Isolation and identification of entomopathogenic nematode bacterial metabolites for biological control of *Fusarium* head blight of wheat and wheat aphids

by

JULIUS LEUMO KGOSIEMANG

submitted in accordance with the requirements
for the degree of

MASTER OF SCIENCE

in the subject

AGRICULTURE

at the

UNIVERSITY OF SOUTH AFRICA

SUPERVISOR: DR T RAMAKUWELA

CO-SUPERVISOR: DR S FIGLAN

Dedication

I dedicate this work to my daughter, my mother and my grandmother.

"With God all things are possible", Matthew 19:26

Declaration

I **Julius Leumo Kgosiemang** hereby declare that the dissertation, with the title: "**Isolation and identification of entomopathogenic nematode bacterial metabolites for biological control of *Fusarium* head blight of wheat and wheat aphids"** that I hereby submit in accordance with the requirements for the degree of MSc in Agriculture at the University of South Africa, it is own and it has never been submitted any other degree at any institution.

I declare that all figures and data from other people's work have being correctly paraphrased and acknowledged, and quotaion marks have being used where exact words are used.

I declare that I have received ethics approval to conduct and gathere data from all the experiements of this study.

I declare that the content of my dissertation has been electronically submitted to Turnitin for plagiarism detection before the final submission for examination.

Student signature: ___________________ Date: ________________

Acknowledgements

My sincere gratitude goes to the following institutions and people for being part of the success of this study:

- Agricultural Research Council for allowing me to use their facilities to conduct research and for accommodating me throughout my studies.
- Winter Cereal Trust and National Research Foundation for funding the study.
- My supervisors Dr Tshimangadzo Ramakuwela (Agricultural Research Council) and Dr Sandiswa Figlan (University of South Africa) for support and guidance, and for reviewing and editing this dissertation.
- Nicolene Thiebaut for statistical analysis of results generated in this study and Dr Lisemelo Motholo for inputs and edits on this dissertation.
- I would like to convey my sincere gratitude and appreciation to Amos Mosia, Motlotleng Thipe, Lebohang Moloi, Buyisiwe Ngubane and Sinethemba Zulu for technical support and social outings.
- My family for believing in me and for the unconditional love I get from them.

Table of contents

Dedication ii

Declaration iii

Acknowledgements iv

Table of contents v

List of figures x

List of tables xii

List of abbreviations used xiii

Abstract xiv

Chapter 1 1

Introduction 1

1.1 Background 1

1.2 Problem statement 2

1.3 Hypothesis 3

1.4 Aim and objectives 4

1.4.1 Aim 4

1.4.2 Objectives 4

1.5 Research questions 4

1.6 Chapter layout 4

1.7 Benefits of the study 5

1.8 Limitations of the study 5

1.9 Ethical consideration 5

1.10 COVID 19 guidelines 5

1.11 Outputs from the study 6

1.11.1 Publications 6

1.11.2 Oral presentation 6

Chapter 2 7

Literature review 7

2.1 Wheat (*Triticum aestivum* L.) (*Poaceae*). 7

2.1.1 Global wheat production 7

2.1.2 South African wheat production 7

2.2 Abiotic stressors of wheat 8

2.2.1 Salinity 8

2.2.2 Temperature 8

2.2.3 Drought 9

2.3 Fungal pathogens found in wheat 9

2.3.1 *Fusarium* head blight 9

2.4 Wheat Aphids 11

2.4.1 Russian wheat aphid, (*Diuraphis noxia)* 12

2.4.2 Life cycle of Russian wheat aphid 12

2.5 Chemical pesticides 13

2.5.1 Chemical pesticides used for FHB management 14

2.5.2 Chemical pesticides used for wheat aphids management 14

2.6 Integrated pest management (IPM) 15

2.7 Biological control 15

2.7.1 Biological control of FHB 16

2.7.2 Biological control of wheat aphids and RWA 16

2.7.3. Entomopathogenic nematodes as biocontrol agents 16

2.8 The symbiotic bacteria of EPN, *Xenorhabdus* and *Photorhabdus* 17

2.8.1 Phase variation 18

2.9 The life cycle entomopathogenic nematodes and their symbiotic bacteria 18

2.10 Bacterial secondary metabolites 19

2.10.1 Insecticidal compounds produced by *Xenorhabdus* and *Photorhabdus* spp. 20

2.10.2 Antifungal compounds produced by *Xenorhabdus* and *Photorhabdus* spp. 20

2.11 Chromatography 21

2.11.1 High performance liquid chromatography 21

2.11.2 Mass spectrometry 22

2.11.3 Liquid chromatography- Mass spectrometry (LC-MS)......................22

2.12 Gap in literature 23

Chapter 3...24

Materials and Methods..24

3.1 Source of wheat cultivar, cultures and insects...24

3.1.1 Wheat cultivar..24

3.1.2 *Fusarium* sp. cultures 24

3.1.3 Russian wheat aphid biotypes...24

3.1.4 Rearing of *Galleria mellonella*..24

3.2 Isolation and culturing of EPNs symbiotic bacteria...................................24

3.3 Production of bacterial metabolites 25

3.4 Preparation of metabolite treatments...26

3.5 Evaluation of bacterial metabolites on FHB causing *Fusarium* sp. *Fusarium graminearum*. 26

3.5.1 Efficacy of bacterial metabolites on *F. graminearum* mycelial growth.........26

3.5.2 Efficacy of bacterial metabolites on *F. graminearum* spore germination.....27

3.5.3 Statistical analysis on the efficacy of the bacterial metabolite against *F. graminearum* mycelial growth and spore germination...27

3.6 Efficacy of bacterial metabolites on RWA biotypes 28

3.6.1 Evaluation of bacterial metabolites against five RWA biotypes 28

3.6.2 Statistical analysis on the effect of bacterial metabolites against five RWA biotypes 28

3.7 DNA extraction and amplification of two effective isolates 28

3.8 Profiling and identification of bacterial metabolites from two best isolates 29

3.8.1 Sample preparation 29

3.8.2 Liquid Chromatography-Quadruple Time-of-Flight Tandem Mass Spectrometry (LC-MS/MS) analysis 29

3.8.3 Data processing and molecular networking for identification of metabolites 30

Chapter 4 31

Results 31

4.1 Evaluation of bacterial metabolites against FHB causing *Fusarium* sp. *F. graminearum*. 31

4.1.1 Efficacy of bacterial metabolites on *F. graminearum* mycelial growth 31

4.1.2 Efficacy on *Fusarium graminearum* spore germination 37

4.2 Effect of bacterial metabolites from EPN bacterial symbionts against five RWA biotypes 39

4.3 Liquid chromatography- Mass spectrometry (LC-MS) analysis 41

4.4 DNA extraction and amplification 42

Chapter 5 44

Discussion 44

5.1 Inhibitory effect of bacterial metabolites against FHB 44

5.1.1 Efficacy of bacterial metabolites on *Fusarium graminearum* mycelial growth. 44

5.1.2 Inhibitory effect on *F. graminearum* spore germination. 46

5.2 Efficacy of bacterial metabolites on Russian wheat aphid biotypes. 46

5.3 Liquid chromatography- Mass spectrometry 47

5.4 Effective isolates and their bacterial symbionts..48
Chapter 6...49
Conclusions and recommendations..49
6.1 Conclusions 49
6.2 Recommendations..49
References..51

List of Figures

Figure 1. *Fusarium graminearum* life cycle. Adapted from Shude et al., (2020). 11

Figure 2. Life cycle of Russian wheat aphid (Botha, 2020) 13

Figure 3. A typical example of a mutualistic association between entomopathogenic nematodes and their bacteria symbionts (Stock, 2019). 19

Figure 4. The LC-MS mobile phase separation conditions throughout 20 min cut-off time. The mobile phase composition was 95% solvent A and 5% solvent B at 0 min; and ramped to 95% solvent B and 5% solvent A at 12-16 min. 30

Figure 5. Overall (all isolates; three and seven days of incubation) mean inhibition activity of different bacterial metabolites treatments compared control (P < 0.0001). 32

Figure 6. *Fusarium graminearum* mycelia growth treated with different bacterial metabolite treatments of *Steinernema beitlechemi* SGI 197 compared to control after three days of incubation. 32

Figure 7. Mean mycelial growth inhibition activity of autoclaved metabolites compared to control after three days of incubation (P < 0.0002). 33

Figure 8. Mean mycelial growth inhibition activity of autoclaved metabolites compared to control after seven days of incubation (P = 0.0063). 34

Figure 9. Mean mycelial growth inhibition activity of crude metabolites compared to control after three days of incubation (P < 0.0001). 35

Figure 10. Mean mycelial growth inhibition activity of crude metabolites compared to control after three days of incubation (P < 0.0001). 35

Figure 11. Mean mycelial growth inhibition activity of filtered metabolites compared to control after three days of incubation (P < 0.0001). 36

Figure 12. Mean mycelial growth inhibition activity of filtered metabolites compared to control after seven days of incubation (P < 0.0001). 36

Figure 13. The overall mean mycelial growth inhibition activity of different isolates compared to control (P < 0.0001). 37

Figure 14. Mean *Fusarium graminearum* spore germination percentages in potato dextrose broth (PDB) treated with 20% filtered metabolites of different EPN symbiotic bacteria cultures compare to control (P < 0.0001). 38

Figure 15. Russian wheat aphid Biotype 1 mortality rates (α= 0.05). 39

Figure 16. Russian wheat aphid Biotype 2 mortality rates (α= 0.05). 40

Figure 18. Russian wheat aphid Biotype 4 mortality rates (α= 0.05). 40

Figure 21. LC-MS chromatogram of *Heterorhabditis bacteriophora* SGI 170. 41
Figure 22. LC-MS chromatogram of *Steinernema beitlechemi* SGI 197. 42
Figure 24. Gel image from PCR products with amplified ITS and 28S regions. Well M: 100bp DNA ladder, Well 1-4: ITS region, Well 5-8: 28S region, Well 1-2 and 5-6: *Heterorhabditis bacteriophora* SGI 170, Well 3-4 and 7-4: *Steinerrnema beitlechemi* SGI 197...43

List of tables

Table 1. Selected indigenous EPN isolates for isolation of their symbiotic bacteria............ 25

Table 2. Mycelial growth inhibition rates (%) of all isolates in both incubation periods. 31

Table 3. Mean inhibition rate (%) of *Fusarium graminearum* spore germination. Inhibition rate = 100 × (spores germinated in control – spores germinated in treatment)/ spores germinated in control (Hazir et al., 2016) 38

List of abbreviations used

ARC-SGI	Agricultural Research Council- Small Grain Institute
MBA	Mung Bean Agar
PDA	Potato Dextrose Agar
NBTA	Nutrient Bromothymol blue Triphenyltetrazolium chloride Agar
TSB	Trypric Soy Broth
PDB	Potato Dextrose Broth
ANOVA	Analysis of Variance
LSD	Least Significant Difference
RWA	Russian Wheat Aphid
DNA	Deoxyribonucleic acid
LC-MS	Liquid Chromatography- Mass Spectrometry
qTOF	Quadruple Time of Flight
HMDB	Human Metabolome Database
FHB	Fusarium Head Blight
Dn gene	*D. noxia* resistance source
ERC	Ethics Review Committees
FAO	Food and Agriculture Organization

Abstract

Fungal diseases such as *Fusarium* head blight (FHB) and a complex of wheat aphids are significant biotic stressors, negatively affecting wheat production and quality. The use of chemicals and resistant cultivars is continuously challenged by the development of resistance, while harsh chemical pesticides pose environmental and human/animal health risks. This study explored the pesticidal activity of bacterial metabolites, produced by the bacterial symbionts of entomopathogenic nematodes (EPNs), against FHB causing *Fusarium* sp. *Fusarium graminaerum* and Russian wheat aphid biotypes. To achieve this aim, metabolites were isolated from the symbiotic bacteria derived from the EPNs collection at the Agricultural Research Council-Small Grain (ARC-SG). Metabolites were screened against *Fusarium graminaerum* and RWA biotypes, followed by the identification of nematodes, bacteria and metabolites. Mycelial growth and spore germination of *F. graminaerum* were differently inhibited by metabolites produced by bacterial symbionts of the nine selected EPNs isolates. Isolate SGI 197 and SGI 170 produce the highest overall mycelial growth inhibition rates of 96.25% and 95.79%, respectively. Out of the three types of metabolite treatments tested against mycelial growth, crude metabolites were the most effective compared to other metabolite treatments in all isolates. *Xenorhabdus* isolates displayed higher spore germination inhibition activity compared to *Photorhabdus* isolates. Among *Xenorhabdus* isolates, isolate SGI 257 had the highest spore germination inhibition rate of 96.29%, which was higher than that of other *Xenorhabdus* isolates. Crude metabolites were also screened for aphicidal activity on five RWA biotypes. The results depicted that resistance to metabolites increased with the order of the biotypes (from Biotype 1 to 5). Isolate SGI 197 had the highest overall aphicidal activity when compared to other isolates and the control. LC-MS analysis and molecular networking computational tools detected and confirmed the presence of two previously described antimicrobial compounds, Anthraquinones (m/z 255/285) and stilbene precursor 3,5-dihydroxy-4-isopropyl-trans-stilbene (Isopropylstilbene) (m/z 255); and a number of other unidentified compounds. The results from this study are based on *in vitro* assays. More studies are needed to evaluate the antifungal and insecticidal activities of these bacterial metabolites on *in vivo* conditions. Among the selected isolates, Isolate SGI 197 seemed to be the best candidate for use as a biopesticide. These are baseline steps towards the development of cheaper and more eco-friendly pest control products based on the metabolites.

Key words: Entomopathogenic nematodes, *Xenorhabdus*, *Photorhabuds*, *Fusarium* head blight, Russian wheat aphids, bacterial metabolites.

Chapter 1
Introduction

1.1 Background

The economic worth of wheat and its contribution as food to humans and livestock cannot be questioned (Shewry and Hey, 2015). An estimated 20% of the total calories humans consume come from wheat, and it gives more protein when compared to other food sources (Appels *et al*., 2018). To cater for the growing human population, there is a demand for wheat research and breeding to speed up the genetic gain and increase and protect wheat quality and yield (Appels *et al*., 2018). A ceaseless drive to produce high yields and good quality grains is faced with many challenges. Decreasing availability of good farmland, climate change and other irregular abiotic and biotic factors continue to threaten wheat production locally and globally (Figueroa *et al*., 2017). Out of all threating wheat diseases, fungal diseases are the most significant threat in increasing the gap between actual and usable yield (Goutam *et al*., 2015). Figueroa *et al*. (2017) reviewed few of the causal fungal wheat diseases, of which wheat rusts, blotch diseases, and *Fusarium* head blight (FHB) are highlighted. Apart from fungal diseases, other biotic stressors of economic importance are pests belonging to the family *Aphididae*, which is made up of about 5,000 aphids species, distributed all over the world (Shavit *et al*., 2018).

Fusarium head blight is a significant floral disease of grains and also poses a serious health hazard to humans and animals by poisoning grains with hazardous mycotoxins (Jimenez-Garcia *et al*., 2018; Dilks *et al*., 2019). The disease is primarily caused by an *Ascomycete* fungus, *Fusarium graminearum* (*Gibberella zeae* Petch.) (*Hypocreales: Nectriaceae*) (Figueroa *et al*., 2017). *Fusarium* head blight disease leads to early aging of wheat heads, resulting in approximately 80% reduction of both grain quality and yield (Torres *et al*., 2019). Effects of FHB disease have two parts: reduce grain yield and quality, which jeopardize the overall harvest and delays commerciality; furthermore, the production of various sesquiterpenoid trichothecene mycotoxins [such as the type B toxin, deoxynivalenol (DON)] in the grains causes a serious food safety risk and health hazard to humans, animals and natural ecosystems (Figueroa *et al*., 2017). Dweba *et al*. (2017) reported that it is not easy to actively manage the diseases using a single control method due to their limitations such as development of resistance. Several methods are used to control the disease, including crop rotation, cultural practices, planting less-susceptible cultivars, chemical and biological management strategies (Torres *et al*., 2019).

Wheat aphids are devastating invasive pests to small grains and other cultivated grass species (Tulpová *et al*., 2019). Symptoms that result from aphid feeding may be leaf rolling, leaf chlorosis, plant desiccation and plant stunting. The damage eventually leads to yield reduction (Luna *et al*., 2018). Damage by aphids to cereal crops can be severe, especially in wheat. Commonly, 8% to 34% yield losses can be reached with aphid infestation (Yahya *et al*., 2017), while even higher through feeding by Russian wheat aphids (RWA). Some of the wheat aphids with high economic importance have been described in detail by Blackman and Eastop (2000). Problematic aphids include the Bird cherry-oat aphid, English Grain aphid, Rose-grain aphid, Greenbug and RWA (Blackman and Eastop, 2000; Shavit *et al*., 2018). The common approaches to control aphid populations in crops is through breeding for resistance and insecticidal treatments. However, management strategies, such as the use of insect-resistant breeds, can lead to the development of new biotypes (Jankielsohn, 2013) and/or the development of resistance against insecticides (Simon and Peccoud, 2018), for these reasons, it must be addressed in the long-term with sustainable solutions.

Entomopathogenic nematodes (EPNs) are a subgroup of insect-parasitic nematodes that are used in augmentative, classical and conservation biological control as alternatives or supplements to chemical pesticides (Lu *et al*., 2017). *Xenorhabdus* and *Photorhabdus* are gram-negative bacterial symbiont of EPNs (Yooyangket *et al*., 2018). Entomopathogenic nematodes bacterial symbionts are responsible for killing the host insect, and also prevent scavengers and contaminants from eating or spoiling the cadaver (serving as food source for the EPNs) (Hazir *et al*., 2016). Shan *et al*. (2019) hypothesized that EPNs symbiotic bacteria produce volatile and non-volatile metabolites that can act as deterrents against many soil microbes. Therefore, these specialized metabolites have the potential to be developed as biopesticides, targeting both plant pathogens and insect pests. This study explored the potential of these bacterial metabolites, which are generated by the EPN symbiotic bacteria, as biological control of FHB and RWA.

1.2 Problem statement

Fusarium head blight (FHB) is an economically important disease of small grains (Wegulo *et al*., 2015). Unlike other foliar diseases that indirectly affect grain yield, FHB develops directly on wheat spikes, which can cause severe losses of grain yield during serious epidemics (Su *et al*., 2019). Moreover, the production of mycotoxins such as nivalenol (namely deoxynivalenol), zearalenone, zearalene, T-2 toxin, zearalene and diacetoxyscirpenol can be harzadous to both humans and animals (Jimenez-Garcia *et al*., 2018). Worldwide, the disease can result in losses

amounting to billions of dollars per year, and majority of these losses comes from wheat and barley (Wegulo *et al*., 2015). *Fusarium* head blight is best controlled by integrating different methods such as genetic resistance, crop rotation, a timely fungicide application and tillage practices (Paul *et al*., 2018). The complex genetics of *Fusarium* species and fungicide residues highlight the need for alternative measures.

Wheat aphids are responsible for major damage in wheat fields resulting in serious yield losses (Li *et al*., 2019), and are said to cause hundreds of millions of dollars in worldwide crop losses annually (Ali *et al*., 2018). Aphids cause a serious economic loss of grains by directly feeding on the plant phloem sap and indirectly by vectoring plant viruses [such as barley yellow dwarf virus (BYDV)] in between plants and crops (Shah *et al*., 2017). As they feed, aphids remove plant photo-assimilates, resulting in longitudinal streaking around the main leaf vein, plant chlorosis, considerable biomass reduction and head trapping, in worse infestations, plant death (Tulpová *et al*., 2019). Currently, chemical pesticides are widely used for the control of aphids (Zhao *et al*., 2018). Due to the safety and development of resistance concerns over synthetic chemical application, it is urgent to look for alternative control measures that are safe and environmentally sound (Shah *et al*., 2017).

Entomopathogenic nematodes (EPNs) have been applied successfully as biological control agents of a range of economically important insects found in different habitats (Garcia-del-Pino *et al*., 2018). Their application seems to be cantered on the soil-based stages in the life cycles of insect pests, as EPNs are adapted to soil environments as their natural habitat (Platt *et al*., 2018). Research on the use of EPN to control foliage-based insect life stages has been less successful and rarer because of harsh environmental conditions above ground (Platt *et al*., 2019). EPN symbiotic bacteria are responsible for killing the host insect, and prevent scavengers and contaminants from eating or spoiling the cadaver by producing secondary metabolites. Therefore, specialised metabolites have the potential for biological control of both plant pathogens and insect pests.

1.3 Hypothesis

Entomopathogenic nematodes symbiotic bacteria from the genera *Xenorhabdus* and *Photorhabdus* produce secondary metabolites with antifungal and insecticidal activities with the potential to control FHB and RWA infestations.

1.4 Aim & Objectives

1.4.1 Aim

The aim of the study was to isolate and identify potential secondary metabolites produced by EPN symbiotic bacteria for safe and environmentally friendly biocontrol of FHB and RWA biotypes.

1.4.2 Objectives

Objectives of the study were:

1.4.2.1 To test bacterial metabolites treatments against FHB and RWA.

1.4.2.2 Extract and identify secondary bacterial metabolites from effective strains/isolates.

1.4.2.3 To identify or characterise the effective nematode and their symbiotic bacterium.

1.5 Research questions

1.5.1 Do bacterial metabolites from EPN bacterial symbionts have inhibitory effects on mycelial growth and spore germination of FHB causing *Fusarium* sp.?

1.5.2 What effect do bacterial metabolites from EPNs bacterial symbionts have on five RWA biotypes?

1.5.3 What kind of secondary metabolites are produced by EPN bacterial symbionts?

1.6 Chapter layout

1.6.1 Chapter 1 is an introductory chapter; it includes the background of the topic and the problems that the work addresses with the objectives that will lead to the achievement of the aim.

1.6.2 Chapter 2 is a review chapter; it highlights all the knowledge that is in the public domain for all the keywords used and all the variables of this study.

1.6.2 Chapter 3 includes the materials and methods; this is where all the protocols, instruments and setting of conducted experiments are described in detail.

1.6.3 Chapter 4 comprises results; this is where the results obtained from this study are presented.

1.6.4 Chapter 5 is made up of a discussion of results, which includes the meaning and significance of the results using referenced literature as evidence backing up the meaning or findings, and a set of conclusions made from the results for all objectives and recommendations for future studies.

1.7 Benefits of the Study

Firstly, this study holds potential for prototype development based on the metabolites and the EPN symbiotic bacteria species for use by both emerging and commercial farmers. Both these bioproducts can be incorporated into integrated pest management (IPM) programmes. Ultimately, increasing crop production in support of the national food security and nutrition policy and climate-smart agriculture for sustainable food production.

Secondly, the research may result in a decrease in the use of chemical pesticides. Currently, South Africa has more than 3000 registered pesticides (DAFF) and is one of the four largest importers of pesticides in sub-Saharan Africa (Quinn *et al*, 2011). Lastly, the outcome of this project contributes to the pool of knowledge about the safe and sustainable use of biological pesticides.

