops are mostly attacked by these diseases?

1. Rape	2. Cabbage
3. Tsunga	4. Covo
5. Spinach	6. Onions
7. Garlic	8. Leeks
9. Beans	10. Tomatoes
11. Green pepper	12. Red pepper
13. Chillies	14. Cucumbers
15. Pumpkins	16. Watermelons
17. Kiwino	18. Mapodzi

19.Other.....Specify.....

9. When the crops mentioned in question 3 are not available in the field, which alternative crops are attacked by these diseases?

Crop	**Alternative crops**
Leafy vegetables	
Bulbs	
Legumes	
Fruits	
Cucurbits	

10. Besides alternative plants, name the weeds or plants that act as alternative hosts of these diseases?

Crop	**Alternative weeds**
Leafy vegetables	
Bulbs	
Legumes	
Fruits	
Cucurbits	

11. In which season are these diseases most problematic?

1. Summer [Aug – Oct] **2**. Rainy [Nov – Feb]
3. Winter [May – July] **4**. Spring [March – April]
5. All year round [persistent]

12. Is there a change in the frequency or intensity of the disease attacks and number of disease attacks depending upon the prevailing weather conditions?
    1. There is an increase **2.** There is a decrease **3**.There is no change

13. If there is an increase in the frequency/intensity and number of diseases, which diseases are the most troublesome?
    1. Fungal
    2. Bacterial
    3. Other .....Specify.....

The Disease cycle, biology or physiology

14. How do you perceive the problem of diseases with respect to the following attributes in the past 10 years:
    a. Rate of infection of diseases
        1. Extreme to very severe infections
        2. Moderate to Mild infections
        3. Low to very low infections
        4. Very little to no infections
    b. Specify the disease and the symptoms they cause

Disease	**Symptoms**		
1. Fungal	Chlorosis and/ necrosis	Whitish blisters	Powdery patches
	Stunted growth	Clubbed roots	Cottony fungal growth
	Water-soaked rotting	Wilting of the leaves	Greying or reddening of the leaves
2. Bacterial	Water- soaked lesions	Greasy looking leaves	Yellowing V-shaped lesions

	Cankers	Wet, slimmy, soft rots	Dying of the leaves
	Small dark spots surrounded by yellow halos	Reddish or tan spots	Disagreeable odours
3. Other specify			

c. State amount of losses caused
    1. Major losses
    2. High to very high
    3. Moderate to low
    4. Insignificant to none

d. How many different types/forms of the most problematic diseases highlighted in question 4 have you noticed in your fields during the past 10 years?

Problem Disease	**Different signs/symptoms of the disease**
a)	
b)	
c)	
d)	
e)	
f)	

15. Which measures do you use to control the problem diseases?
    1. Chemical insecticides
    2. Biological methods
    3. Natural remedies
    4. Mechanical methods
    5. Cultural control.....specify.....

16. If you are using chemical insecticides which chemicals do you use and on which crops and how often do you apply them?

Crops/ Plants Protected	**Insecticide used**
a)	
b)	

c)	
d)	
d)	
e)	
f)	

17. Are the chemical insecticides effective in the control of these diseases?
    1. Yes ☐ 2. No ☐

18. Give reasons to your answer on question 16

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19. How frequent do you spray these chemical insecticides?
    1. Once/month ☐ 2. Once /week ☐ 3.Twice/week ☐ 4.Three times/week ☐
    5. Other, please specify.....

20. Is there a difference in spraying frequency from what you used to do in the past 10 years?
    1. Yes ☐ 2. No ☐

21. Do you rotate these chemical insecticides? 1. Yes ☐ 2. No ☐

22. Give reasons to your answer on question 19

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23. If the answer to question (19) is yes, highlight the sequence of chemical rotation

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24. Giving names, explain the role played by named natural remedies in the control of these diseases

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25. Are these natural remedies effective or their efficiency has been reduced over the past years?

1. They are effective ☐ 2. They are less efficient ☐

26. What do you think could be the reason for a reduction in efficiency of these natural remedies?

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27. Besides the problem diseases you have mentioned earlier on, how do you view the problem of insect pests in your area?

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28. Which are the most important insect pest, in which crops are the insect pests most prevalent?

Field Crops
a)
b)
c)
d)

e)
Horticultural Crops
a)
b)
c)
d)
e)

29. What corresponding diseases or symptoms are caused by these insect pests?
    1. Yellowing
    2. Mottling/mosaic symptoms
    3. Brown spots
    4. Stunted growth
    5. Curling of crop leaves
    6. Rotting
    7. Death of crops

29.Can you comment on the responses of diseases to a changing climate in Zimbabwe.....
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.....

Thank You for your Cooperation and assistance!!!!