1.8 Limitations of the study

The aim of this study was to extract and identify secondary metabolites produced by EPN symbiotic bacteria for safe and environmentally friendly biocontrol of FHB and RWA biotypes. Metabolite treatments were successfully screened against FHB causing *Fusarium* sp. *F. graminearum* but metabolites that were responsible for inhibiting mycelial growth and spore germination were not individually identified. Metabolome databases used in this study were not solely for bacterial metabolites. As a result, most metabolites or compounds could not be identified or matched with compounds originating from bacteria, or it could be that these compounds are new and, therefore, more work should be done to characterise them. The source of Russian wheat aphids and other insects used in this study was limited since these insects were used in many projects, hence they were not always available when needed.

1.9 Ethical consideration

UNISA-CAES health research committee approved the ethical clearance application for this study. Clearance approval was valid from 05/11/2020 to 31/10/2023 (Appendix A). The work in this study was conducted at Agricultural Research Council-Small Grain (ARC-SG), Insect Pathology laboratory under qualified personnel supervision. All culture plates (bacterial and fungal plates) were autoclaved before disposal.

1.10 COVID 19 guidelines

The study continued without Ethics Review Committees (ERC) notifications (Appendix C). Activities were postponed during level 5 of the lookdown or when a positive case was reported

within the institute, and all safety and productive measures were taken throughout the study period.

1.11 Outputs from the study

1.11.1 Publications

- Kgosiemang, J.L., and Ramakuwela, T., (2021, September). Nematodes as potential biofungicide for *Fusarium* head blight. SA Graan/Grain, page 39. (Appendix D)

1.11.2 Oral presentation

- Kgosiemang, J.L., Ramakuwela, T., and Figlan, S., 2021. Antimicrobial effect of symbiotic bacterial metabolites from entomopathogenic nematodes for control of *Fusarium* Head Blight of wheat. The 23rd Symposium of the Nematological Society of Southern Africa, Cape Town, Western Cape province, South Africa, 19-23 September.

Chapter 2
Literature review

2.1 Wheat (*Triticum aestivum* L.) (*Poaceae*).

Wheat is one of the most important food grain used around the world as food to humans and feed to livestock (Alikina *et al*., 2016). Furthermore, wheat grains contain high nutritional value, have an acceptable taste, and can be used to make many processed food products, namely bread, pizza, pasta, bulgur, and couscous or drinks such as beer (Cianferoni, 2016). Its first cultivation was around 10 000 years ago, during the 'Neolithic Revolution', and the first forms of cultivated wheat were diploid and tetraploid wheats (Shewry and Hey, 2015). Their genetic relationships show that they originally came from the south-eastern parts of Turkey (Shewry, 2009). Now, the main species of wheat that has been cultivated throughout the globe is (*Triticum aestivum* L.) (*Poaceae*)., a hexaploid form known as "common" or "bread" wheat (Shewry and Hey, 2015). This is a hybrid species from two unrelated species, cultivated tetraploid wheat *T. turgidum* subsp. *durum* and wild grass *Triticum tauschii*; and it can be found only in cultivation (Shewry, 2009). Since its existence, bread wheat (*Triticum aestivum* L.) has become the most preferred staple crop, not only due to its ability to survive in different photoperiod and prolonged cold temperatures but also due to the fact that it grows better than its ancestral parents under low pH, salt, aluminium, and freezing conditions (Li *et al*., 2018). There are two forms of wheat, based on their sowing season: spring wheat and winter wheat (Khanfri *et al*., 2018).

2.1.1 Global wheat production

Wheat (*Triticum aestivum* L.) is the world's third most grown crop, following maize and rice. Food and Agriculture Organization (FAO) (2014) reported that the crop occupies 4% of agricultural fields globally, which is 218.5 million hectares, with an average yield of 3.26 tons per hectare (Kajla *et al*., 2015). Since 1961, global wheat production has increased at a rate of 21.8% per year (Pardey, 2011).

2.1.2 South African wheat production

South Africa (SA) has the second-largest wheat production area (0.5 Mha), following Ethiopia (1.7 Mha) in Sub-Sahara Africa (Tadesse *et al*., 2019). Still, SA is the largest contributor in wheat production, followed by Ethiopia in sub-Sahara Africa (Asela *et al*., 2020). South African areas with high contributions to wheat production include the Western Cape, the Free State, and Northern Cape provinces. Other areas ranked as minor contributors in South African

wheat production this includes Gauteng and the North West province (Nhemachena and Kirsten, 2017). South Africa's wheat (*T. aestivum*) production dropped significantly from 2.5 million tonnes, harvested from 974 000 ha in 2002, to roughly 1.7 million tonnes, harvested from 500 000 ha in 2013 (DAFF,(2014); Sosibo *et al*., 2017). Sadly, the decrease in wheat production occurs when the demand is progressively increasing annually at a rate of 1.6%, and it is estimated that almost 60% more wheat will be required by 2050 (Singh *et al*., 2015). Negative factors such as climate change, drought, poor soil fertility and incidences of pests and diseases are responsible for the sudden decrease in SA wheat production (Gqozo *et al*., 2020).

2.2 Abiotic stressors of wheat

Wheat is grown in sub-tropical and tropical parts of the world that experience different abiotic stresses (Poudel and Poudel, 2020). Abiotic stresses continue to be a global threat to wheat production (Faran *et al*., 2019). According to world estimates, approximately 50% of yield losses in agricultural crops are attributed to abiotic factors such as temperature (27%), salinity (10%), drought (9%) and other abiotic forms of stresses (4%) (Kajla *et al*., 2015). The severity of two or more abiotic stresses occurring together is more than of one abiotic stress occurring alone (Kajla *et al*., 2015).

2.2.1 Temperature

Wheat is very susceptible to heat stress. Estimates show that for every 1°C increase in temperature, global wheat production decreases by 6% (Poudel and Poudel, 2020). Heat stress negatively affects wheat growth and development at different stages, causing an increase in yield loss. At the flowering stage, heat stress limits nutrient uptake to developing grain, resulting in small-sized grains and reduced yields (Ullah et al., 2022).

2.2.2 Salinity

Agricultural fields, including the ones for wheat cultivation, are exposed to different abiotic stresses, including salinity (Miransari and Smith, 2019). Salinity stress is among the main abiotic stresses that reduce crop yield by negatively affecting plant growth (Afridi *et al*., 2019). Salinity affects nutrient uptake and enzyme activity, protein synthesis, photosynthesis, assimilation and hormone metabolism of the plant (Ahmad *et al*., 2019). Elevated salt concentrations in agricultural fields result in ionic and osmotic stress, causing noticeable growth changes such as necrosis, reduced leaf area and abscission (Ahanger *et al*., 2019), and subsequently low yield and grain quality (Miransari and Smith, 2019).

2.2.3 Drought

Drought affect plant growth and production more than any other abiotic stress (Keyvan *et al*., 2010). Depending on the onset time, period and severity of the stress, drought can reduce wheat productions by up to 92%. During drought, leaf aging is accelerated, and the process of photosynthesis declines resulting in decreased grains size and numbers (Saeidi and Abdoli, 2015).

2.3 Fungal pathogens found in wheat

Fungal diseases form part of the most important biotic stressors and often cause unneglectable losses in wheat production globally (Jighly *et al*., 2016). Although other fungal species are important in promoting plant growth by associating with the host plant's roots in a mycorrhizal association, almost 20 000 fungal species are parasites and are able to cause diseases in crops (Ray *et al*., 2017), and others are endophytic causing symptomless infections within healthy plant tissues (Larran *at el*., 2007). Wheat diseases from fungal pathogens are more threatening to crop yields and grain quality than diseases resulting from bacteria and viruses (Goutam *et al*., 2015). Among fungal diseases of wheat, rusts (*Puccinia* spp.), powdery mildew (*Blumeria graminis*), blotches (*Mycosphaerella graminicola*), and head blight/scab (*Fusarium* spp.) are the current highlighted threats (Qi *et al*., 2019). From the mentioned, *Fusarium* spp. are of interest simply because they secrete bioactive secondary metabolites of which include mycotoxins (Hertz *et al*., 2016).

2.3.1 *Fusarium* head blight

Fusarium head blight is the most significant wheat ear disease; it can cause total or partial early ear aging and therefore reduces both crop yield and grain quality (Scarpino *et al*., 2015). In 1884, W.G. Smith was the first to describe the disease in England and six years later F.D. Chester and J.C. Arthur (Wegulo *et al*., 2015) reported the disease in the United States. In SA, FHB was first found in 1980 along the Vaal River in the North West province (Minnaar-Ontong *et al*., 2017). The second outbreak report of FHB in SA was in KwaZulu-Natal during the 1985/86 production season (Minnaar-Ontong *et al*., 2017).

The main FHB causing *Fusarium* species are *Fusarium graminearum* group II (also known as *Gibberella zeae* sexual stage), *F. poae*, *F. avenaceum* and *F. culmorum* (Matny, 2015), where *F. graminearum* and *F. culmorum* predominate the list and are the most destructive (Wegulo *et al*., 2015). Infection from FHB is mainly influenced by air, weather and atmospheric humidity during flowering and early stages of seedling development (Qi *et al*., 2016). Various

mycotoxins such as deoxynivalenol (DON) and zearalenone (ZEA) which are toxic to both humans and animals are secreted by these *Fusarium* species (Dweba *et al*., 2017). The main toxin producers belonging to genus *Fusarium* are *F. fujikuroi* (Sawada) and *F. graminearum* species complexes (Van der Lee *et al*., 2015), of which the latter is a pathogen of wheat.

2.3.2 *Fusarium graminearum* Group II (*Nectriaceae*)

Fusarium graminearum (*Gibberella zeae*) is the primary pathogen causing FHB disease in South African wheat (Shude *et al*., 2020), and the presence of its mycotoxins in human food and livestock feeds have long been a pandemic (Pleadin *et al*., 2013; da Rocha *et al*., 2014; Dweba *et al*., 2017). In addition to causing FHB in wheat, the pathogen is also a causal agent for other diseases such as crown root rot and ear rot of wheat, barley, and maize (Lu and Edwards, 2018). *Fusarium graminearum* is ranked the fourth most significant plant fungal pathogen based on the pathogen's scientific and economic importance (Ntushelo *et al*., 2019). Unlike other plant pathogenic fungi, e.g. *Magnaporthe oryzae* and *Colletotrichum orbiculare*, *F. graminearum* does not use appressoria to enter cells of its host cells (Qiu *et al*., 2019). In wheat, the pathogen enters at anthesis (flowering) stage because at this stage wheat anthers open to release pollen creating an opening to the pathogen (Shude *et al*., 2020). The pathogen overwinters (in some cases over-summers) in crop debris, and at this stage it produces asexual macroconidia and sexual ascospores, both of which are responsible for causing FHB disease on cereal heads in the next crop season (Manstretta and Rossi, 2015). Normally, infestation inoculums are either by airborne ascospores or water-splashed conidia (Wang *et al*., 2015). Under laboratory conditions, the entire *F. graminearum* life cycle takes about two weeks (Manstretta and Rossi, 2015). Figure 1 adapted from Shude *et al*. (2020) below, illustrate the life cycle of *F. graminearum.*

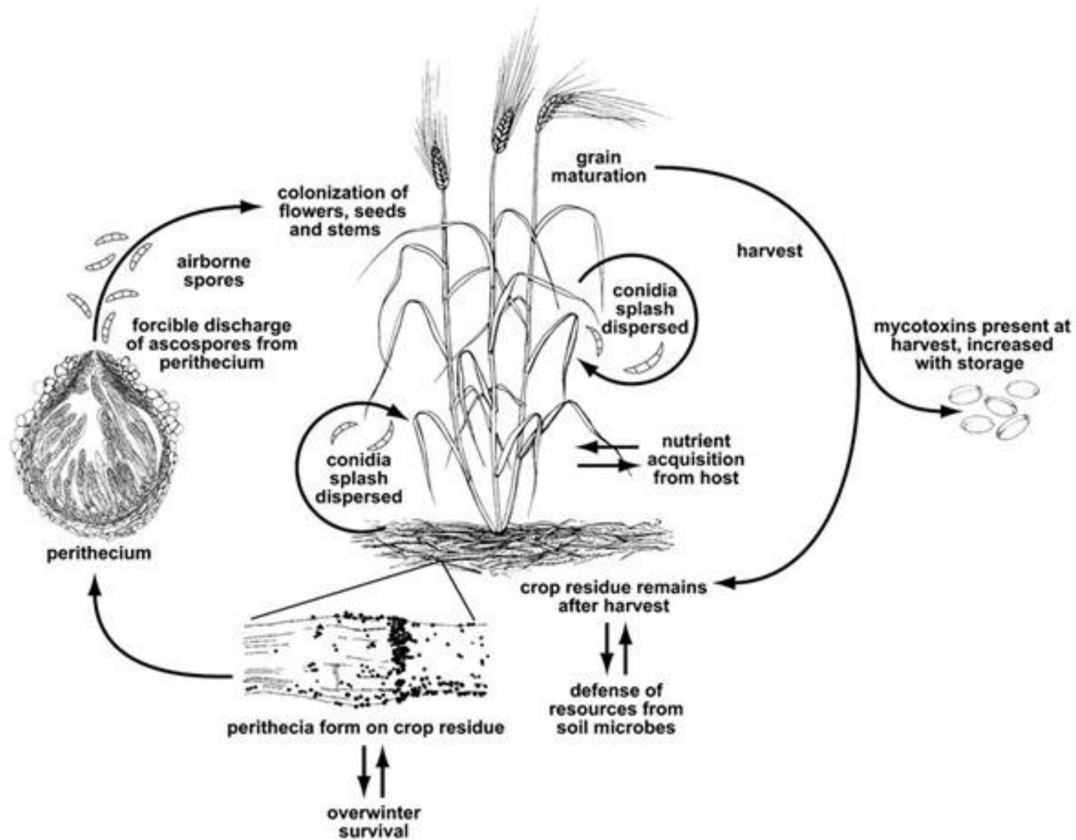


Figure 1. *Fusarium graminearum* life cycle. Adapted from Shude *et al.,* (2020).

2.4 Wheat aphids *Diuraphis noxia* (Mordvilko)(*Aphididae)*

Wheat aphids are devastating invasive pests of small-grain cereals and many cultivated grass species (Tulpová *et al*., 2019). They belong to a large family called *Aphididae*, Order: *Hemipterans,* which is composed of approximately 50,000 extant insect species (Burger and Botha, 2017). Signs of feeding by aphids may be leaf chlorosis, leaf rolling, plant stunting, and plant desiccation (Luna *et al*., 2018). Damage by aphids to cereal crops can be severe, especially in wheat. Commonly, yield losses of 8 to 34% can be reached with aphid infestation (Yahya *et al*., 2017). Wheat aphids are likely to cause hundreds of millions of dollars in worldwide crop losses annually (Ali *et al*., 2018). Wheat aphids are known as vectors of plant viruses such as the barley yellow dwarf virus (BYDV) from crop to crop. The honeydew produced by the aphids inhibits plant respiration and photosynthesis by covering the surface of leaves, which results in declining yield and poor-quality wheat (Zhang *et al*., 2016). Blackman and Eastop (2000) have extensively described some of the wheat aphids with high economic significance. Problematic aphids include the English grain aphid, Greenbug, Bird cherry-oat aphid, Rose-grain aphid and RWA (Shavit *et al*., 2018).

2.4.1 Russian wheat aphid (*Diuraphis noxia)*

Russian wheat aphid, *Diuraphis noxia* Kurdjumov (*Hemiptera: Aphididae*) is a significant pest of cereal crops globally, which infests over 140 species of graminaceous plants (Kirkland *et al*. 2018). Despite its ubiquity, infestation rates are low with irregular occurrences and constricted to areas in the central interior and the Western Cape regions of SA (Kisten *et al*., 2020). These relatively small, light, greenish, phloem-feeding aphids can be found living in different habitats and conditions, making them extremely successful and invasive (Botha, 2021). There is no evidence that RWA is carrying viruses or pathogens, but it can inject its salivary proteins into a plant while feeding on phloem, causing a serious systemic phytotoxic effect (Avila *et al*., 2019). Russian wheat aphid originates from central Asia (Jankielsohn, 2017), but its first record away from its native origins was in South Africa in the late 1970s (Jankielsohn, 2016). They can change and adapt in response to changing environments resulting in biotypes with varying virulence patterns (Jankielsohn, 2018). Ever since the first report of RWA in SA in 1978's, RWA has experienced a number biotypification events as there are now five different biotypes described in SA (Burger and Botha, 2017; Jankielsohn, 2018). Biotypification is when an aphid population develops resistance to previously established resistance within wheat (Burger and Botha, 2017). The difference between these biotypes is their *D. noxia* resistance source (Dn gene) (Botha, 2021), and they can be easily distinguished by the susceptibility of wheat carrying different RWA resistance genes (Jankielsohn, 2021). Different from the US biotype numbers that only follow the order of collection, not virulence, South African biotype numbers follow both the order of virulence and collection: SA1 (1978) < SA2 (2006) < SA3 (2009) < SA4 (2011) < SA5 (2018) (Jankielsohn, 2018; Botha, 2021).

2.4.2 Life cycle of Russian wheat aphid

Russian wheat aphids are holocyclic (i.e. they are able to reproduce by both sexual and asexual reproduction) in some countries (Kirkland *et al*., 2018). In other parts of the world, RWA have a sexual cycle where oviparous females lay overwintering eggs (i.e. holocyclic eggs) during autumn (Avila *et al*., 2019). In SA, RWA populations are mostly anholocyclic (i.e. with no males present) (Avila *et al*., 2019). In an anholocyclic lifecycle, a single female aphid can produce close to 70 nymphs/daughters in their lifetime (Kirkland et al., 2018), and the population overwinters as viviparous parthenogenetic females (Avila et al., 2019). Russian wheat aphid populations produce viviparous females with wings when the environmental conditions are unfavourable or when nutrients are depleted (Chemeda *et al*., 2015). Botha (2021) illustrated a typical RWA life/sexual cycle/ (Fig. 2) during all four seasons.

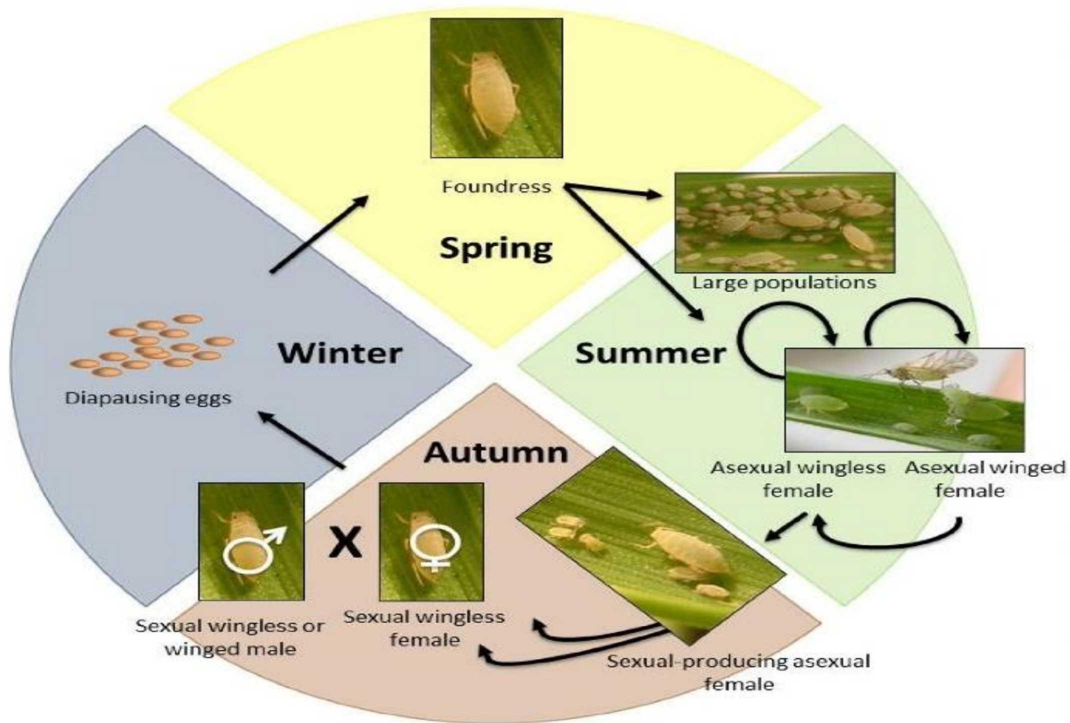


Figure 2. Life cycle of Russian wheat aphid (Botha, 2021)

2.5 Chemical pesticides

Chemical pesticides are chemical substances that are mainly used to control plant pests and diseases (Guler *et al*., 2010). Some examples of chemical pesticides are fungicides, rodenticides, insecticides, herbicides, and plant growth regulators (Nicolopoulou-Stamati *et al*., 2016). The application of chemical pesticides in the agricultural sector is important to combat different types of pests that could reduce crop yields and increase the quality of the food produced (Guler *et al*., 2010). Despite their broad application and their significant role in constantly boosting agricultural production (Kolberg *et al*., 2011), the majority of chemical pesticides have been identified to have environmental and health issues (Nicolopoulou-Stamati *et al*., 2016). A significant amount of laboratory and epidemiological evidence suggests that some chemical pesticides are proven to cause carcinogenesis, immunotoxicity, behavioural disturbance, neurotoxicity, infertility, developmental disabilities, endocrine disruption, skin conditions and respiratory diseases, such as asthma (Guler *et al*., 2010). Chemical pesticides can be classified into different classes, the most common ones include organochlorines, carbamates, organophosphates, neonicotinoids, pyrethroids and triazines (Nicolopoulou-Stamati *et al*., 2016).

2.5.1 Chemical pesticides used for FHB management

Fusarium head blight occurs in most wheat cultivars. In order to control FHB, chemical pesticides have been applied for years (Kheiri *et al*., 2016). Different active ingredients, such as triazoles and imidazoles, are reported to have good efficacy against FHB, causing *Fusarium* spp. (Tini *et al*., 2020). Moreover, the DeMethylation Inhibitors (DMI) (sterol biosynthesis inhibitors) or triazoles (tebuconazole, metconazole and prothioconazole) chemical pesticides are the most commonly used active ingredients against *Fusarium* spp. (Amarasinghe *et al*., 2013). In the triazole family, tebuconazole and metconazole are commonly used active ingredients for FHB control, whereas prothioconazole is used to control numerous fungal diseases, including FHB (Tini *et al*., 2020). Wheat farmers have been applying benzimidazole chemical pesticides, mainly carbendazim, to control FHB for the past decades (Li *et al*., 2019) due to their low price when compared to other classes of chemical pesticides (Feksa *et al*., 2019). Although chemical pesticides application forms a critical part in integrated FHB management (Xiao *et al*., 2020), foliar application of fungicides such as tilt at anthesis [the optimum growth stage for chemical pesticides application for FHB control (Paul *et al*., 2018)] might provide some protection. Still, they destroy natural antagonists of plant pathogens and induce pathogen populations resistant to chemicals pesticides (Kheiri *et al*., 2016). Furthermore, resistance to carbendazim can result in the enhancement of DON biosynthesis in the infected wheat (Li *et al*., 2019). On the other hand, QoI (Quinone outside inhibitor) class of chemical pesticides, e.g. the strobilurins, can cause an increase in DON accumulation, even though they partially reduce FHB outbreaks (Feksa *et al*., 2019).

2.5.2 Chemical pesticides used for wheat aphid's management

Crop protection from aphids is based mostly on the application of insecticides derived from active ingredients of the following classes: carbamates, neonicotinoids, pyrethroids and insect growth regulators (Ikbal and Pavela, 2019). In SA, registration of active substances is limited. Registered insecticides in SA include thiamethoxam, chlorpyrifos, acetamiprid, chlorpyrifos + cypermethrin, imidacloprid, demeton-S-methyl, parathion, dimethoate and prothiofos (Jankielsohn, 2021). In the Western Cape province of SA, chlorpyrifos, dimethoate, imidacloprid and thiametoxam are the most commonly used active substances (Jankielsohn, 2021). Additionally, chlorpyrifos is widely used in the Western United States of America (Jankielsohn, 2021). Apart from the above-mentioned insecticides, results obtained from Hassan *et al*. (2018) suggest that imidacloprid and thiamethoxam prepared with tebuconazole for seed treatment can potentially limit the risk of a delayed spray by preventing infestation

outbreaks and increasing crop yield. However, the application of some chemical insecticides or their residues may have a pesticidal effect on non-target organisms, including humans (Zhang *et al.*, 2018).

2.6 Integrated pest management (IPM)

The concept of IPM came from entomologists faced with extensive use of broad-spectrum insecticides and insect outbreaks that were induced by the elimination of natural antagonists and the development of resistance to insecticides (Barzman *et al.*, 2015). The concept and definition of IPM differ in implementation depending on a number of factors that include the level of education, social and economic status, environmental awareness, rational thinking, moral values/ethics, regulatory aspects, government acts and policies, availability of IPM tools, extension education, marketability and consumer preference (Dara, 2019). "The late RJ Prokopy (2003) defined integrated pest management (IPM) as a decision-based process involving the coordinated use of different tactics to optimize the control of all classes of pests (insects, pathogens, weeds, vertebrates) in an ecologically and economically friendly manner" (Ehler, 2006). Therefore, IPM strategy is channelled to keep strengths within the ecosystems and direct the pest populations to manageable or toleratable levels rather than eliminate them (Ehi-Eromosele *et al.*, 2013). Integrated pest management is a strategy to minimising the reliance on chemical pesticides (Muriithi *et al.*, 2016). However, IPM is not a concept that strictly and uniformly suits all situations but a philosophy that can be applicably applied by the practitioner to help them make a decision for their situation (Barzman *et al.*, 2015). Dara (2019) listed and discussed some common control strategies that can be employed at different stages of crop production to prevent, reduce, or treat pest infestations as part of an IPM program. These common strategies include host plant resistance, chemical control, cultural control and Biological control (Dara, 2019)

2.7 Biological control

Biological control is the use of biological agents (organisms that suppresses the pest or pathogen) or their products on a host animal or plant to prevent or control the development of disease or stress caused by a pathogen or pest (O'Brien, 2017). The agents or their products that negatively affect crop pests and pathogens include macroorganisms, microorganisms, chemical mediators, and natural substances (substances originating from animals, microorganisms or plants) (Lecomte *et al.*, 2016). O'Brien (2017) mentioned that the level of disease suppression achieved by the application of biocontrol agents to a plant can be the same as that achieved by the application of chemical pesticides. Biological control can be categorized

into four different categories: natural, conservation, classical, and augmentative biological control (Van Lenteren *et al*., 2017).

2.7.1 Biological control of FHB

The employment of biological control agents such as bacteria and fungi can provide protection against *F. graminearum* and associated toxin secretion (Dweba *et al*., 2017). Endophytic fungi have been evaluated for potential use as biological control agents of FHB. For example, *Sarocladium zeae* an endophyte of corn can secrete secondary antifungal metabolites with an inhibitory effect on *F. graminearum* (Kemp *et al*., 2020). Besides *Sarocladium zeae,* other fungal enemies include *Trichoderma* spp. (Matarese *et al*., 2012), *Aureobasidium pullulans* (Wachowska and Glowacka, 2014), *Clonostachys rosea* (Xue *et al*., 2014), and *Cryptococcus spp.* (Schisler *et al*., 2011). Bacterial species that have been reported to have antagonistic activity against *Fusarium* spp. causing FHB include *Pseudomonas* spp., *Streptomyces* spp., *Lysobacter enzymogenes* and *Bacillus* spp. (Wegulo *et al*., 2015). To date, none of the reported biological control agents has achieved complete control of FHB, and the results are collected from a small number of field experiments (Dweba *et al*., 2017). These biological control agents are currently not available commercially (Powell and Vujanovic, 2021).

2.7.2 Biological control of wheat aphids and RWA

The biological control of agricultural pests depends mostly on the introduction of natural enemies and parasites/parasitoids, or on the release of pest pathogens and competitors (Van Rijn and Sabelis, 2005). In agriculture, natural enemies/predators play a significant part in controlling arthropod pest density and preventing them from reaching economic thresholds (Ali *et al*., 2018). Ladybugs are natural enemies of aphids, and their introduction can partially control the population density of aphids. However, when they are used in integration with cultural, genetic or chemical methods, they are proven to have improved activity (Du Toit, 1987). Two wasp species, *Aphidius colemani* and *Diaeretiella rapa* have also been reported to specialise in parasiting numerous aphid spp. including RWA (Starý, 1999). In 1989, Aalbersberg *et al*. (1989) reported that the use of natural predators/parasites to control the RWA is not effective in susceptible cultivars.

2.7.3. Entomopathogenic nematodes as biocontrol agents

Entomopathogenic nematodes (EPNs) are obligate parasites of insects (Nowrin *et al*., 2018). Entomopathogenic nematodes from *Steinernematidae* and *Heterorhabditidae* gained more attention due to their many attributes as effective biological control agents for insects (Lacey,

and Georgis, 2012). Entomopathogenic nematodes from the *Steinernema* and *Heterorhabditis* have been commercialised in several continents (Lu *et al*. 2016) and used to control a wide range of economically important insect pests (Mahar *et al*. 2005), aided by their bacterial symbionts (Shapiro-Ilan *et al*., 2017). They have a free-living, third-stage infective juvenile (IJ) that can search for their host insect (Gumus *et al*. 2015), using carbon dioxide secretions, vibrations and other chemical cues (Dlamini *et al*., 2020). The IJs of EPN can be cultured easily using *in vitro* and *in vivo* protocols (Nowrin *et al*., 2018) for mass production. Commonly IJs are sprayed in aqueous suspensions for field applications (Gumus *et al*. 2015).

In contrast to their obvious potential, commercial successes of EPN application are rare (Kim *et al*., 2014), and a number of biotic and abiotic factors affect EPN pest control efficacy (Lacey *et al*., 2015). An example of biotic factor is that applied EPNs might result in undesirable side effects on non-targeted beneficial arthropods or the existence of arthropod predators can deplete the population of EPN by directly eating free-living EPNs, nematode-killed insects or by damaging the integrity of nematode-killed insects resulting in unfavourable environment for nematode development (Belien, 2018). Furthermore, abiotic environmental stresses can negatively affect EPN survival and persistence. In particular, EPN survival and biocontrol efficacy can be negatively impacted by exposure to temperature extremes, UV radiation and desiccation (Gulzar *et al*., 2020).

2.8 The symbiotic bacteria of EPN, *Xenorhabdus* and *Photorhabdus*

Xenorhabdus and *Photorhabdus* from *Morganellaceae* family (www.ncbi.nlm.nih.gov/), are etomopathogenic bacteria which cause septicaemia (blood poisoning by bacteria) and toxemia (toxic bacterial toxins) in their insect hosts (Mollah and Kim, 2014). In nature, *Photorhabdus* and *Xenorhabdus* are found in symbiosis with *Heterorhabbditis* and *Steinernema* respectively (Heinrich *et al*., 2017). Entomopathogenic nematodes of the genus *Steinernema* harbour their symbiotic bacteria (*Xenorhabdus*) in a receptacle vesicle located in the anterior part of the gut which is specialised for the bacteria, while *Heterorhabditis* use their intestinal lumen to carry their symbiotic bacteria (*Photorhabdus*) (Sajnaga and Kazimierczak, 2020). There are currently only 29 *Xenorhabdus* species and 20 *Photorhabdus* species (Yimthin *et al*., 2021). *Xenorhabdus* and *Photorhabdus* bacteria are classified as Gram-negative, facultatively anaerobic, non-spore forming rods (Sajnaga and Kazimierczak, 2020). Despite their different evolutionary origins, *Xenorhabdus* and *Photorhabdus* species have a similar life cycle (Da Silva *et al*., 2020). These two bacterial genus can easily be differentiated by catalase test and

bioluminescence which are only reserved traits for *Photorhabdus* (Sajnaga and Kazimierczak, 2020).

2.8.1 Phase variation

Xenorhabdus spp. and *Photorhabdus* spp. exhibit a phase variation that affects their relationship with their nematode hosts, *Steinernema* and *Heterorhabditis* spp. respectively (Smigielski *et al*., 1994). The two phases of *Xenorhabdus* and *Photorhabdus* spp. with distinct phenotypes have been characterized as phase I (primary phase) and phase II (secondary phase) (Han and Ehlers, 2001). However, it is still unclear what stimulates them and what is the role of the switch between two cell types in the life cycle of these bacteria (Sajnaga and Kazimierczak, 2020). However, there are reports that phase variation has some consequences on the success of nematode production (Han and Ehlers, 2001). Phase II variants occur suddenly during *in vitro* culture or during the production of nematodes on artificial diets (Boemare *et al*., 1996). Naturally, the phase I variant is the one been carried by the IJs and supports reproduction of the nematode within its host insect better than phase II (Smigielski *et al*., 1994). To distinguish between phase I and phase II variants of these bacteria, the nutrient bromothymol blue triphenyltetrazolium chloride agar (NBTA) test based on absorption of bromothymol blue can be conducted (Aiswarya *et al*., 2017). Only phase I can absorb the dye. Additionally, phase I variants have protoplasmic paracrystalline inclusions, secret lecithinase and can have fimbriae (Moureaux *et al*., 1995).

2.9 The life cycle Entomopathogenic nematodes and their symbiotic bacteria

Entomopathogenic nematodes and their symbiotic bacteria have a species-specific symbiotic relationship, in which *Steinernema* species are specifically associated with bacteria from genus *Xenorhabdus* and *Heterorhabditis* species are similarly associated with bacteria from the genus, *Photorhabdus* (Park *et al*., 2017). The IJ stage of EPN with their symbiotic bacteria in their midguts can be found in the soil of distinct ecological systems (Yooyangket *et al*., 2018). Bacterial symbionts secrete secondary metabolites that assist in creating and maintaining favourable conditions for nematode reproduction by overcoming the host insect's immune defence, killing the insect, and inhibiting the growth of various fungal and other bacterial competitors (Kary *et al*., 2017). The nematodes feed upon the bacteria and dead insect carcass and can stay one to three generations in the host cadaver (Muangpat *et al*., 2017). After depletion of food, the nematodes retain bacterial symbiont cells in its guts before leaving the depleted cadaver as IJs in search for another insect host (Kepenekci *et al*., 2015). A typical example of this mutualistic association (Fig. 2) adapted from Stock (2019) can be found below.

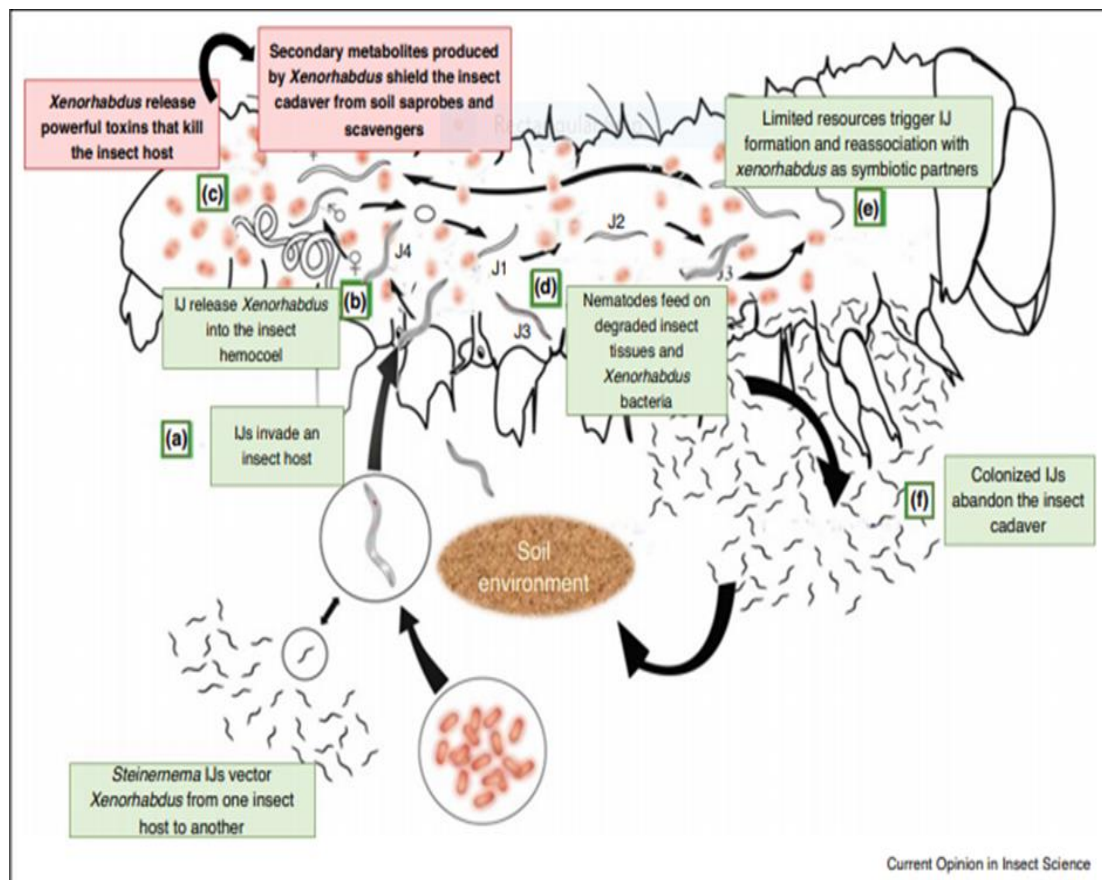


Figure 3. A typical example of a mutualistic association between entomopathogenic nematodes and their bacteria symbionts (Stock, 2019).

2.10 Bacterial secondary metabolites

Secondary metabolites are small molecules that are not important for life, although these molecules are likely to confer an evolutionary benefits to the producer organism (O'Connor, 2015). Bacterial secondary metabolites have very diverse chemical structures which display a vast range of biological activities, hence acquiring actual or potential industrial use in different industrial fields (Bibián *et al*., 2020). Secondary metabolites are commercially important as pesticides, drugs, fragrances and flavour, pigments or dyes, and food additives (Ingle and Narkhede, 2019).

Photorhabdus and *Xenorhabdus* are great secretors of structurally different secondary metabolites that are important for their symbiotic lifestyle with nematodes, killing of insect host and protection of the host cadaver against sacavangers (Engel *et al*., 2017). They secrete many types of proteins, including lipases, phospholipases, proteases, and peptides which are known to synthesis novel secondary metabolites with diverse biological activities (Eroglu *et al*., 2019). Notably, *Photorhabdus* and *Xenorhabdus* can produce a number secondary

metabolites including antimicrobial and insecticidal compounds e.g. phenethylamines, benzylideneacetone, xenocoumacins, indole and 3,5-dihydroxy-4-isopropylstilbene (Muangpat *et al.*, 2017). Hazir *et al.* (2016) reported that secondary metabolites have no phytotoxic activity when applied to different plant species in the glasshouse.

2.10.1 Insecticidal compounds produced by *Xenorhabdus* and *Photorhabdus* spp.

Some secondary metabolites secreted by *Xenorhabdus* and *Photorhabdus* play an important role during their association with their symbiotic nematode hosts, while some are essential for pathogenesis specifically targeting insect's immune defences (Mollah and Kim, 2020). An isocyanide-containing molecule, rhabducin [a functionalised tyrosine product of amidoglycosyl- and vinyl-isonitrile (Crawford *et al.*, 2012)] produced by both *Xenorhabdus* and *Photorhabdus* species, can inhibit the activity of phenoloxidase (main component of the insect's natural immune system) in *Galleria mellonella* (Linnaeus)(*Lepidoptera: Pyralidae*) larvae (Mollah and Kim, 2020; Crawford *et al.*, 2012). Stilbenes from *Photorhabdus* are also involved in inhibiting phenoloxidase in *Manduca sexta* larvae (Eleftherianos *et al.*, 2007). Another phenoloxidase inhibitor, benzaldehyde, was reported by Ullah *et al.* (2015) using *G. mellonella* larvae as a model system. Oxindole and benzylideneacetone produced by *Xenorhabdus nematophila* and *Photorhabdus temperata* can act as phospholipase A2 (catalyse the production of mediators of the immune response and eicosanoid biosynthesis in insects) inhibitors in insects (Da Silva *et al.*, 2020). Furthermore, previous studies have illustrated that proteins and secondary metabolites such as iodine, benzylideneacetone, phenethylamides, xenooxides, xenorhabdins, indol derivatives and xenocoumacins that are produced by a *Xenorhabdus nematophila* are effective in the management of culicids (Da Silva *et al.*, 2020).

2.10.2 Antifungal compounds produced by *Xenorhabdus* and *Photorhabdus* spp.

Li *et al.* (1995) were the first study to report the antifungal activity of isolated and identified compounds produced from *Xenorhabdus* and *Photorhabdus* spp. They observed strong antifungal activity of these compounds against several medical and agricultural important fungal species. From an *in vitro* and *in vivo* study conducted by Zhang *et al.* (2019), Nematophin a compound produced by *X. nematophila* exhibited antifungal properties against *Rhizoctonia solani*. Two stilbene derivatives produced by a *Photorhabdus temperate* showed strong antifungal activity against a plant pathogenic fungi *Pythium aphanidermatum* (Shi *et al.*, 2012). Also produced by *Photorhabdus* sp. *P. temperate*, an aromatic aldehyde, benzaldehyde inhibited the growth of three economically important fungal phytopathogens, *R. solani, Phytophthora capsici* and *Corynespora cassiicola* in *in vitro* experiments (Ullah *et al.*,

2015). Xenociumacin produce by *X. nematophilus* var. *pekingensis* exhibited strong antifungal activity against several *Phytophthora* species and 95.7% inhibitive activity against *Rhizoctonia solani* (Yang *et al*., 2011). Additionally, *Fusarium* sp. *F. oxysporum* was significantly inhibited by Cabanillasin, a compound produced by *Xenorhabdus cabanillasii* (Houard *et al*., 2013).

2.11 Chromatography

Chromatography is a biophysical technique used to separate, quantify, identify and purify individual components of a mixture for qualitative and quantitative analysis (Coskun, 2016). The technique's principle is based on separating mixture components according to their differences between two phases (Yashin and Yashin, 2012). One of these phases is the stationary phase, which is made up of a sorbent; the other is the mobile phase which is normally made up of liquid or gaseous component (Coskun, 2016). The technique was developed in 1903 by Mikhail Tswett, a botanist from Russia; He used a calcium carbonate column to separate plant pigments (Parasuraman, *et al*., 2014). The word chromatography was formed from Greek words chroma (colour) and graphein (writing) which means 'colour writing' (Parasuraman, *et al*., 2014). Since the first development, other types of chromatography methods have been developed including paper chromatography, column liquid chromatography, gas chromatography, thin-layer chromatography, gel permeation chromatography, high-pressure liquid chromatography, ion exchange chromatography and affinity chromatography (Coskun, 2016).

2.11.1 High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is a leading analytical technique employed for the separation, identification and quantification of each constituent of mixture (Thammana, 2016). High performance liquid chromatography is an updated form of column liquid chromatography and, is currently one of the most needed analytical chemistry tools (Bhardwaj *et al*., 2015). Instead of allowing a solvent to move through a column under gravity, high pressures of up to 400 atmospheres is being apply to accelerate the solvent movement (Meher *et al*., 2018). The tool only to separate, quantify and identif the molecules that are found in any sample that can be dissolved into a liquid. High performance liquid chromatography separation of each chemical component from the sample mixture is based on its unique affinity towards the adsorbent material in the column and the mobile phase, causing different components of the sample mixture to leave the column at different time and separate (Sahu *et al*, 2018).

2.11.2 Mass spectrometry

Mass spectrometry (MS) is an analytical tool that measures the mass-to-charge ratio of ions generated by the sample, allowing quantitative determination and qualitative identification of the sample. A physicist Joseph J. Thomson (1856 – 1940) and chemist Francis W. Aston (1877 - 1945) (Noda *et al.*, 2016; Chong *et al.*, 2018) put the first mass spectrometry device into functional form in 1912. The discovery of electrons [the ground-breaking work on cathode rays (Thomson, 1897)] was not the only discovery by Joseph J. Thomson. Him together with F. W. Aston used mass spectrometry to demonstrate the presence and to measure the mass isotopes of elements (Thomson, 1911; Aston, 1920; Lössl *et al.*, 2016). In the early 20th century, mass spectrometry was mainly used to measure masses of existing isotopes and masses of atoms (Griffiths, 2008). The technique analyse the molecular components of a biological analyte by ionizing the sample components and then measuring the resulting ions mass-to-charge ratios (Martens *et al.*, 2011). These charged ions are formed in the ion source. In other cases, the ion source also helps in transferring liquid-phase or solid-phase sample into the gas phase. The gas-phase ions are then received by the mass analyser. After receiving the gas-phase ions, the mass analyser set the charges—in space or time—in accordance to their mass-to-charge ratios (m/z) (Ubarn, 2016).

2.11.3 Liquid Chromatography- Mass spectrometry (LC-MS)

Mass spectrometry (MS) without preparation have been used as an analytical tool for metabolomics, but MS alone has been proven to have some limitations as a comprehensive tool for the characterization of metabolites end groups and copolymer composition sequences (Crotty *et al.*, 2016). Some years later after the MS analysis of proteins/metabolites was discovered, there were attempts to couple/combine it with a more advanced separation tool, such as electrophoresis and liquid chromatography (LC) for sample preparation (Lössl *et al.*, 2016). The coupling of chromatographic separation with mass spectrometric detection has emerged as the number one technology for multiparallel analysis of low molecular weight compounds in metabolomics (Tautenhahn *et al.*, 2008). Liquid chromatography coupled with mass spectrometry provides more selectivity and identity confirmation compared to traditional chromatography that were coupled to one-dimensional detector e.g. UV absorbance (Kruve *et al.*, 2015). The success of the LC–MS technique comes from its capability to give three-dimensional data. First, it separates the compounds in relation to time by LC. Secondly, ions produced in the ionization source are then sorted according to their mass to charge (m/z) ratios in the mass analyzer of MS. Finally, the MS detector measures the concentration of each ion

(Kruve *et al*., 2015). The coupling of LC to MS is a functional analytical technique, and many different types of LC-MS techniques were developed over the past 20 years making LC-MS coupling techniques important in metabolite characterization and dedicating them for sophisticated metabolite analysis (Crotty *et al*., 2016).

2.12 Gap in literature

The current literature is focused on studies from other parts of the world. Secondary metabolites from EPN symbionts have been previously evaluated for their many bioactive activities against pests and pathogens. However, none of these studies evaluated their potential for management of FHB of wheat and RWA biotypes. Moreover, there is no study available for evaluation of indigenous EPN symbionts for the management of these biotic stressors. This study is the first to extract and identify bacterial metabolites from the ARC EPN collection, and to evaluate their bioactive activities against FHB causing *Fusarium* sp. *Fusarium graminearum* and RWA biotypes.

Chapter 3

Materials and Methods

The study was conducted at the Insect Pathology Laboratory of Agricultural Research Council-Small Grain Institute (ARC-SGI), Bethlehem, Free State province, South Africa.

3.1 Source of wheat cultivar, cultures and insects

3.1.1 Wheat cultivar

Seeds of a RWA susceptible wheat cultivar (Duzi) were obtained from ARC-SG and they were kept in a dry cabinet at room temperature for later use.

3.1.2 *Fusarium* sp. cultures

Fusarium graminearum cultures (isolate D52) were provided by the *Fusarium* laboratory at ARC-SGI and they were successively subcultured onto plates of potato dextrose agar (PDA) (Merck Co., http://www.merck.com/) and mung bean agar (MBA). The plates were then incubated at 25 ±1˚C for four days and seven days respectively. After incubation, *Fusarium* spores were confirmed under a light microscope (Model U-LHLEDC, Olympus Co., www.Olympus-global.com) at 40 x magnification and plates were kept at ±10 °C for later use.

3.1.3 Russian wheat aphid biotypes

Five South African RWA biotypes were obtained from caged clone colonies at ARC-SGI, reared in a glasshouse cubicle at 25 ±1°C with a photoperiod of 14:10h on planted wheat seedlings/plants.

3.1.4 Rearing of *Galleria mellonella*

The great wax moth, *G. mellonella* were reared in 3L Consol glass bottles containing artificial feed (Birah *et al*., 2008) at 25 ±2 °C in an insect rearing room at ARC-SG insect pathology laboratory. The last-instar larvae were harvested when needed for experiments.

3.2 Isolation and culturing of EPN symbiotic bacteria

Nine EPN isolates (Table 1) from the collection at ARC-SGI were used for this study. Isolation of the symbiotic bacteria was achieved using the combination of Kaya and Stock (1997) and Muangpat *et al*. (2017) protocols with some modifications. For each isolate, three last instar *G. mellonella* larvae were infected and incubated for 48h at 25±1°C in the dark. Under the laminar flow hood, symbiotic bacteria were isolated from the hemolymph by dissecting the cadavers of *G. mellonella* larvae with a sterile scalpel blade, and the hemolymph inoculum were streaked

onto selective medium. MaConkey agar (Merck Co., http://www.merck.com) medium was used for *Heterorhabditis* isolates and NBTA (nutrient agar, 0.0025% bromothymol blue, and 0.004% triphenyltetrazolium chloride medium) medium was used for *Steinernema* isolates. Inoculated plates were incubated at 25±1 °C for 72 h in the dark. Morphological identification of bacterial colonies was achieved by visually observing characteristics of the colonies and by making a slide and observing cell morphology as described by Akhurst (1980). To obtain pure bacterial cultures, single colonies of the desired morphology and characteristics were picked and plated successively onto a new MaConkey agar or NBTA plates until a pure culture was obtained. For stock preservation, bacteria were suspended in 15% glycerol and stored at -80 °C until needed for experiments (Boemare and Akhurst, 2006, Eroglu *et al*., 2019).

Table 1. Selected indigenous EPN isolates for isolation of their symbiotic bacteria.

Isolate	Nematode	Symbiotic bacteria
SGI 35	*Steinernema innovationi*	*Xenorhabdus* sp.
SGI 170	*Heterorhabditis bacteriophora*	*Photorhabdus luminescens (Bai et al., 2013; Ciche, 2007*
SGI 197	*Steinernema beitlechemi*	*Xenorhabdus khoisanae (Çimen et al., 2016)*
SGI 208	*Steinernema* sp.	*Xenorhabdus* sp.
SGI 220	*Steinernema* sp.	*Xenorhabdus* sp.
SGI 245	*Heterorhabditis bacteriophora*	*Photorhabdus* sp.
SGI 246	*Steinernema biddulphi*	*Xenorhabdus* sp.
SGI 257	*Steinernema* spp.	*Xenorhabdus* sp.
ROOI 161	*Steinernema khoisanae*	*Xenorhabdus* sp.

3.3 Production of bacterial metabolites

Production of secondary metabolites was performed as described by Eroglu *et al*. (2019) with some modifications. Each bacterial isolate was grown on MaConkey agar/NBTA for 72 h at 25±1°C in the dark. Then for each isolate a loop of bacterial cells from a single colony was harvested and transferred into 100 ml trypric soy broth (TSB) (Merck KGaA,

http://www.merck.com) in a 250 ml Erlenmeyer flask. Three flasks were inoculated for each isolate. The liquid cultures were covered in foil and incubated on a rotary shaking incubator at 180 rpm for seven days at 25±1°C.

3.4 Preparation of metabolite treatments

Seven days incubated bacterial liquid cultures were used as the first treatment and these were referred to as crude metabolites. The second type of treatment was prepared by centrifuging (Hermle Z200A, http://www.merck.com) bacterial liquid cultures at 6,000 rpm for 20 min, followed by filtering the supernatants through 0.22 µm Millipore filter discs (LASEC, http://www.lasec.co.za) and these were referred to as filtered metabolites. The last type of treatment was prepared by autoclaving the supernatants at 121 °C for 20 min and they were referred to as autoclaved metabolites. All three types of bacterial metabolites treatments were transferred into 50ml falcon tubes and kept at ±10°C for later use (overnight for mycelial growth experiment and four days for the spore germination experiment).

3.5 Evaluation of bacterial metabolites on FHB causing *Fusarium* sp. *Fusarium graminearum*.

3.5.1 Efficacy of bacterial metabolites on *F. graminearum* mycelial growth

All three types of treatments were used to test the efficacy of bacterial metabolites of the nine isolates against *F. graminearum* mycelial growth. The three types of metabolite treatments were incorporated into PDA at 20% following the procedure by Hazir *et al*. (2016). Before autoclaving PDA, 20% of the prescribed distilled water was omitted when preparing the media for subsequent addition of metabolite treatment suspensions. After preparation, 40 ml of the media was poured into 100 ml Erlenmeyer flasks and autoclaved. After autoclaving the flasks were allowed to cool to 45-50 °C by placing them in a water bath, then 20% (10ml) of bacterial metabolites treatments were added and mixed thoroughly before pouring into 65 mm Petri dish plates and 10ml sterile distilled water was added for control treatments. Using a sterile cork borer, the centre of the PDA plates were welled, creating a well of 5 mm in diameter. These wells were plugged with 5 mm diameter pieces of *F. graminearum* infected PDA (previously incubated for 4 days at 25±1°C). After filling the wells, the plates were incubated at 25±1°C for 7 days in the dark. Two mean growth diameter (measurements taken perpendicular to each other using a ruler) were measured per plate after 3 and 7 days of incubation. The inhibition rate of mycelial growth was calculated using the formula: inhibition rate = 100 × (colony diameter in control – colony diameter in treatment)/colony diameter in control (Hazir *et al*.,

2016). The experiment had three replicates per treatment and was repeated twice on different dates with a different batch of bacterial metabolites.

3.5.2 Efficacy of bacterial metabolites on *F. graminearum* spore germination

Suppression of spore germination was performed as described by Hazir *et al*. (2016). *Fusarium* cultures were sub-cultured onto MBA plates (90mm) and incubated at 25±1°C for 7 days. After 7 days, spores were harvested from the plates by suspending them in sterile water. Then 0.1 ml of the conidia suspensions were added to 0.7 ml potato dextrose broth (PDB) (Sigma-Aldrich, https://www.sigmaaldrich.com/) in 2 ml Eppendorf tubes containing 0.2 ml (20 % v/v) filtered metabolites treatments of each isolate. For control, PDB (0.9 ml) alone and 0.1 ml conidial suspension without metabolites were used. The tubes were incubated at 25±1°C for 3 days, followed by loading spore suspensions on the hemocytometer slide and counting the first 100 conidium under the compound microscope (Model U-LHLEDC, Olympus Co., http://www.olympus-global.com/) at 40× magnification to determine percentage spore germination. The inhibition rate of spore germination was calculated using the formula: inhibition rate = 100 × (spores germinated in control – spores germinated in treatment)/ spores germinated in control (Hazir *et al*., 2016). Each treatment had three replicates, and the experiment was repeated twice at different dates with a different batch of bacterial metabolites.

3.5.3 Statistical analysis on the efficacy of the bacterial metabolite against *F. graminearum* mycelial growth and spore germination

Analysis of variance (ANOVA) ($\alpha = 0.05$) was used to detect the significance of the treatments on mycelial growth and spore germination. Trial repeats of both experiments were assessed to determine whether there were significant differences between experiment repeats. For the spore germination experiment, the data were combined, as there were no significant differences between trials ($P>0.1215$). The standardized residuals were normally distributed (Shapiro-Wilk test) and therefore the means of spore germination percentages were separated using Fisher's unprotected t-test (least significant difference; LSD) at $\alpha=0.05$ (Snedecor and Cochran, 1980). For mycelial growth, growth inhibition data from repeated trials were pooled. The trials were checked for homogeneity using Levene's Test and Bartlett's Test before pooling the data. The means of the growth inhibition diameters were separated using Fisher's unprotected t-test (least significant difference; LSD) at $\alpha=0.05$ (Snedecor and Cochran, 1980).

3.6 Efficacy of bacterial metabolites on RWA biotypes

3.6.1 Evaluation of bacterial metabolites against five RWA biotypes

Crude metabolites from symbiotic bacteria of five EPN isolates (*H. bacteriophora* SGI 170, *H. bacteriophora* SGI 245, *S. beitlechemi* SGI 197, *Steinernema* sp. SGI 257 and *S. khoisanae* ROOI 161) were prepare as in section 3.5 and used to test their efficacy against five South African RWA biotypes. Wheat leaves infested with the five biotypes were collected from the glasshouse and transported to the laboratory in Petri dishes. Roots of non-infested wheat seedlings (Duzi) at two-leaf stage were covered with small wet pieces of cotton wool wrapped with foil and the leaves were dipped for 5 seconds in metabolite treatments. There were three plants per treatment for each biotype. For control, three plants per biotype were dipped in water for 5 seconds. After allowing the plants to dry, the plants were placed individually in Petri dishes (90 mm) and infested with five adult aphids per petri dish for each biotype (three plates for each treatment). Petri dishes were left on the laboratory bench at 25±2˚C. For mortality data, aphids were assessed at 24h interval by checking each aphid's ability to show movement in response to touch with a small brush. Results were expressed as mean cumulative percentage mortalities at 48h. Schneider- orelli's formula was used to correct the mortality rates. The experiment was repeated twice, seven days apart.

3.6.2 Statistical analysis on the effect of bacterial metabolites against five RWA biotypes

Forty-eight hours trial data was used in applying a combined ANOVA after the three trials were tested for homogeneity of trial variances using Levene's test (Levene, 1960). For each biotype and per biotype separately (factorial analysis). The trial variances were acceptable homogeneous (P>0.001). The standardized residuals were normally distributed (dead aphid residual percentages for combined data; P>0.2) (Shapiro-Wilk test) (Shapiro and Wilk, 1965) and therefore the means of mortality rates were separated using Fisher's unprotected t-test (least significant difference; LSD) at α=0.05 (Snedecor and Cochran, 1980)

3.7 DNA extraction and amplification of two effective isolates

Deoxyribonucleic acid (DNA) of two EPN isolates that displayed a high overall inhibitory effect on *F. graminearum* mycelial growth was extracted following Knot *et al*. (2020) procedure. DNA was extracted from a single IJ by rupturing the nematode on a 15µl drop of sterile distilled water and 10µl of the nematode-smash solution (ruptured nematode and distilled water) was transferred into 1.5ml Eppendorf tube. The cells were lysed at 56°C overnight (Knot *et al*., 2020) using the lysis solution from the GeneJET Genomic DNA Purification Kit (ThermoFisher Scientific Ltd, https://www.thermofisher.com/). Digestion and

Purification were conducted according to GeneJET Genomic DNA Purification Kit manufacturer's instructions.

The internal transcribed spacer regions (ITS) of both isolates were amplified using primers 18S: 5-TTGATTACGTCCCTGCCCTTT-3 (forward), and 28S: 5-TTTCACTCGCCGTTACTAAGG-3 (reverse) (Cimen *et al*., 2016). The Polymerase Chain Reaction master mix was made up of nuclease free water (7.25 µl), 10× PCR buffer (1.25 µl), dNTPs (1 µl), primers (0.75 µl each), polymerase (0.1 µl) and 1 µl of the gDNA. The PCR profiles were used as follows: 1 cycle of 94°C for 7 min followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1 min and a final extension at 72°C for 7 min (Cimen *et al*., 2016). Polymerase Chain Reaction was followed by electrophoresis of 2% TAE buffered agarose gel (45 min, 120 V) of 2 µl of PCR product stained with safe dye (20 µl safe dye per 100 ml of gel).

3.8 Profiling and identification of bacterial metabolites from two best isolates

3.8.1 Sample preparation

The homogenised samples of each of the two isolates of bacteria (SGI 170 and SGI 197) were measured (30ml; 50ml; 100ml) and 70% LC-MS methanol/H2O was added at a ratio 1:1 m/v. The mixtures were vortexed for 30 seconds and sonicated using the BRANDSON 1800 (Branonic, www.Bransonic.com), for 10 min. After sonication, the samples were centrifuged at 10 000 revolutions per minute (rpm) for 5 min. The supernatants were filtered using 0.22 µm nylon filters into 1.5ml glass vials with 500 µL inserts.

3.8.2 Liquid Chromatography-Quadruple Time-of-Flight Tandem Mass Spectrometry (LC-MS/MS) analysis

Samples were analyzed on a Shimadzu LC-MS-9030 Q-TOF Mass Spectrometer (Shimadzu, https://www.ssi.shimadzu.com/) using a 100 mm × 2.1 mm with a particle size of 2.7 µm C18 column (Shimadzu, https://www.ssi.shimadzu.com/) at 55°C for 20 min at a flow rate of 0.4 mL/min following the separation conditions displayed on Figure 1. For each sample, 15 µL was injected and run on a binary mobile phase gradient, which comprises of solvent A: 0.1% formic acid in Milli-Q water and solvent B: Methanol with 0.1% formic acid. The chromatographic effluents were further analysed utilizing the qTOF high-definition mass spectrometer set to acquire negative electrospray ionisation data.

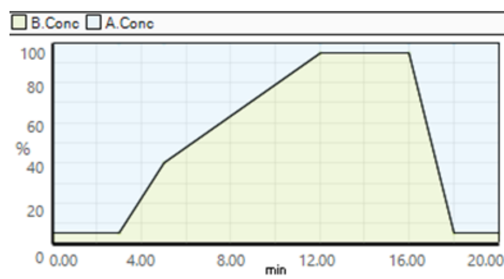


Figure 4. The LC-MS mobile phase separation conditions throughout 20 min cut-off time. The mobile phase composition was 95% solvent A and 5% solvent B at 0 min; and ramped to 95% solvent B and 5% solvent A at 12-16 min.

3.8.3 Data processing and molecular networking for identification of metabolites

Data from LC-MS/MS analysis was first converted into an mzML file before uploading it into computational tools. Lab solutions and Lab Solutions Insight were the first tools to be used to calculate the peaks masses and molecular formula, respectively. For better molecular formula results, SIRIUS software (https://bio.informatik.uni-jena.de) was used to confirm the results obtained from Lab Solutions Insight. Using the molecular formula, compounds names were obtained from the following databases:

- KNAPSACK: http://www.knapsackfamily.com/knapsack_core/top.php
- Pubchem: https://pubchem.ncbi.nlm.nih.gov/
- Chemspider: https://www.chemspider.com/
- HMDB: https://hmdb.ca/

Chapter 4

Results

4.1 Evaluation of bacterial metabolites against *Fusarium* head blight (FHB) causing *Fusarium* sp. *F. graminearum*.

Bacterial metabolites from bacterial symbionts of nine EPN isolates resulted in varying antifungal activity against FHB causing *Fusarium* sp. *F. graminearum* (isolate D52).

4.1.1 Efficacy of bacterial metabolites on *F. graminearum* mycelial growth

The ANOVA presented varying significant inhibitions of mycelial growth from the three different metabolites treatments (crude, filtered and autoclaved metabolites) of each isolate. Crude metabolite treatments exhibited the highest growth inhibition activity when compared to the other two metabolite treatments (filtered metabolites and autoclaved metabolites) while autoclaved metabolites exhibited the lowest growth inhibition activity when compared to the other two metabolite treatments (crude metabolites and filtered metabolites) in all isolates ($F=2855.75$; $df = 3$; $P<0.0001$) (Figure 5). Crude metabolites mean inhibition rates of all isolates ranged from 64.12 to 86.76% while filtered metabolites mean inhibition rates ranged from 17.65 to 60.88% and autoclaved metabolites mean inhibition rates ranged from 0 to 21.72% after three days of incubation. After seven days of incubation, mean inhibition rates ranged from 42.88 to 96.25% for crude metabolites, 0 to 55.9% for filtered metabolites and 0 to 5.4% for autoclaved metabolites (Table 2).

Table 2. Mycelial growth inhibition rates (%) of all isolates in both incubation periods.

	3 days incubation			7 days incubation		
Isolates	**Crude**	**Filtered**	**Autoclaved**	**Crude**	**Filtered**	**Autoclaved**
Xenorhabdus sp. SGI 35	77.65	58.82	14.30	47.33	29.59	4.52
Photorhabdus sp. SGI 170	73.82	60.88	7.35	95.79	55.90	1.69
Xenorhabdus sp. SGI 197	83.53	42.06	21.72	96.25	15.49	5.37
Xenorhabdus sp. SGI 208	76.18	38.53	18.27	61.66	2.06	1.69
Xenorhabdus sp. GI 220	68.82	39.12	11.76	42.88	2.43	4.52
Photorhabdus sp. SGI 245	86.76	17.65	0.00	88.39	28.37	1.69
Xenorhabdus sp. SGI 246	64.12	20.29	0.00	54.30	0.00	0.00
Xenorhabdus sp. SGI 257	74.12	30.00	15.88	85.77	14.42	0.85
Xenorhabdus sp. ROOI 161	81.76	31.18	9.71	81.46	1.87	0.00

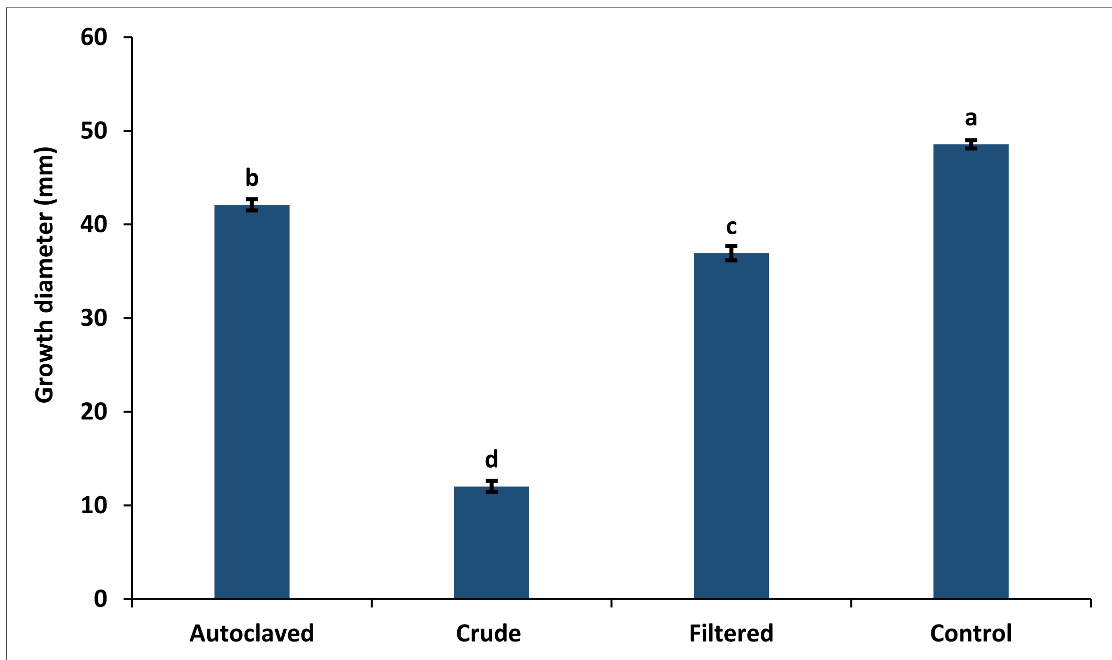


Figure 5. Overall (all isolates; three and seven days of incubation) mean inhibition activity of different bacterial metabolite treatments compared to control (P<0.0001).

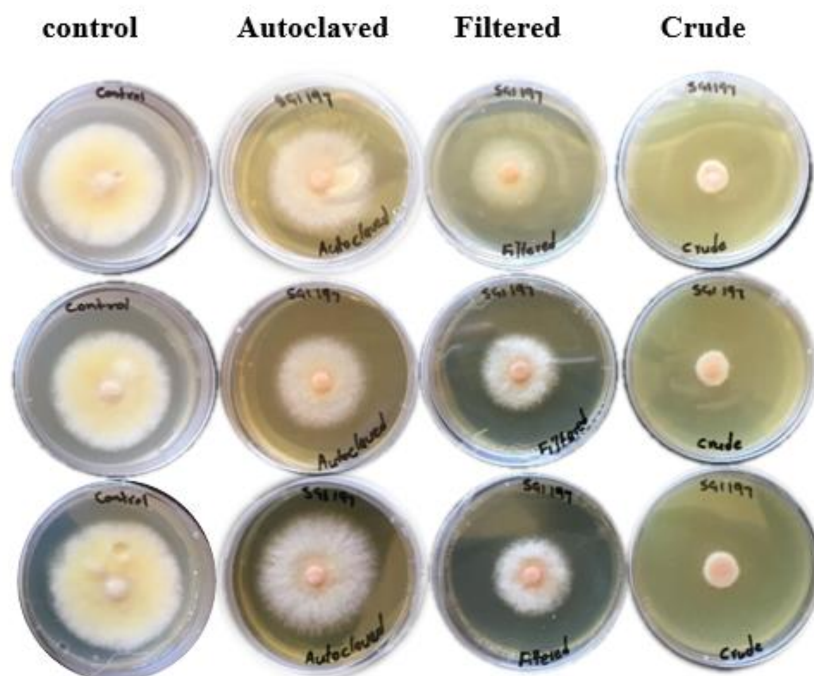


Figure 6. *Fusarium graminearum* mycelia growth treated with different bacterial metabolite treatments of isolate SGI 197 compared to control after three days of incubation.

Autoclaved metabolites displayed varying significant inhibition after three days (F = 4.74; df = 9; $P < 0.0002$) (Figure 7) and seven days (F = 3.50; df = 9; $P = 0.0063$) (Figure 8) of incubation. From all the isolates, SGI 197 had the lowest growth diameter when compared with control and other isolates in both incubation periods.

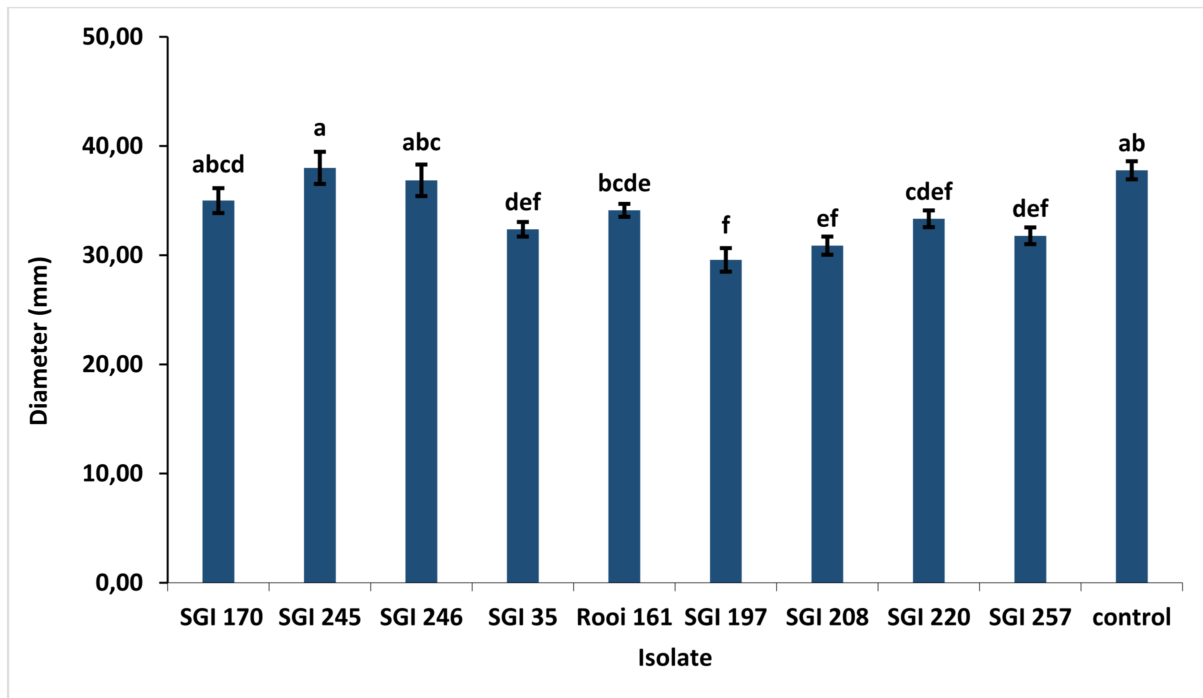


Figure 7. Mean mycelial growth inhibition activity of autoclaved metabolites compared to control after three days of incubation ($P < 0.0002$).

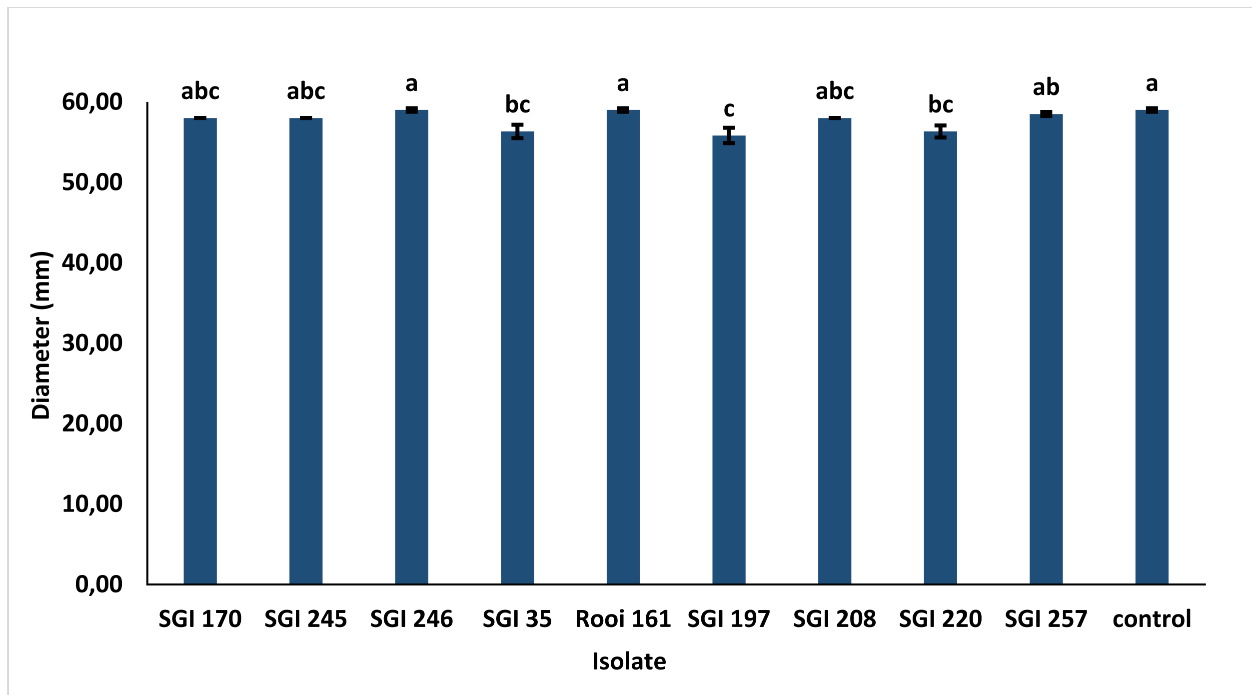


Figure 8. Mean mycelial growth inhibition activity of autoclaved metabolites compared to control after seven days of incubation (P = 0.0063).

Although crude metabolite treatments were the best, mycelial growth inhibition activity of the different isolates also varied after three days of incubation (F = 177.19; df = 9; P < 0.0001) (Figure 9) and seven days of incubation (F =123.15; df = 9; P < 0.0001) (Figure 10). After three days of incubation, isolate SGI 245 had the lowest growth diameter followed by SGI 197. After seven days of incubation, SGI 197 followed by SGI 170 had the lowest mycelial growth diameters which were not significant from each other.

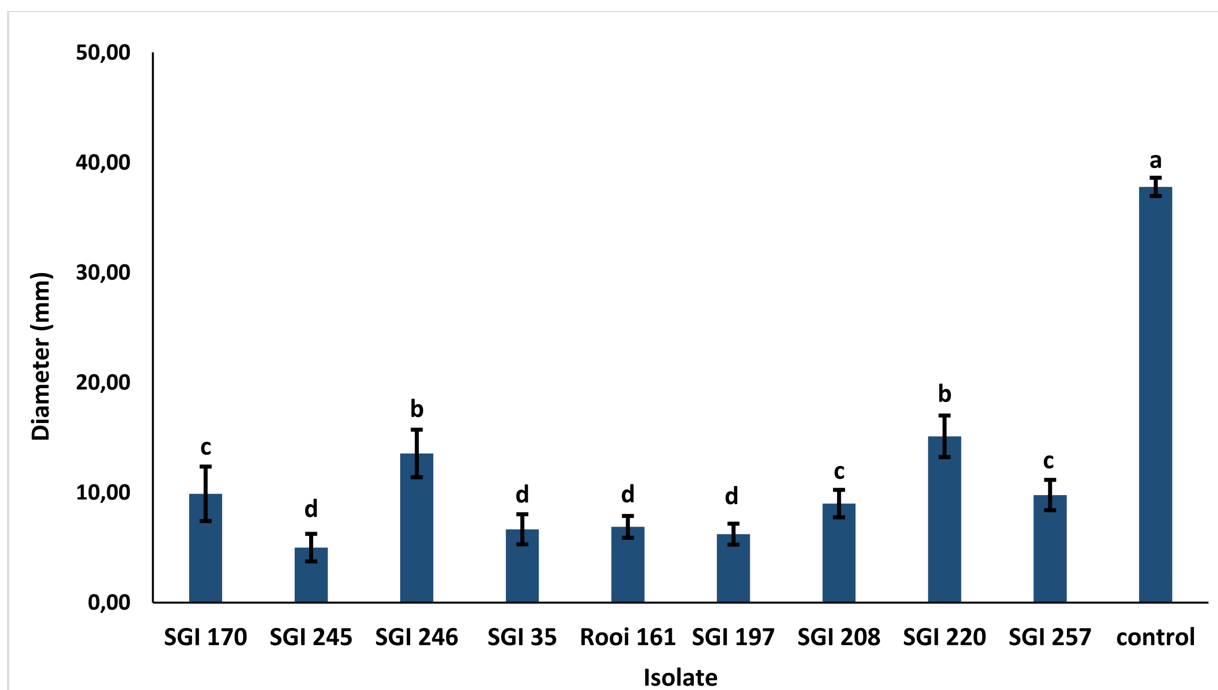


Figure 9. Mean mycelial growth inhibition activity of crude metabolites compared to control after three days of incubation ($P < 0.0001$).

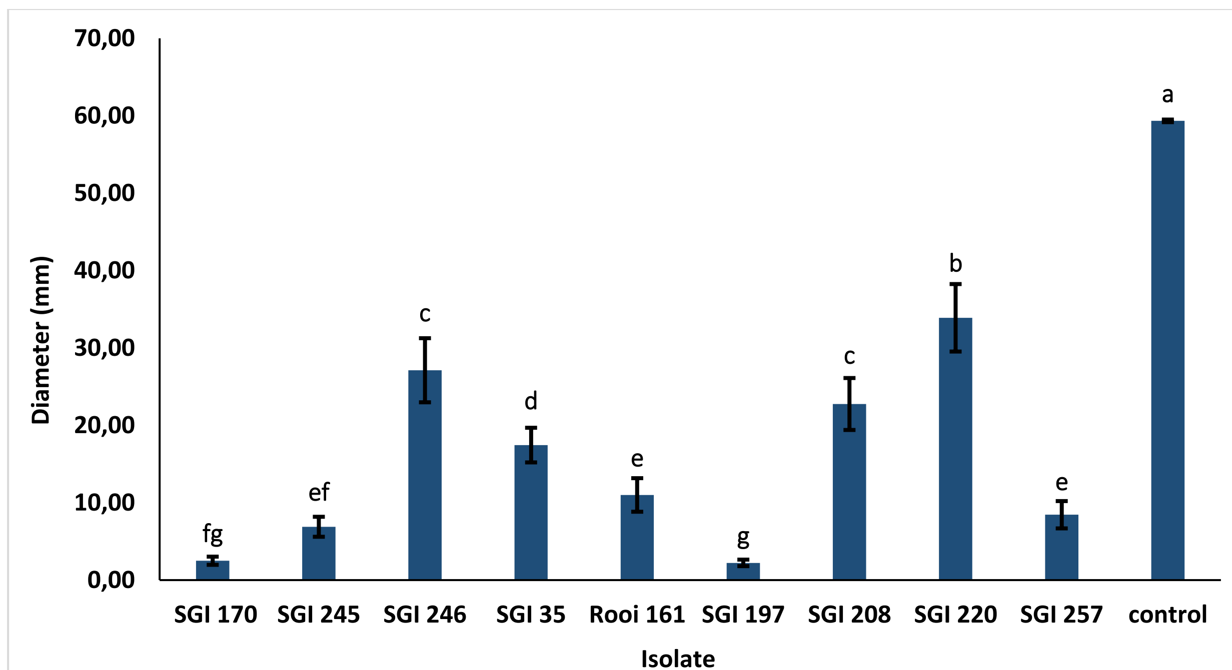


Figure 10. Mean mycelial growth inhibition activity of crude metabolites compared to control after seven days of incubation ($P < 0.0001$).

Filtered metabolites also had varying significant inhibition activity after three days of incubation ($F = 51.49$; $df = 9$; $P < 0.0001$) (Figure 11) and seven days incuation ($F = 122.29$; $df = 9$; $P < 0.0001$) (Figure 12). After three days of incubation, SGI 170 had the lowest growth diameter which was not significantly different to that of SGI 35. The lowest growth diameter came from SGI 170 after seven days of incubation.

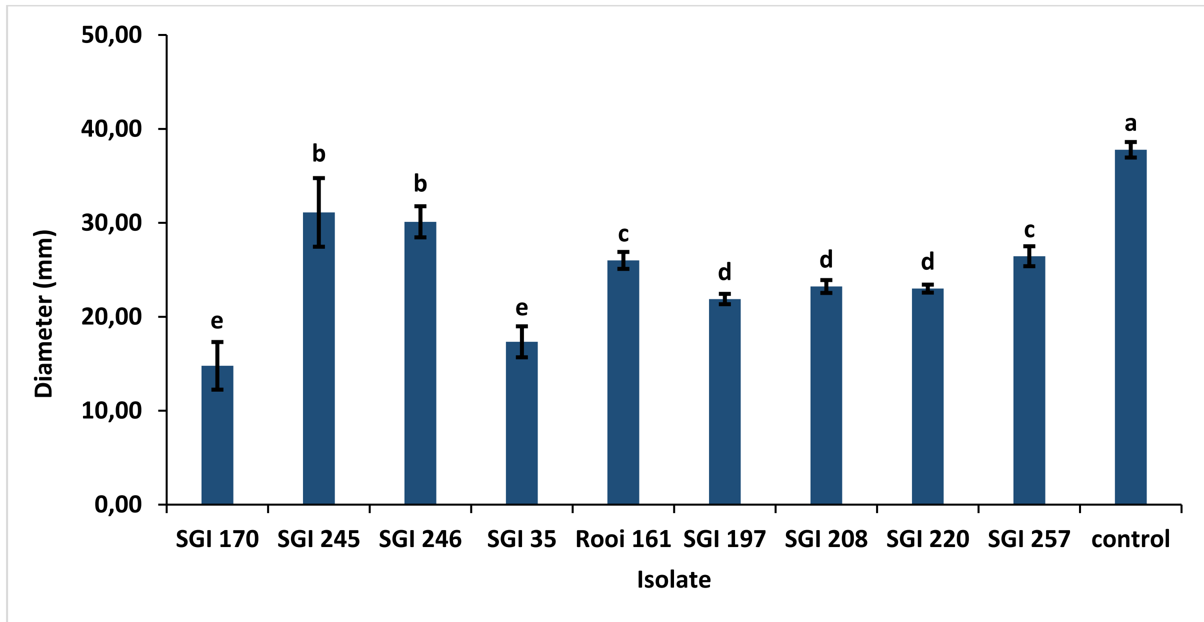


Figure 11. Mean mycelial growth inhibition activity of filtered metabolites compared to control after three days of incubation ($P < 0.0001$).

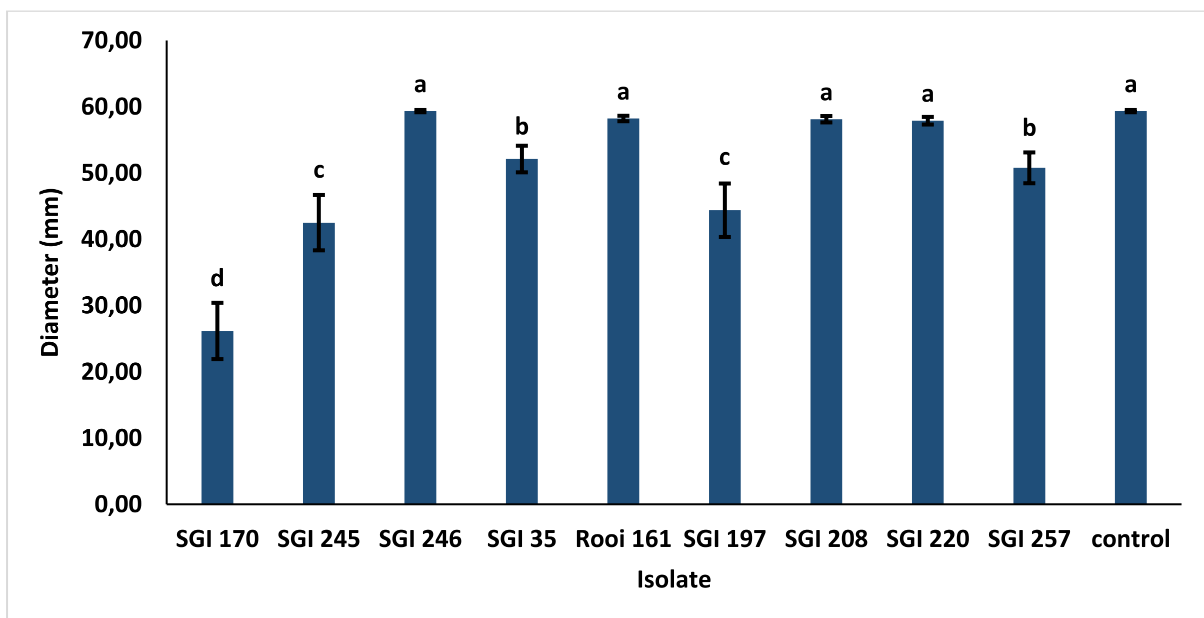


Figure 12. Mean mycelial growth inhibition activity of filtered metabolites compared to control after seven days of incubation ($P < 0.0001$).

Overall inhibitory effect of metabolites seemed to differ per isolate irrespective of the genus of the bacteria that associate with *Heterhorhabditis* and *Steinernema* (F = 321.02; df = 9; $P < 0.0001$) (Figure 13). Metabolites of the bacteria associated with *H. bacteriophora* SGI 170 and *S. beitlechemi* SGI 197 had the highest overall inhibitory effect on mycelial growth compared to control and all other isolates. Interestingly, inhibitory effect of all isolates was significantly different from the control.

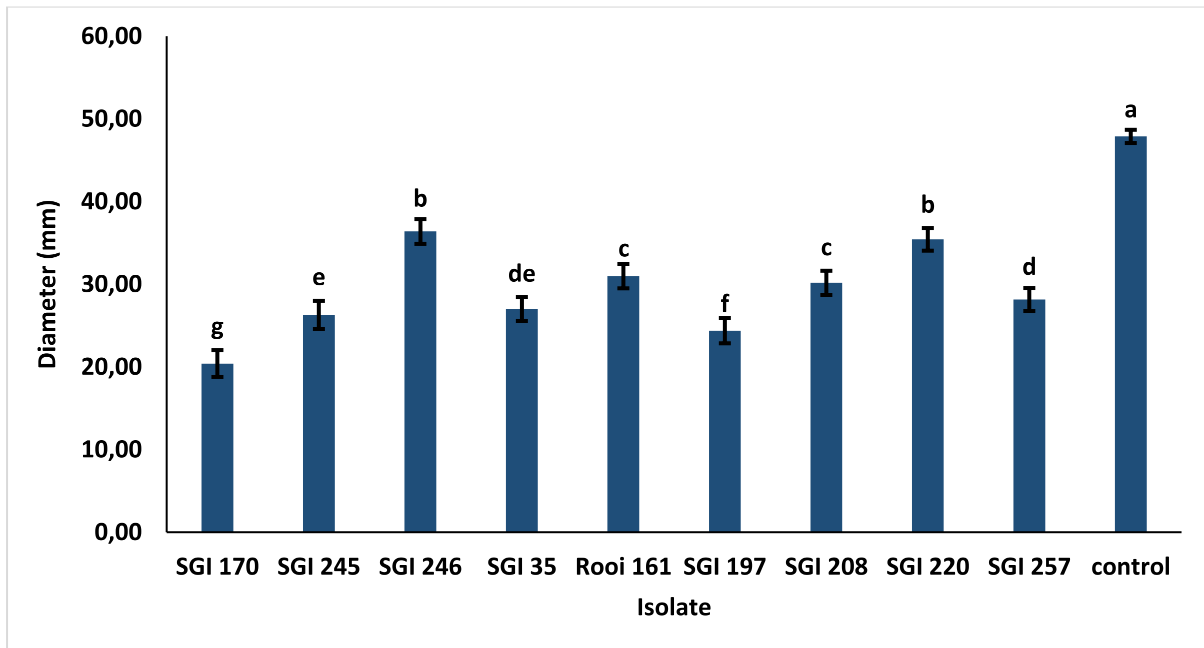


Figure 13. The overall mean mycelial growth inhibition activity of different isolates compared to control ($P < 0.0001$).

4.1.2 Efficacy on *Fusarium graminearum* spore germination

For spore germination experiment, three trials were conduct on different dates and all three trials exhibited a similar pattern of inhibition of spore germination after ANOVA analysis of the results (F = 2.15; df = 2; $P = 0.1258$). The control exhibited the highest percentage of 80.78% spore germination, which was higher than that of all the isolates tested. *Xenorhabdus* isolates had higher inhibition when compared with *Photorhabdus* isolates (F = 407.46; df = 9; $P < 0.0001$). Amongst *Xenorhabdus* isolates, SGI 257 had the highest spore germination inhibition percentage when compare with other *Xenorhabdus* isolates (F = 540.30; df = 7; $P < 0.0001$) (Figure 14). The table 3 below illustrates the inhibition rate (%) of the bacterial metabolite treatments when compared to the control.

Table 3. Mean inhibition rate (%) of *Fusarium graminearum* spore germination. Inhibition rate = 100 × (spores germinated in control – spores germinated in treatment)/ spores germinated in control (Hazir et al., 2016).

Isolates	Spore germination percentage	Inhibition rate
Xenorhabdus sp. SGI 35	13,00	83,91
Photorhabdus sp.SGI 170	75,89	6,05
Xenorhabdus sp. SGI 197	6,44	92,02
Xenorhabdus sp. SGI 208	7,44	90,78
Xenorhabdus sp. SGI 220	9,50	88,23
Photorhabdus sp.SGI 245	68,86	14,74
Xenorhabdus sp. SGI 246	4,78	94,09
Xenorhabdus sp. SGI 257	3,00	96,29
Xenorhabdus sp. ROOI 161	4,56	94,36

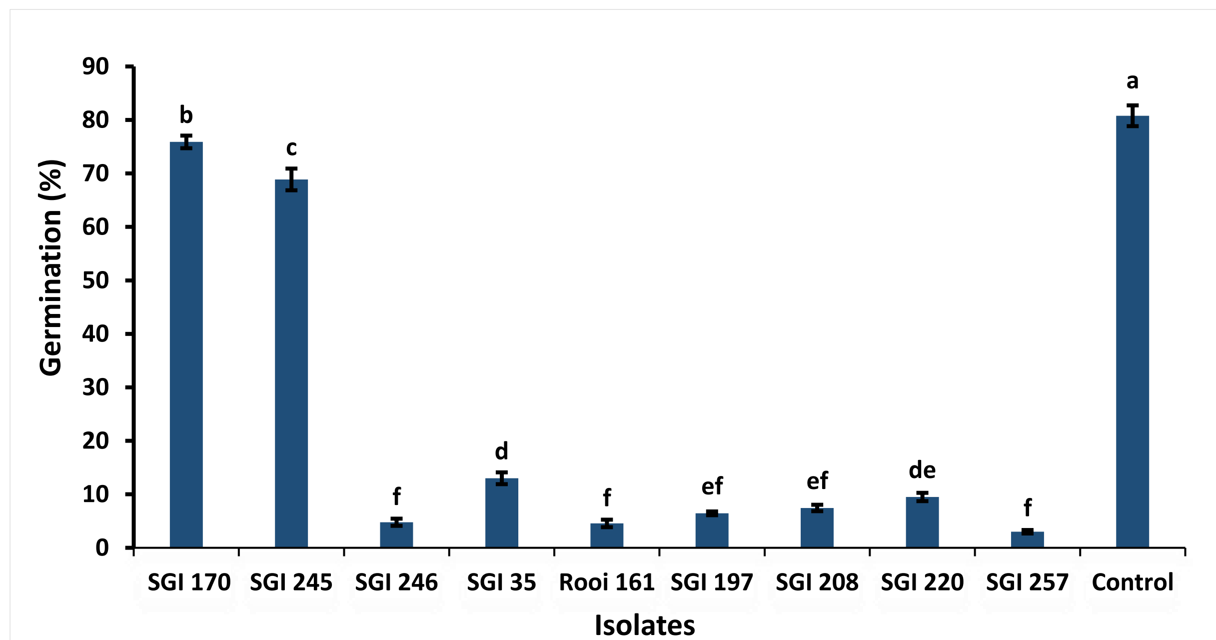


Figure 14. Mean *Fusarium graminearum* spore germination percentages in potato dextrose broth (PDB) treated with 20% filtered metabolites of different EPN symbiotic bacteria cultures compare to control ($P < 0.0001$).

4.2 Effect of bacterial metabolites from EPN bacterial symbionts against five RWA biotypes

Analysis of variance (ANOVA) revealed varying aphicidal activity of bacterial metabolites produced by bacterial symbionts of the five selected EPN isolates. Homogeneity of the three trials variances resulted in a combined ANOVA per biotype in a factorial analysis of isolates as the first factor and biotype as the second factor. *Xenorhabdus* sp. SGI 257 induced the highest mortality on RWA Biotype 1, with close to 40% mortality rate, which was significantly higher than that of the control (Figure 14). The rest of other treatments had intermediate aphicidal activity in Biotype 1 when compared to control (F= 2.07; df= 5; $P > 0.1190$). Among the isolates, SGI 197 aphicidal activity on Biotype 2 was significantly higher (Figure 15) than that of the other isolates (F=1.18; df= 5; $P > 0.3434$). Analysis of variance displayed no significant difference between isolates and control mortality rates for biotype 3 (F= 0.62; df= 5; $P > 0.6839$). SGI 197 also gave a significantly higher mortality rate in biotype 4 when compare to other isolates and control (Figure 17) (F=3.94; df= 5; $P > 0.0082$). Biotype 5 mortality rates were not significant from other (F= 0.18; df= 5; $P > 0.9644$). Overall, SGI 257 was the best isolate for Biotype 1 and SGI 197 performed better for Biotype 2 and Biotype 4.

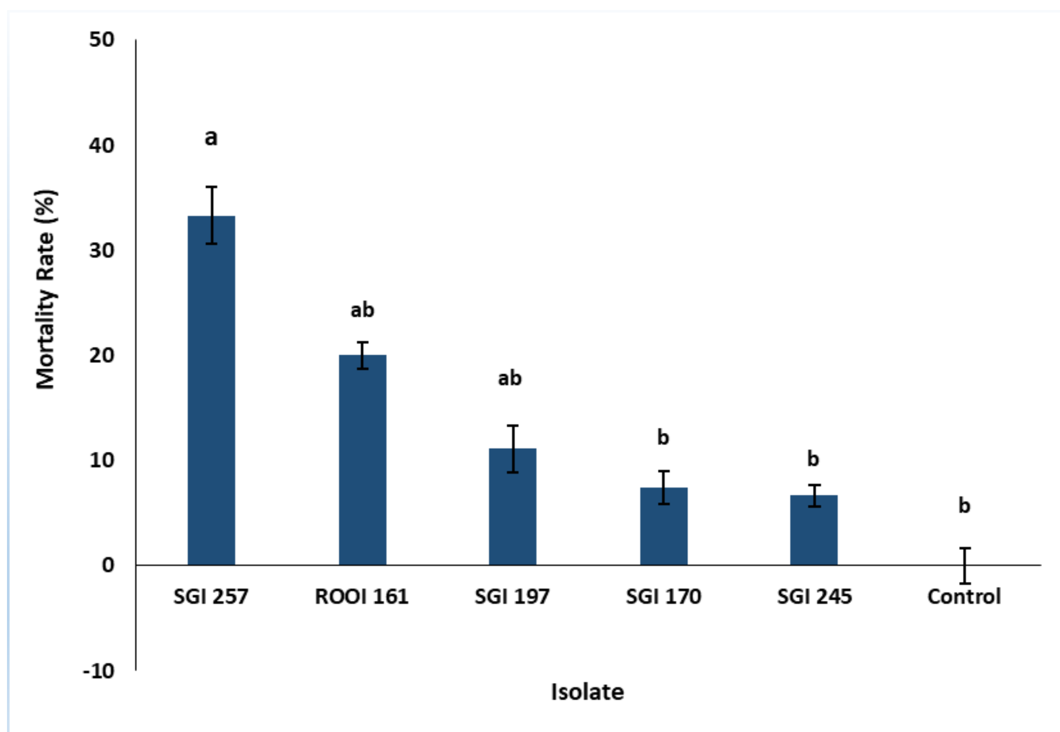


Figure 15. Russian wheat aphid Biotype 1 mortality rates from different isolates metabolite treatments compared to control (α= 0.05).

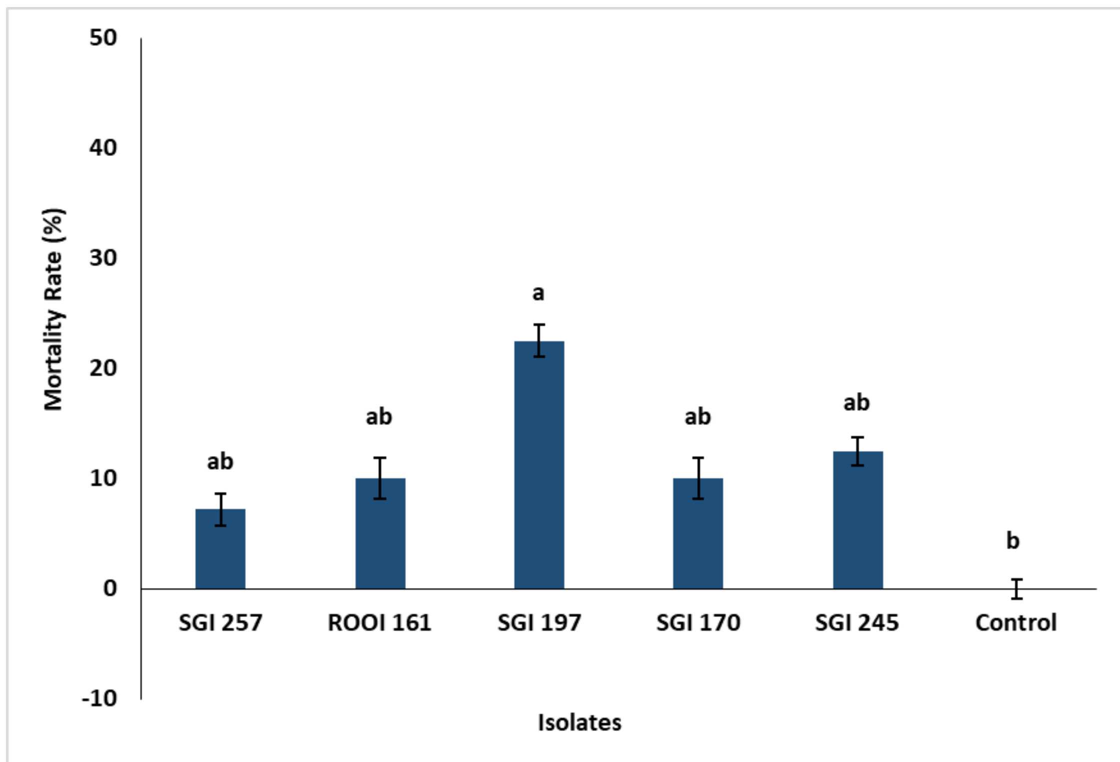


Figure 16. Russian wheat aphid Biotype 2 mortality rates from different isolates metabolite treatments compared to control (α= 0.05).

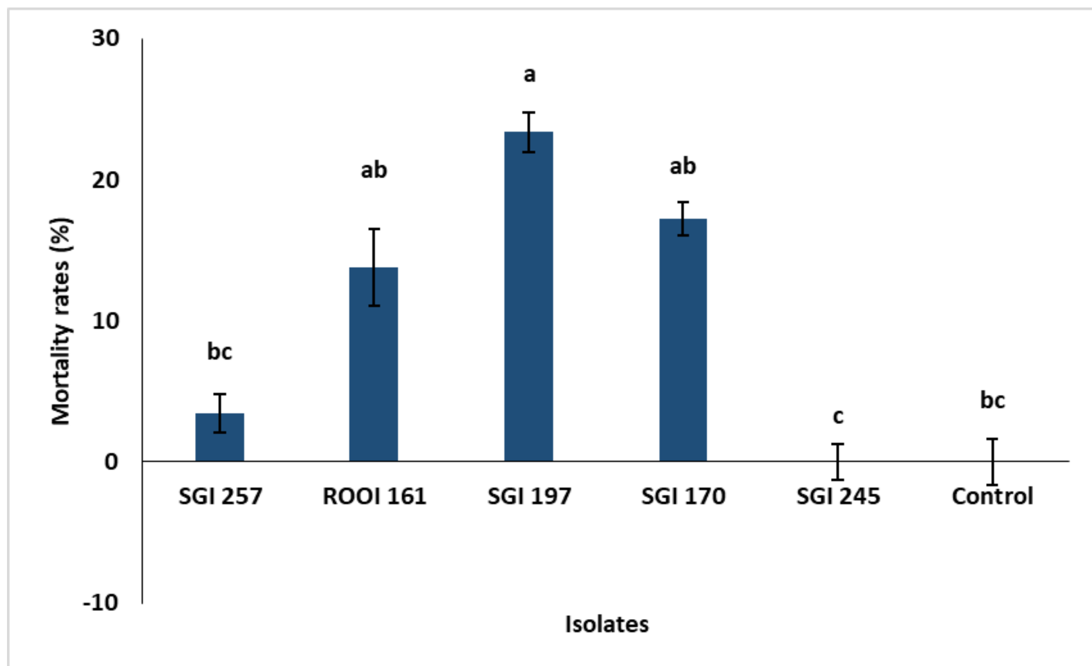


Figure 17. Russian wheat aphid Biotype 4 mortality rates from different isolate metabolite treatments compared to control (α= 0.05).

4.3 Liquid Chromatography- Mass Spectrometry (LC-MS/MS) analysis

Liquid Chromatography- Mass Spectrometry (LC-MS) analyses displayed varying metabolome profiles of the extracts of the two selected isolates (based on FHB efficacy data), *Photorhabdus* sp. SGI 170 and *Xenorhabdus* sp. SGI 197. Difference in concentration of the samples had no significant effect on the metabolite profiles of both isolates. The two replicates of SGI 197 had the same profile. The two isolates produced many corresponding m/z peaks namely 633, 285, 724, 600, 950, 311, 293, 255, and 283. One compound at m/z 584 which had the highest intensity was only seen in chromatograms of SGI 170 around retention time of 12.5 min. Just after the retention time of 12.5 min, isolate SGI 197 produced a peak of m/z 379 that was not identified in SGI 170. Isolate SGI 197 chromatograms also displayed a peak at a retention time of 15 min of m/z 491. Based on their m/z, some of these compounds have been previously identified as Anthraquinones m/z 255 and m/z 285 (Orozco *et al*., 2016), and stilbene precursor 3,5-dihydroxy-4-isopropyl-trans-stilbene (isopropylstilbene) m/z 255 (Wollenberg *et al*., 2016; Orozco *et al*., 2016).

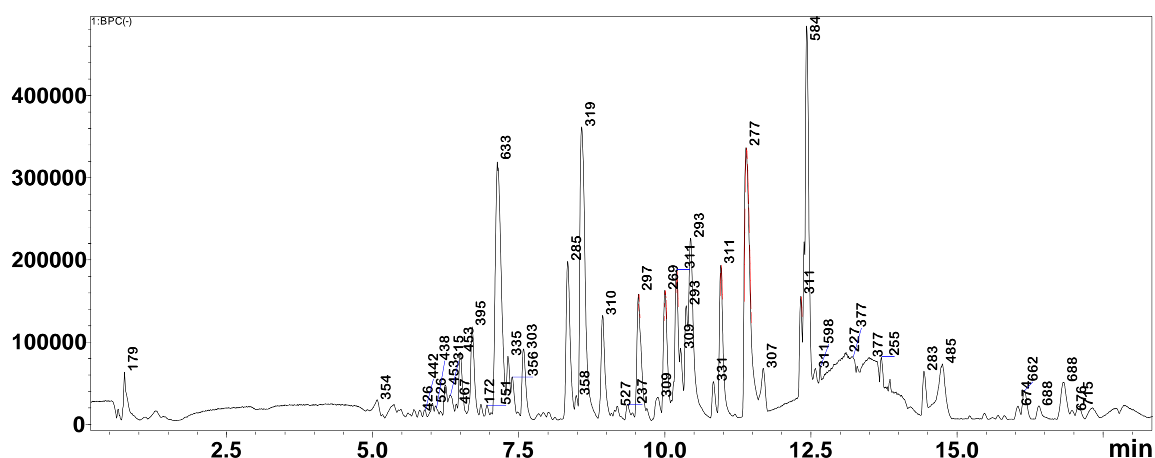


Figure 18. LC-MS chromatogram of *Photorhabdus* sp. SGI 170.

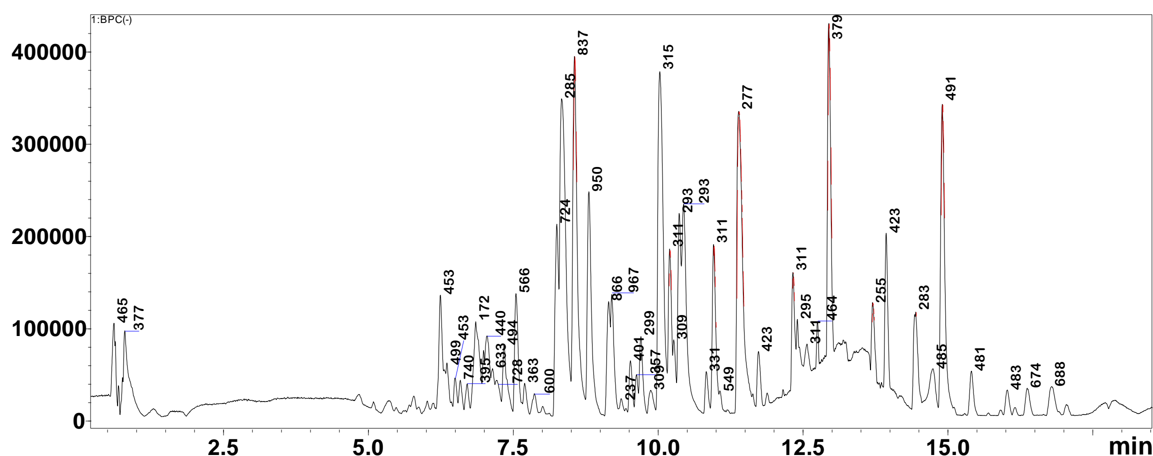


Figure 19. LC-MS chromatogram of *Xenorhabdus* sp. SGI 197.

SIRIUS software (https://bio.informatik.uni-jena.de/) revealed molecular formulae of most of the peaks in relation to their masses (m/z). The peak of mass 311m/z produced by both isolates's molecular formula is $C_{17}H_{26}O_5$ (SIRIUS) and matched with 13 compounds on KNAPSACK database; the most relevant from the list is 7-O-Demethyl-18-hydroxy-albocycline Oxycineromycin B previously produced by *Streptomyces griseoviridis* strain FH-S 1832 (Schneider et al., 1996). The most relevant molecular formula for 584 m/z peak is $C_{32}H_{49}N_5O_5$ (SIRIUS) and matched with 147 lactam compounds on Pubchem database. For 379 m/z peak, molecular formula $C_{19}H_{38}O_3S_2$ was the most scored by SIRIUS software. Peak of mass of 277 m/z most relevant molecular formula is $C_{13}H_{26}O_6$ (SIRIUS). Peak of mass of 491 m/z most SIRIUS scored molecular formula is $C_{29}H_{48}O_5$ and HMDB identified the name of the compound as Homodolicholide.

4.4 DNA extraction and amplification

Genomic DNA of both isolates was successfully extracted and their ITS regions were amplified and depicted on an agarose gel (figure 24). Both effective isolates have previously been characterised (Çimen *et al.*, 2016; Hatting *et al.*, 2009).

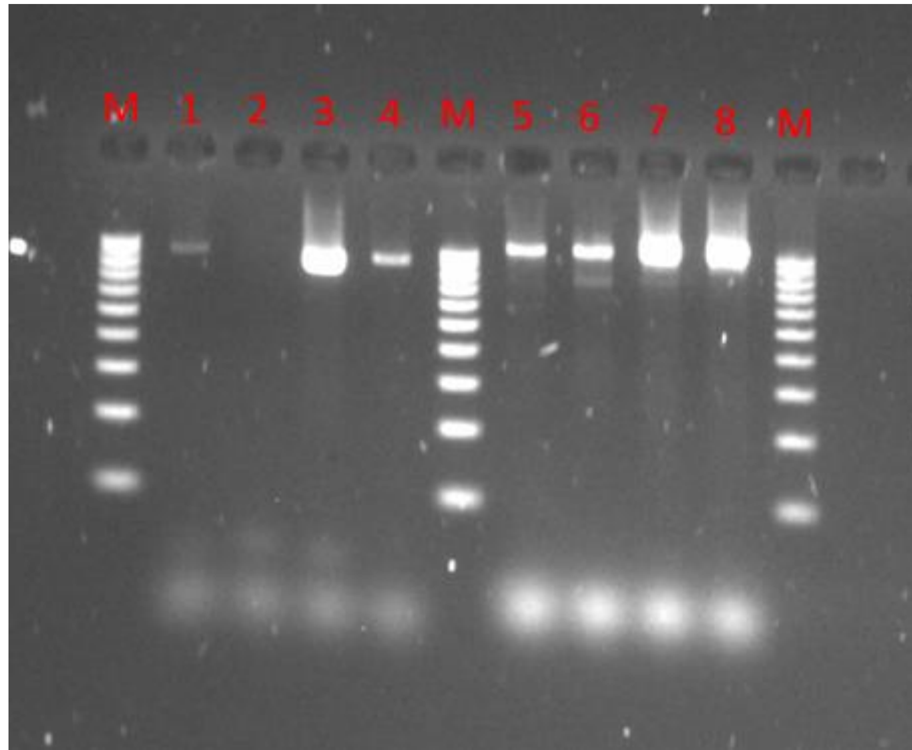


Figure 20. Gel image from PCR products with amplified ITS and 28S regions. Well M: 100bp DNA ladder, Well 1-4: ITS region, Well 5-8: 28S region, Well 1-2 and 5-6: *Heterorhabditis bacteriophora* SGI 170, Well 3-4 and 7-4: *Steinernema beitlechemi* SGI 197.

Chapter 5

Discussion

5.1 Inhibitory effect of bacterial metabolites against FHB

The results show different levels of inhibition on the mycelial growth and spore germination of *F. graminearum* in all tested metabolites treatments and isolates. The level of inhibition varied significantly among isolates and treatments. Hazir et al. (2016) reported similar results when they investigated the relative potency of secondary metabolites in various *Photorhabdus* and *Xenorhabdus* supernatants on diverse fungal phytopathogens. Additionally, secondary metabolites from EPNs symbionts *Photorhabdus* and *Xenorhabdu*s have also been reported to have varying antifungal activities in previous studies (Block *et al*., 2012; Sharma *et al*., 2016; Hazir *et al*., 2016). However, this is the first report of secondary metabolites from symbiotic bacteria of EPNs against FHB causing *Fusarium* spp. *F. graminearum* group II.

5.1.1 Efficacy of bacterial metabolites on *Fusarium graminearum* mycelial growth.

Shan *et al.* (2020) mentioned that antimicrobial activity of symbiotic bacteria derived from EPNs against mycelial growth of plant pathogens depends on the species of the symbiotic bacteria. The highest mycelial growth mean inhibition rate (96.25%) came from a *Xenorhabdus* sp. isolated from SGI 197 crude metabolite treatments after seven days of incubation. Noticeably, the mean inhibition rate from *Xenorhabdus* sp. SGI 197 crude metabolite treatments was the highest compared to other *Xenorhabdus* isolates in all incubation periods. *Photorhabdus* sp. Isolated from SGI 170 followed *Xenorhabdus* sp. isolate SGI 197 as the second highest performing isolate by 95.79% mean inhibition rate after seven days of incubation. Besides this South African based study, *Xenorhabdus* spp. displayed antifungal activity on a number of fungal phytopathogens other than *F. graminearum* in other parts of the world. Indian *Xenorhabdus* spp. (*Xenorhabdus assam*, *X. indica* and *X. gujarat*) displayed antifungal activity against *Fusarium oxysporum*, *Macrophomina phaseolina*, *Sclerotium rolfsii* and *Rizoctonia solani* (Sharma *et al*., 2016). From their results, *X. assam* resulted in 78.1–82.2 % inhibition rate of *S. rolfsii*, *F. oxysporum* and *R. solani* and complete inhibition of *M. phaseolina,* while *X. indica* had the most activity against *F. oxysporum* and least activity against *M. phaseolina*. Chinese *Xenorhabdus* sp. *X. nematophila* displayed antifungal activity against maize fungal pathogens *Bipolaris maydis* and *Curvularia lunata* with inhibition rates of 66.7% and 69.1%, respectively (Wang *et al*., 2014). The results we obtained from our *Xenorhabdus* sp. SGI 197 crude metabolite treatments seem to support antifungal activity

results obtained in other parts of the world, but with higher inhibition 96.25% against *F. graminearum*.

Photorhabdus spp. are famously known as key producers of *trans*-cinnamic acid (TCA) (Block *et al*., 2014). *Trans*-cinnamic acid is said to have antifungal properties (Block *et al*., 2014; Hazir *et al*., 2016; Shan *et al*., 2020). Lalramchuani *et al*. (2020) reported raising inhibition rate from a *Photorhabdus* sp. *P. akhurstii* which displayed a 50 to 60% inhibition rate of *F. oxysporum* myceial growth after 48h that went up to 76 to 79% inhibition rate after 96h and raised again to 87% after 192h of incubation. Similarly, in this study, crude metabolite treatments inhibition rates of both *Photorhabdus* spp. isolates SGI 170 and SGI 245 increased from 73.82% to 95.79% and 86.76% to 88.39% after three days and seven days of incubation, respectively. The increase in inhibition rate of these two isolates may have something to do with the antifungal compound, TCA. An increase in inhibition rate also resulted from two *Xenorhabdus* isolates [SGI 197 (83.53 to 96.25%) and SGI 257 (74.12 to 85.77%)] while other *Xenorhabdus* isolates had a decrease in inhibition rate after three days and seven days of incubation. The varying antagonistic effect between *Xenorhabdus* isolates may be attributed to the varying production levels of their antifungal compounds.

Overall, crude treatments were the most effective on *F. graminearum* mycelial growth. The overall mean inhibition rate from crude treatments was 75.25%, which was higher than that from filtered treatments (23.93%) and autoclaved treatments (13.32%). The lower inhibition activity of autoclaved treatments may be attributed to the autoclaving temperature (121 °C) and the time of the autoclaving cycle (15 min). Wang *et al*., (2014) also reported a decline in inhibition activity of the metabolites against *Bipolaris maydis* after their exposure to high temperatures (50°C and 100°C). However, the same metabolites did not lose their inhibition activity against *Curvularia lunata* after exposure to high temperatures. Additionally, Hazir *et al*. (2017) Highlighted that metabolites produced by *Xenorhabdus szentirmaii* did not lose their antifungal activity after autoclaving them at 121 °C for 15 min. From their results, autoclaved metabolites treatment from *X. szentirmaii* displayed similar inhibition activity against *Monilinia fructicola* as filtered metabolites treatment, but higher inhibition activity against *Glomerella cingulata* than filtered metabolites treatment. Nevertheless, it is worth noting that these studies, in contrast to the current study did not compare autoclaved metabolites to crude metabolites. This is contrary to our results where we found that autoclaved metabolites lost inhibitory activity against *F. graminearum*. Metabolites from *Xenorhabdus* and *Photorhabdus*

have different stability when exposed to high temperatures and their antifungal activity seem to depend on the species of the fungi being treated.

5.1.2 Inhibitory effect on *F. graminearum* spore germination.

Xenorhabdus isolates from the genera *Steinernema* displayed higher inhibition rates of *Fusarium graminearum* spore germination compared to *Photorhabdus* isolates from *Heterorhabditis*. Approximately over 83% inhibition rate of *Fusarium* spore germination resulted in all *Xenorhabdus* isolates from the genera *Steinernema* while *Photorhabdus* isolates from *Heterorhabditis* ranged below 15% compared to control. Hazir *et al*. (2016) reported a similar trend when they tested seven bacterial isolates (four *Xenorhabdus* spp. and three *Photorhabdus* isolates) to determine the inhibitory effect of metabolites on spore germination of *Fusicladium carpophilum* and *Fusicladium effusum* spores. From their results, germination was lower in treatments with *Xenorhabdus* spp. compared with *Photorhabdus* isolates for both *F. carpophilum* and *F. effusum*, except metabolites from *Xenorhabdus nematophila* that displayed intermediate results when tested on *F. carpophilum* spores. In contrast, *Xenorhabdus* and *Photorhabdus* metabolites inhibitory effect seemed to depend on the isolate tested not on the genera when they were tested against *Pythium myriotylum* spores (Shan *et al*., 2020). Spore germination is a critical step in the development FHB disease in wheat seedlings. From our results, bacterial metabolites from *Xenorhabdus* spp. isolated from the *Steinernema* isolates have potential as candidates in integrated FHB management.

5.2 Efficacy of bacterial metabolites on Russian wheat aphid biotypes.

This study explored the efficacy of bacterial secondary metabolites produced by bacterial symbionts of EPNs on the mortality of RWA biotypes. Metabolites treatments presented different aphicidal activities on RWA biotypes. From the results, aphicidal activity of the metabolites treatments seemed to decrease with an increase in the order of the biotypes. Even though insecticides or resistant cultivars are commonly used to control RWA infestations, some studies reported aphicidal activity from different biological control agents (Mesquita *et al*., 1997; Motholo *et al*., 2020; Abebe *et al*., 2020). Some of these biocontrol agents include *Beauveria bassiana* (Motholo *et al*., 2020), botanical extracts (Neem) *Azadirachta indica* L. (Abebe *et al*., 2020), *Aphelinus asychis* (Mesquita *et al*., 1997). Although, aphicidal activities from bacterial metabolites produced by bacterial symbionts of EPNs have been reported previously on cotton aphids (*Aphis gossypii* Glov.) (Hemiptera: Aphididae) (Iqbal *et al*., 2020), this study is the first to evaluate the aphicidal activity of crude bacterial metabolites for EPN bacterial symbionts against five South African RWA biotypes.

Efficacy of the isolates seemed to depend on the biotype and isolate tested. *Xenorhadus* sp isolated from SGI 257 produced the highest mean mortality rate of biotype 1, but biotype 2 mortality rate was better induced by *Xenorhabdus* sp. from SGI 197. Biotype 3 and 5 were resistant to all treatments, this may be attributed by the *D. noxia* resistance source (Dn gene) that differs between the biotypes. *Xenorhabdus* sp. SGI 197 had the highest overall mean mortality rate. The isolate was also the only one to produce the highest mortality rate in two different biotypes.

5.3 Liquid Chromatography- Mass Spectrometry

One approach for the development of new control products is the evaluation of microorganisms and the bioactive molecules they produce (Orozco *et al*., 2016). The study aimed at analysing metabolome profiles of two isolates of EPN symbiotic bacteria (*Photorhabdus* sp. SGI 170 and *Xenorhabdus* sp. SGI 197). Liquid Chromatography-Mass Spectrometry (LC-MS/MS) analyses of the two isolates had varying metabolome profiles. Liquid Chromatography-Mass Spectrometry chromatograms displayed several peaks that were shared between the two isolates. Correspondence between the two profiles may be because *Xenorhabdus* and *Photorhabdus* have a similar life cycle and they are phylogenetically related (Sajnaga and Kazimierczak, 2020).

Two previously described peaks were detected by LC-MS analyses of both isolates. The peak m/z 285 was described as Anthraquinones (Orozco *et al*., 2016), and m/z 255 was described as stilbene precursor 3,5-dihydroxy-4-isopropyl-trans-stilbene (Isopropylstilbene) (Wollenberg *et al*., 2016; Orozco *et al*., 2016; Park and Crawford, 2015). Most of the peak masses and molecular formulae did not match with other compounds on different databases. Orozco *et al*., (2016) reported similar results from two *Photorhabdus sonorensis* strains (Caborca and CH35). Their LC-MS analysis only confirmed the identity of stilbenes, anthraquinone, and cinnamic acid (not detected in our results) and a number of unidentified compounds. More knowledge in the biosynthesis and structures of these compounds is needed to elucidate the identification of these compounds.

The stilbenes identified in this study are non-flavonoid bioactive compounds that are commonly produced by plants. These compounds exist as *cis* or *trans* stereoisomers, but their natural forms are *trans* (Valletta *et al*., 2021). Stilbenes are made up of a 14 Carbon skeleton and their bioactive activities include antibacterial, anti-inflammation, anti-cancer and cardioprotective effects (Shahidi and Yeo, 2018). Stilbene derivative 3,5-dihydroxy-4-

isopropyl-*trans*-stilbene was detected in both isolates. 3,5-dihydroxy-4-isopropyl-trans-stilbene is thought to play a crucial role in symbiosis. Furthermore, 3,5-dihydroxy-4-isopropyl-*trans*-stilbene have antifungal, antimicrobial, anticancer, antioxidant activities (Hapeshi *et al*., 2019). *Photorhabdus* bacteria was the first to be reported to produce a bacterial stilbene 3,5-dihydroxy-4-isopropyl-trans-stilbene (Park *et al*., 2017). Anthraquinones are known to be produced by bacteria, but their production is not common (Li *et al*., 1995). Previous studies suggest that Anthraquinones are responsible for the reddish pigmentation presented by the insect cadaver (Forst and Nealson, 1996). Industrially, Anthraquinones are been used in cosmetic, dyes and food (Yang *et al*., 2018).

5.4 Effective isolates and their bacterial symbionts

In this study *Photorhabdus* sp. SGI 170 and *Xenorhabdus* sp. SGI 197 isolates symbionts were the best candidates' producers of bioactive metabolites. *Heterorhabditis bacteriophora* is known to be mutually associated with the enteric bacterium, *Photorhabdus luminescens* (Bai *et al*., 2013; Ciche, 2007). *Heterorhabditis bacteriophora* was first described by Poinar in 1975 in a new genera, species, and family (*Heterorhabditidae*) of *Rhabditida* and its bacterial symbiont *Photorhabdus luminescens* was first described by G.M Thomas and G.O.J Poinar in 1979 (Thomas and Poinar, 1979). *Photorhabdus luminescens* is now known as *Photorhabdus luminescens* subspecies *luminescens* (Fischer-Le Saux *et al*., 1999). *Steinernema beitlechemi* was discovered in Bethlehem, Free State province, South Africa and molecular characterisation of its bacteria symbiont closely matched with *Xenorhabdus khoisanae* (Çimen *et al*., 2016). Dreyer *et al*., (2017) reported that *Xenorhabdus khoisanae* was first isolated from *S. khoisanae* and was also found in association with unrelated nematodes clades (between clades III and V), namely *Steinernema jeffreyense* and *Steinernema sacchari*. *Heterorhabditis bacteriophora* SGI 170 isolate was collected from Fouriesburg in the Free State province in a survey conducted by Hatting *et al*., (2009). The authors also identified the isolate as *H. bacteriophora* and all other collected *Heterorhabditis* isolates were identified as *H. bacteriophora.*

Chapter 6

Conclusions and Recommendations

6.1 Conclusions

To conclude on bacterial metabolites against FHB causing *Fusarium* sp. *F. graminearum*, it was found that bacterial metabolites from bacterial symbionts of EPNs had varying antimicrobial effect on two important development stages (mycelial growth and spore germination) of *F. graminearum*. It is important to note that these results are from an *in vitro* approach. Although field efficacy against FHB is still not studied, the significant inhibition of mycelial growth and spore germination suggest that application of secondary metabolites at flowering stage may provide protection of plants against infection or spread of *F. graminearum*. Further studies are needed to evaluate their efficacy under glasshouse or field conditions to address their effects on DON accumulation and beneficial organisms. A locally produced bio-fungicide of this nature will be more economic in price. Additionally, after addressing the above-mentioned issues, the product will be an alternative eco-friendly product to synthetic chemical products available in the market.

Aphicidal activity of the metabolites depended on both the biotype and treatment. Biotype 3 and 5 were resistant to all the tested treatments. *Xenorhabdus* sp. SGI 197 metabolite treatments are the best candidates in the control of RWA. Wheat aphid control by biopesticides, based on entomopathogenic bacteria and their bioactive metabolites, can be the best alternative to chemicals pesticides. Production of Anthraquinones and bacterial Stilbenes were confirmed in both effective isolates (*Xenorhabdus* sp. SGI 197 and *Photorhabdus* sp. SGI 170) extracts. This study is the first to report the production of Anthraquinones by *Xenorhabdus* bacteria.

6.2 Recommendations

From the results obtained in this study, it is recommended that further studies should be conducted in order to develop bacterial metabolites from EPN bacterial symbionts as an alternative for synthetic chemical use. In order to achieve this, metabolites/compounds that displayed inhibitory activity against mycelial growth and spore germination of *F. graminearum* should be individually identified and their production should also be triggered and upregulated. Furthermore, these antifungal compounds should be tested against other agricultural important fungal pathogens in order to obtain a comprehensive product that will be integrated in the control of numerous fungal pathogens. Russian wheat aphid biotypes immune defence against these bacterial metabolites should also be elucidated. Understanding the defence mechanisms

of these pests will help in the development of biopesticides and in avoiding the development of new biotypes.

References

- Aalbersberg, Y.K., Van der Westhuizen, M.C., and Hewitt, P.H., 1989. Characteristics of the population build-up of the Russian wheat aphid *Diuraphis noxia* and the effect on wheat yield in the eastern Orange Free State. Annals of Applied Biology, 114: 231-242, https://doi.org/10.1111/j.1744-7348.1989.tb02100.x .
- Afridi, M.S., Mahmood, T., Salam, A., Mukhtar, T., Mehmood, S., Ali, J., Khatoon, Z., Bibi, M., Javed, M.T., Sultan, T., and Chaudhary, H.J., 2019. Induction of tolerance to salinity in wheat genotypes by plant growth promoting endophytes: involvement of ACC deaminase and antioxidant enzymes. Plant Physiology and Biochemistry, 139: 569-577, https://doi.org/10.1016/j.plaphy.2019.03.041.
- Ahmad, P., Ahanger, M.A., Alam, P., Alyemeni, M.N., Wijaya, L., Ali, S., and Ashraf, M., 2019. Silicon (Si) supplementation alleviates NaCl toxicity in mung bean [*Vigna radiata* (L.) wilczek] through the modifications of physio-biochemical attributes and key antioxidant enzymes. Journal Plant Growth Regulation, 38(1): 70-82, https://doi.org/10.1007/s00344-018-9810-2 .
- Aiswarya, D., Raja, R., Gowthaman, G., Deepak, P., Balasubramani, G., and Perumal, P., 2017. Antibacterial activities of extracellular metabolites of symbiotic bacteria, *Xenorhabdus* and *Photorhabdus* Isolated from entomopathogenic nematodes. International Biological and Biomedical Journal, 3(2): 80-88, http://ibbj.org/article-1-110-en.html.
- Akhurst, R.J., 1980. Morphological and functional dimorphism in *Xenorhabdus spp*., bacteria symbiotically associated with the insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. Microbiology Society, 121(2): 303-309, https://doi.org/10.1099/00221287-121-2-303.
- Ali, A., Desneux, N., Lu, Y., and Wu, K., 2018. Key aphid natural enemies showing positive effects on wheat yield through biocontrol services in northern China. Agriculture, Ecosystems and Environment, 266: 1-9, https://doi.org/10.1016/j.agee.2018.07.012 .
- Alikina, O., Chernobrovkina, M., Dolgov, S., and Miroshnichenko, D., 2016. Tissue culture efficiency of wheat species with different genomic formulas. Crop Breeding and Applied Biotechnology, 16(4): 1984-7033.

- Amarasinghe, C.C., Tamburic-Ilincic, L., Gilbert, J., Anita L. Brûlé-Babel, A.L., and Fernando, W.G.D., 2013. Evaluation of different fungicides for control of *Fusarium* head blight in wheat inoculated with 3ADON and 15ADON chemotypes of *Fusarium graminearum* in Canada. Canadian Journal of Plant Pathology, 35 (2): 200-208, https://doi.org/10.1080/07060661.2013.773942 .
- Appels, R., Eversole, K., Stein, N., Feuillet, C., and Keller, B., 2018. Shifting the limits in wheat research and breeding using a fully annotated reference genome. The International Wheat Genome Sequencing Consortium, 361(6403): 719-728, https://doi.org/10.1126/science.aar7191 .
- Asela, K., Alemayehu, C., and Elfinesh, S., 2020. Fungi Associated with Wheat (*Triticum* spp.) in South East Ethiopia under Storage Conditions. Academic Research Journal of Agricultural Science and Research, 8(2): 109-117.
- Aston, F.W., 1920. Isotopes and atomic weights. Nature, 105: 617–619
- Avila, G., Davidson, M., Van Helden, M., and Fagan, L., 2019. The potential distribution of the Russian wheat aphid (*Diuraphis noxia*): An updated distribution model including irrigation improves model fit for predicting potential spread. Bulletin of Entomological Research, 109(1): 90-101, https://doi.org/10.1017/S0007485318000226 .
- Bai, X., Adams, B.J., Ciche, T.A., Clifton, S., Gaugler, R., Kim, K., Spieth, J., and Sternberg, P.W., 2013. A Lover and a Fighter: The Genome Sequence of an Entomopathogenic Nematode *Heterorhabditis bacteriophora*. PLoS ONE, 8(7): e69618, https://doi.org/10.1371/journal.pone.0069618.
- Barzman, M., Bàrberi, P., and Birch, A.N.E., 2015. Eight principles of integrated pest management. Agronomy for Sustainable Development, 35: 1199-1215. https://doi.org/10.1007/s13593-015-0327-9.
- Belien, T., 2018. Entomopathogenic nematodes as biocontrol agents of insect pests in orchards.CAB Reviews, 13: 51-58, doi: 10.1079/PAVSNNR201813058.
- Bhardwaj, S. K., Dwivedi, K., and Agarwal, D. D., 2015. A Review: HPLC Method Development and Validation." Univers. Res. Publ.
- Bibián, M.E., Pérez-Sánchez, A., Mejía, A., and Barrios-González, J., 2020. Penicillin and cephalosporin biosyntheses are also regulated by reactive oxygen species. Applied Microbiology and Biotechnology, 104(4): 1773-1783, https://doi.org/10.1007/s00253-019-10330-2 .

- Blackman, R.L. and Eastop, V.F., 2000. Aphids on the World's crops: An Identification and Information Guide, 2nd edition. John Wiley, Chitchester, UK: Wiley, page 476.
- Block, C. H., Shapiro-Ilan, D. I., Wedge, D. E., and Cantrell, C. L., 2014. Identification of the antifungal compound, *trans*-cinnamic acid, produced by *Photorhabdus luminescens*, a potential biopesticide against pecan scab. Journal of Pest Science, 87(1): 155–162, https://doi.org/10.1007/s10340-013-0519-5 .
- Boemare, N., Laumond, C., and Mauleon, H., 1996. The entomopathogenic nematode-bacterium complex: Biology, life cycle and vertebrate safety. Biocontrol Science and Technology, 6: 333-346, https://doi.org/10.1080/09583159631316 .
- Boemare, N.E., and Akhurst, R.J., 2006. The genera *Photorhabdus* and *Xenorhabdus*. Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H. Stackebrandt, E. (Eds.), the Prokaryotes, Springer Science+Business Media Inc, New York, Pages 451-494.
- Botha, A., 2021. Fast developing Russian wheat aphid biotypes remains an unsolved enigma. Current Opinion in Insect Science, 45: 42-52, https://doi.org/10.1016/j.cois.2020.12.001 .
- Burger, N.F.V., and Botha, A., 2017. Genome of russian wheat aphid an economically important cereal aphid. Standards in Genomic Sciences, 12:90. https://doi.org/10.1186/s40793-017-0307-6.
- Chemeda, T.F., 2015. A Review on Distribution, Biology and Management Practice of Russian Wheat Aphid *Diuraphis noxia* (Mordvilko) (*Hemiptera:Aphididae*), in Ethiopia. Journal of Biology, Agriculture and Healthcare, 5(17): 70-81.
- Chong, Y., Ho, C., Leung, S., Lau, S.K.P., and Woo, P.C.Y., 2018. Clinical Mass Spectrometry in the Bioinformatics Era: A Hitchhiker's Guide. Computational and Structural Biotechnology Journal, 16: 316-334, https://doi.org/10.1016/j.csbj.2018.08.003.
- Cianferoni, A., 2016. Wheat allergy: diagnosis and management. Journal of asthma and allergy, 9: 13, https://doi.org/10.2147%2FJAA.S81550 .
- Ciche, T.A., 2007. The biology and genome of *Heterorhabditis bacteriophora*. (February 20, 2007). In: WormBook (Ed.), The *C. elegans* Research Community. WormBook, http://dx.doi.org/10.1895/wormbook.1.135.1.
- Çimen, H., Půža, V., Nermuť, J., Hatting, J., Ramakuwela, T., Faktorová, L., and Hazir, S., 2016. *Steinernema beitlechemi* n. sp., a new entomopathogenic nematode

(Nematoda: Steinernematidae) from South Africa, Nematology, 18(4): 439-453, https://doi.org/10.1163/15685411-00002968.

- Coskun, O., 2016. Separation techniques: Chromatography. North Clin Istanbul, 3(2): 156, https://doi.org/10.14744%2Fnci.2016.32757 .
- Crawford, J.M, Portmann, C., Zhang, X., Roeffaers, M.B., and Clardy, J., 2012. Small molecule perimeter defense in entomopathogenic bacteria. Proceedings of the National Academy of Sciences of the United States of America, 109(27): 10821-10826, https://doi.org/10.1073/pnas.1201160109 .
- Crotty, S., Gerişlioğlu, S., Endres, K.J., Wesdemiotis, C., and Schubert, U.S., 2016. Polymer architectures via mass spectrometry and hyphenated techniques: A review. Analytica Chimica Acta, 932: 1-21, https://doi.org/10.1016/j.aca.2016.05.024.
- Da Rocha, M. E. B., Freire, F. D. C. O., Maia, F. E. F., Guedes, M. I. F., & Rondina, D., 2014. Mycotoxins and their effects on human and animal health. Food Control, 36(1): 159-165, https://doi.org/10.1016/j.foodcont.2013.08.021 .
- Da Silva, W.J., Pilz-Júnior, H.L., and Heermann, R., 2020. The great potential of entomopathogenic bacteria *Xenorhabdus* and *Photorhabdus* for mosquito control: a review. Parasites Vectors, 13: 376-381, https://doi.org/10.1186/s13071-020-04236-6.
- Dara, S.K., 2019. The new integrated pest management paradigm for the modern age. Journal of Integrated Pest Management, 10(1): 1-9, https://doi.org/10.1093/jipm/pmz010.
- Dilks, T., Halsey, K., De Vos, R.P., Hammond-Kosack, K.E., and Brown, N.A., 2019. Non-canonical fungal G-protein coupled receptors promote *Fusarium* head blight on wheat. PLoS Pathogens, 15(4): e1007666, https://doi.org/10.1371/journal.ppat.1007666.
- Dlamini, T.M., Allsopp, E., and Malan. A.P, 2020. Application of *Steinernema yirgalemense* to control *Frankliniella occidentalis* (*Thysanoptera: Thripidae*) on blueberries. Crop Protection, 128: 105016, https://doi.org/10.1016/j.cropro.2019.105016.
- Dreyer, J., Malan, A.P., and Dicks, L.M.T., 2017. Three novel *Xenorhabdus–Steinernema* associations and evidence of strains of *X. khoisanae* switching between different clades. Current Microbiology, 74: 938-942. https://doi.org/10.1007/s00284-017-1266-2.

- Du Toit, F., 1987. Resistance in wheat (*Triticum aestivum*) to *Diuraphis noxia* (Hemiptera: *Aphididae*). Cereal Research Communications, 15: 175.
- Dweba, C.C., Figlan, S., Shimelis H.A., Motaung, T.E., Sydenham, S., Mwadzingeni, L., and Tsilo, T.J. 2017. *Fusarium* head blight of wheat: Pathogenesis and control strategies. Crop Protection, 91: 114-122.
- Ehi-Eromosele, C.O., Nwinyi, O.C., and Ajani, O.O., 2013. Integrated Pest Management. Weed and Pest Control - Conventional and New Challenges, http://dx.doi.org/10.5772/54476.
- Ehler, L.E., 2006. Perspective Integrated pest management (IPM): definition, historical development and implementation, and the other IPM. Pest Management Science, 62: 787-789.
- Eleftherianos, I., Boundy, S., Joyce, S.A., Aslam, S., Marshall, J.W., Cox, R.J., Simpson, T.J., Clarke, D.J., and Reynolds, S.E., 2007. An antibiotic produced by an insect-pathogenic bacterium suppresses host defenses through phenoloxidase inhibition. Proceedings of the National Academy of Sciences, 104: 2419–2424, https://doi.org/10.1073/pnas.0610525104 .
- Engel, Y., Windhorst, C., Lu, X., Goodrich-Blair, H., Bode, H.B., 2017. The Global Regulators Lrp, LeuO, and HexA Control Secondary Metabolism in Entomopathogenic Bacteria. Frontiers in Microbiology, 8(209), doi:10.3389/fmicb.2017.0029,https://www.frontiersin.org/article/10.3389/fmicb.2017.00209.
- Eroglu, C., Cimen, H., Ulug, D., Karagoz, M., Hazir, S., and Cakmak, I., 2019. Acaricidal effect of cell-free supernatants from *Xenorhabdus* and *Photorhabdus* bacteria against *Tetranychus urticae* (*Acari: Tetranychidae*). Journal of Invertebrate Pathology, 160: 61-66, https://doi.org/10.1016/j.jip.2018.12.004 .
- Faran, M., Farooq, M., Rehman, A., Nawaz, A., Saleem, M.K., Ali, N., and Siddique, K.H.M., 2019. High intrinsic seed Zn concentration improves abiotic stress tolerance in wheat. Plant and Soil, 437: 195–213, https://doi.org/10.1007/s11104-019-03977-3.
- Feksa, H.R., Do Couto, H.T.Z., Garozi, R., De Almeida, J.L., Gardiano C.G., and Tessmann, D.J., 2019. Pre- and postinfection application of strobilurin-triazole premixes and single fungicides for control of fusarium head blight and deoxynivalenol mycotoxin in wheat. Crop protection, 117: 128-134, https://doi.org/10.1016/j.cropro.2018.12.003 .

- Figueroa, M., Hammond-Kosack, K.E., and Solomon, P.S., 2017. A review of wheat diseases—a field perspective. Molecular Plant Pathology, 19(6): 1523-1536, https://doi.org/10.1111/mpp.12618 .
- Fischer-Le Saux, M., Viallard, V., Brunel, B., Normand, P., and Boemare N.E., 1999. Polyphasic classification of the genus *Photorhabdus* and proposal of new taxa: *P. luminescens* subsp. *luminescens* subsp. nov., *P. luminescens* subsp. *akhurstii* subsp.nov., *P. luminescens* subsp. *laumondii* subsp. nov., *P. temperata* sp. nov., *P. temperata* subsp. *temperata* subsp. nov. and *P. asymbiotica* sp. nov. International Journal of Systematic Bacteriology, 49: 1645-1656, https://doi.org/10.1099/00207713-49-4-1645 .
- Forst, S., and Nealson, K., 1996. Molecular Biology of the Symbiotic-Pathogenic Bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. Microbiological Reviews, 60(1): 21-43.
- Garcia-del-Pino, F., Morton, A., Shapiro-Ilan, D., 2018. Chapter 12 - Entomopathogenic Nematodes as Biological Control Agents of Tomato Pests. Ed(s): Wakil, W., Brust, G.E., Perring, T.M., Sustainable Management of Arthropod Pests of Tomato, Academic Press, 269-282, https://doi.org/10.1016/B978-0-12-802441-6.00012-7.
- Goutam, U., Kukreja, S., Yadav, R., Salaria, N., Thakur, K., Goyal, A.K., 2015. Recent trends and perspectives of molecular markers against fungal diseases in wheat. Frontiers in Microbiology, 6(861). Doi;10.3389/fmicb.2015.00861.
- Gqozo, M.P., Bill, M., Siyoum, N., Labuschagne, N., and Korsten, L., 2020. Fungal diversity and community composition of wheat rhizosphere and non-rhizosphere soils from three different agricultural production regions of South Africa. Applied Soil Ecology, 151: 103543
- Griffiths, J., 2008. A brief history of mass spectrometry. Analytical Chemistry, 80(15), 5678-5683.
- Guler, G.O., Cakmak, Y.S., Dagli, Z., Aktumsek, A., Ozparlak, H., 2010. Organochlorine pesticide residues in wheat from Konya region, Turkey. Food and Chemical Toxicology, 48: 1218–1221, https://doi.org/10.1016/j.fct.2010.02.013.
- Gulzar, S., Usman, M., Wakil, W., Gulcu, B., Hazir, C., Karagoz, M., Hazir, S., and Shapiro-Ilan, D.I., 2020. Environmental tolerance of entomopathogenic nematodes differs among nematodes arising from host cadavers versus aqueous suspension.

Journal of Invertebrate Pathology, 175: 107452, https://doi.org/10.1016/j.jip.2020.107452.

- Gumus, A., Karagoz, M., Shapiro-Ilan, D., and Hazir, S., 2015. A novel approach to biocontrol: Release of live insect hosts pre-infected with entomopathogenic nematodes. Journal of Invertebrate Pathology, 130: 56-60.
- Han, R., and Ehlers, R., 2001. Effect of *Photorhabdus luminescens* phase variants on the in vivo and in vitro development and reproduction of the entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*, FEMS Microbiology Ecology, 35(3): 239–247, https://doi.org/10.1111/j.1574-6941.2001.tb00809.x.
- Hapeshi, A., Benarroch, J.M., Clarke, D.J., and Waterfield, N.R., 2019. Iso-propyl stilbene: a life cycle signal?. Microbiology, 165: 516–526.
- Hassan, B., Saleem, M., Nadeem, M.A., Hassan, I., Muneer, M.A., and Rasheed, M.R., 2018. Efficacy of pesticide seed treatments against wheat aphid and its effect on coccinellid predator and crop yield. Journal of entomology and zoology studies, 6(1): 189-193.
- Hatting, J., Stock, P.S., and Hazir, S., 2009. Diversity and distribution of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae) in South Africa, Journal of Invertebrate Pathology, 102(2): 120-128, https://doi.org/10.1016/j.jip.2009.07.003.
- Hazir, S., Shapiro-Ilan, D. I., Bock, C. H., and Leite, L. G., 2018. Thermo-stability, dose effects and shelf-life of antifungal metabolite-containing supernatants produced by *Xenorhabdus szentirmaii*. European journal of plant pathology, 150(2): 297-306, https://doi.org/10.1007/s10658-017-1277-7 .
- Hazir, S., Shapiro-Ilan, D.I., Bock, C.H., Hazir, C., Leite, L.G., and Hotchkiss, M.W., 2016. Relative potency of culture supernatants of *Xenorhabdus* and *Photorhabdus* spp. on growth of some fungal phytopathogens. European Journal of Plant Pathology, 146(2): 369-381.
- Heinrich, A.K., Hirschmann, M., Neubacher, N., and Bode, H.B., 2017. LuxS-dependent AI-2 production is not involved in global regulation of natural product biosynthesis in *Photorhabdus* and *Xenorhabdus*. Peer Journal, 5: e3471, https://doi.org/10.7717/peerj.3471.

- Hertz, M., Jensen, I.R., Jensen, L., Thomsen, S.N., Winde, J., Dueholm, M.S., Sørensen, L.H., Wollenberg, R.D., Sørensen, H.O., Sondergaard, T.E., and Sørensen, J.L., 2016. The fungal community changes over time in developing wheat heads. International Journal of Food Microbiology, 222: 30-39, https://doi.org/10.1016/j.ijfoodmicro.2016.01.018.
- Houard, J., Aumelas, A., Noe, T., Givaudan, A., Fitton-Ouhabi, V., Villain-Guillot, P., and Gualtieri, M., 2013. Cabanillasin, a new antifungal metabolite, produced by entomopathogenic *Xenorhabdus cabanillasii* JM26. The Journal of Antibiotics, 66: 617–620.
- Ikbal, C., and Pavela, R., 2019. Essential oils as active ingredients of botanical insecticides against aphids. Journal of Pest Science, 92: 971–986, https://doi.org/10.1007/s10340-019-01089-6.
- Ingle, K.P., and Narkhede, G.W. 2019. In Vitro Techniques hasten the Secondary Metabolite Production in *Azadirachta Indica*. Journal of crop technology and Agricultural science, 1(2): 5-6.
- Iqbal, E.Y., Nahiyoon, A.A., Dawar, S., and Fayyaz, S., 2020. Bioremedy of Cotton Aphid (*Aphis gossypii* Glov.) (Hemiptera: Aphididae) by the Application of Different Fractions of Entomopathogenic Bacteria (*Xenorhabdus* Spp.). Pakistan Journal of Zoology, 52(3): 875-884.
- Jankielsohn, A., 2013. “Host Associations of *Diuraphis noxia* (*Homoptera*: *Aphididae*) Biotypes in South Africa”. Journal of Economic Entomology, 106(6): 2595-2601.
- Jankielsohn, A., 2016. Changes in the Russian Wheat Aphid (*Hemiptera: Aphididae*) Biotype Complex in South Africa, Journal of Economic Entomology, 109(2): 907–912.
- Jankielsohn, A., 2017. Influence of Environmental Fluctuation on the Russian Wheat Aphid Biotype Distribution in South Africa. Acta Scientific Agriculture, 1(3): 1-6.
- Jankielsohn, A., 2018. Evaluation of dryland wheat cultivars on the market in South Africa for resistance against four known rRussian wheat aphid, *Diuraphis noxia*, Biotypes in South Africa. Advances in Entomology, 7: 1-9, https://doi.org/10.4236/ae.2019.71001.
- Jankielsohn, A., 2021. Russian wheat aphid distribution in wheat production areas: consequences of management practices. D.M. Ansari (Ed.), Current Trends in Wheat Research, IntechOpen, London , http://dx.doi.org/10.5772/intechopen.96375 .

- Jighly, A., Alagu, M., Makdis, F., Singh, M., Singh, S., Emebiri, L.C., Ogbonnaya, F.C., 2016. Genomic regions conferring resistance to multiple fungal pathogens in synthetic hexaploid wheat. Molecular Breeding, 36(127), https://doi.org/10.1007/s11032-016-0541-4.
- Jimenez-Garcia, S.N., Garcia-Mier, L., Garcia-Trejo, J.F., Ramirez-Gomez, X.S., Guevara-Gonzalez, R.G., Feregrino-Perez, A.A., 2018. *Fusarium* mycotoxins and metabolites that modulate their production. In: Askun T (ed), Fusarium: plant diseases, pathogen diversity, genetic diversity, IntechOpen limited, London, page 23, http://dx.doi.org/10.5772/intechopen.72874.
- Kajla, M., Yadav, V.K., Khokhar, J., Singh, S., Chhokar, R.S., Meena, R.P., and Sharma, R.K., 2015. Increase in wheat production through management of abiotic stresses: A review. Journal of Applied and Natural Science, 7 (2): 1070 -1080.
- Kary, N.E., Mohammadi, D., Girling, R., 2017. New reports on dixenic associations between the symbionts of entomopathogenic nematodes, *Photorhabdus* and *Xenorhabdus*, and non-symbiotic bacteria. Journal of Crop Protection, 6(4) :497-511.
- Kaya, H.K., and Stock, S.P., 1997. Techniques in nematology. Lacey, L.A. (Ed.), Manual of Techniques in Insect Pathology, Academic Press, San Diego, Pages 281-324.
- Kemp, N.D., Vaughan, M.M., McCormick, S.P., Brown, J.A., and Bakker, M.G., 2020. Sarocladium zeae is a systemic endophyte of wheat and an effective biocontrol agent against Fusarium head blight. Biological Control, 149: 104329, https://doi.org/10.1016/j.biocontrol.2020.104329.
- Kepenekci, I., Hazir, S., Lewis, E.E., 2015. Evaluation of entomopathogenic nematodes and the supernatants of the in vitro culture medium of their mutualistic bacteria for the control of the root-knot nematodes *Meloidogyne incognita* and *M. arenaria*. Pest management science, 72(2): 327-334.
- Keyvan, S., 2010. The effects of drought stress on yield, relative water content, proline, soluble carbohydrates and chlorophyll of bread wheat cultivars. Journal of Animal and Plant Sciences, 8(3): 1051- 1060.
- Khanfri, S., Boulif, M., and Lahlali, R., 2018. Yellow Rust (*Puccinia striiformis*): a Serious Threat to Wheat Production Worldwide. Notulae Scientia Biologicae, 10(3): 410-423.

- Kheiri, A., Moosawi, S.A., Malihipour, A., Saremi, H., and Nikkhah, M., 2016. Application of chitosan and chitosan nanoparticles for the control of Fusarium head blight of wheat (Fusarium graminearum) in vitro and greenhouse. International Journal of Biological Macromolecules, 93(A): 1261-1272, https://doi.org/10.1016/j.ijbiomac.2016.09.072.
- Kim, J., Jaffuel, G., and Turlings, T.C.J., 2014. Enhanced alginate capsule properties as a formulation of entomopathogenic nematodes. Biological Control, 60: 527-535.
- Kirkland, L.S., Pirtle, E.I., and Umina, P.A., 2018. Responses of the Russian wheat aphid (*Diuraphis noxia*) and bird cherry oat aphid (*Rhopalosiphum padi*) to insecticide seed treatments in wheat. Crop and Pasture Science, 69: 966-973, https://doi.org/10.1071/CP18266 .
- Kisten, L., Tolmay, V.L., Mathew, I., Sydenham S.L., and Venter, E., 2020. Genome-wide association analysis of Russian wheat aphid (*Diuraphis noxia*) resistance in Dn4 derived wheat lines evaluated in South Africa. PLoS ONE, 15(12): e0244455, https://doi.org/10.1371/journal.pone.0244455.
- Knot, I. E., Zouganelis, G. D., Weedall, G. D., Wich, S. A., and Rae, R., 2020. DNA barcoding of nematodes using the MinION. Frontiers in Ecology and Evolution, 8: 100, https://doi.org/10.3389/fevo.2020.00100 .
- Kolberg, D.I., Prestes, O.D., Adaime, M.B., and Zanella, R., 2011. Development of a fast multiresidue method for the determination of pesticides in dry samples (wheat grains, flour and bran) using QuEChERS based method and GC–MS. Food chemistry, 125(4): 1436-1442, https://doi.org/10.1016/j.foodchem.2010.10.041.
- Kruve, A., Rebane, R., Kipper, K., Oldekop, M., Evard, H., Herodes, K., Ravio, P., and Leito, I., 2015. Tutorial review on validation of liquid chromatography–mass spectrometry methods: Part I. Analytica Chimica Acta, 870: 29-44, https://doi.org/10.1016/j.aca.2015.02.017.
- Lacey, L. A., and Georgis, R., 2012. Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. Journal of nematology, 44(2): 218–225.
- Lacey, L.A., Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M., and Goettel, M.S., 2015. Insect pathogens as biological control agents: Back to the future. Journal of Invertebrate Pathology, 132: 1-41.

- Lalramchuani, M., Lalramnghaki, H.C., Vanlalsangi, R., Vanramliana and Lalramliana Lalhminglian, E., 2020. Characterization and screening of antifungal activity of bacteria associated with entomopathogenic nematodes from Mizoram, North-Eastern India. Journal of Environmental Biology, 41: 942-950, http://doi.org/10.22438/jeb/4(SI)/MS_1913 .
- Larran, S., Perelló, A., and Simón, M.R., 2007. The endophytic fungi from wheat (*Triticum aestivum* L.). World Journal of Microbiology and Biotechnology, 23: 565-572, https://doi.org/10.1007/s11274-006-9266-6.
- Lecomte, C., Alabouvette, C., Edel-Hermann, V., Robert, F., and Steinberg, C., 2016. Biological control of ornamental plant diseases caused by *Fusarium oxysporum*: A review. Biological Control, 101: 17-30, https://doi.org/10.1016/j.biocontrol.2016.06.004.
- Levene, H., 1960. Robust test for equality of variances. In Olkin, I. (Ed.), Contributions to probability and statistics. Stanford, Calif.: Stanford University Press, 278–292.
- Li, A., Liu, D., Yang, W., Kishii, M., and Mao, L., 2018. Synthetic Hexaploid Wheat: Yesterday, Today, and Tomorrow. Engineering, 4(4): 552-558, https://doi.org/10.1016/j.eng.2018.07.001.
- Li, J., Chen, G., Webster, J. M., and Czyzewska, E., 1995. Antimicrobial metabolites from a bacterial symbiont. Journal of Natural Products, 58: 1081-1086.
- Li, J., Duan, Y., Bian, C., Pan, X., Yao, C., Wang, J., and Zhou, M., 2019. Effects of validamycin in controlling *Fusarium* head blight caused by *Fusarium graminearum*: Inhibition of DON biosynthesis and induction of host resistance. Pesticide Biochemistry and Physiology, 153: 152-160, https://doi.org/10.1016/j.pestbp.2018.11.012.
- Li. R., Wang, R., Xie, C., Liu, L., Zhang, J., Wang, F., and Liu, W., 2019. A coarse-to-fine network for aphid recognition and detection in the field. Biosystems Engineering, 187: 39-52.
- Lössl, P., van de Waterbeemd, M., and Heck, A., 2016. The diverse and expanding role of mass spectrometry in structural and molecular biology. The EMBO Journal, 35: 2634-2657, https://doi.org/10.15252/embj.201694818.
- Lu, D., Baiocchi, T., Dillman, A.R., 2016. Genomics of entomopathogenic nematodes and implications for pest control. Trends Parasitolgy, 32(8): 588-598, https://doi.org/10.1016/j.biocontrol.2021.104662.

- Lu, D., Sepulveda, C., Dillman A.R., 2017. Infective juveniles of the entomopathogenic nematode *Steinernema scapterisci* are preferentially activated by cricket tissue. PLoS ONE, 12(1): e0169410, https://doi.org/10.1371/journal.pone.0169410.
- Lu, S., and Edwards, M.C., 2018. Molecular Characterization and Functional Analysis of PR-1-Like Proteins Identified from the Wheat Head Blight Fungus *Fusarium graminearum*. The American Phytopathological Society, 108(4): 424-435, https://doi.org/10.1094/PHYTO-07-17-0260-RVW.
- Luna, E., van Eck, L., Campillo, T., Weinroth, M., Metcalf, J., Perez-Quintero, A.L., Botha, A., Thannhauser, T.W., Pappin, D., Tisserat, N.A., Lapitan, N.L.V., Argueso, C.T., Ode, P.J., Heck, M.L., and Leach, J.E., 2018. Bacteria Associated with Russian Wheat Aphid (*Diuraphis noxia*) Enhance Aphid Virulence to Wheat. Phytobiomes, 2(3): 151- 164.
- Mahar, A. N., Munir, M., Elawad, S., Gowen, S. R., and Hague, N. G., 2005). Pathogenicity of bacterium, *Xenorhabdus nematophila* isolated from entomopathogenic nematode (*Steinernema carpocapsae)* and its secretion against *Galleria mellonella* larvae. Journal of Zhejiang University. Science. B, 6(6): 457–463. https://doi.org/10.1631/jzus.2005.B0457 .
- Manstretta, V., and Rossi, V., 2015. Effects of Weather Variables on Ascospore Discharge from *Fusarium graminearum* Perithecia. PLoS ONE, 10(9): e0138860, https://doi.org/10.1371/journal.pone.0138860.
- Martens, L., Chambers, M., Sturm, M., Kessner, D., Levander, F., Shofstahl, J., Tang, W.H., Rompp, A., and Neumann, S., 2011. mzML- a Community Standard for Mass Spectrometry Data. Molecular and Cellular Proteomics, 10(1), doi:10.1074/mcp.M111.014381, https://doi.org/10.1074/mcp.R110.000133.
- Matarese, F., Sarrocco, S., Gruber, S., and Seidl-Seiboth, V., and Vannacci, G., 2012. Biocontrol of *Fusarium* head blight: interactions between *Trichoderma* and mycotoxigenic *Fusarium*. Microbiology, 158: 98-106.
- Matny, O.N., 2015. *Fusarium* head blight and crown rot on wheat & barley: losses and health risks. Advances in Plants and Agriculture Research, 2(1): 38–43.
- Meher, M. K., Bhangale, C., Dholas, R., Prashant, V. A., and Pawar, R., 2018. Analytical method development, validation and forced degradation studies of lansoprazole by RP-HPLC. Asian Journal of Pharmaceutical Research and Development, 6(4): 21-29, https://doi.org/10.22270/ajprd.v6i4.387 .

- Mesquita, A. L. M., Lacey, L. A., and Leclant, F., 1997. Individual and combined effects of the fungus, Paecilomyces fumosoroseus and parasitoid, Aphelinus asychis Walker (Hym., Aphelinidae) on confined populations of Russian wheat aphid, *Diuraphis noxia* (Mordvilko)(Hom., *Aphididae*) under field conditions. Journal of Applied Entomology, 121(1-5): 155-163, https://doi.org/10.1111/j.1439-0418.1997.tb01386.x .
- Minnaar-Ontong, A., Herselman, L., and Kriel, WM., 2017. Morphological characterization and trichothecene genotype analysis of a *Fusarium* Head Blight population in South Africa. European Journal of Plant Pathology, 148: 261–269, https://doi.org/10.1007/s10658-016-1085-5.
- Miransari, M., and Smith, D., 2019. Sustainable wheat (*Triticum aestivum* L.) production in saline fields: a review. Critical Reviews in Biotechnology, 39(8): 999-1014, https://doi.org/10.1080/07388551.2019.1654973.
- Mollah, M.M.I., Kim, Y., 2020. Virulent secondary metabolites of entomopathogenic bacteria genera, *Xenorhabdus* and *Photorhabdus*, inhibit phospholipase A2 to suppress host insect immunity. BMC Microbiology, 20:359, https://doi.org/10.1186/s12866-020-02042-9.
- Motholo, L. F., Booyse, M., Tsilo, T. J., Hatting, J. L., & Thekisoe, O. M. M. (2020). Pathogenicity of *Beauveria bassiana* (Hypocreales: *Cordycipitaceae*) against the Russian wheat aphid, *Diuraphis noxia* (Hemiptera: *Aphididae*). African Entomology, 28(2): 455-461, https://hdl.handle.net/10520/EJC-203c35959f .
- Moureaux, N., Karjalainen, T., Alain Givaudan, A., Bourlioux, P., and Boemare, N., 1995. Biochemical Characterization and Agglutinating Properties of *Xenorhabdus nematophilus* F1 Fimbriae. Applied and Environmental Microbiology, 61(7): 2707-2712.
- Muangpat P., Yooyangket T., Fukruksa C., Suwannaroj M., Yimthin T., Sitthisak S., Chantratita N., Vitta A., Tobias N.J., Bode H.B., Thanwisai A., 2017. Screening of the Antimicrobial Activity against Drug Resistant Bacteria of *Photorhabdus* and *Xenorhabdus* Associated with Entomopathogenic Nematodes from Mae Wong National Park, Thailand. Frontiers Microbiology, 8: 1133-1142.
- Muriithi, B.W., Affognon, H.D., Diiro, G.M., Kingori, S.W., Tanga, C.M., Nderitu, P.W., Mohamed, S.A, Ekesi, S., 2016. Impact assessment of Integrated Pest

Management (IPM) strategy for suppression of mango-infesting fruit flies in Kenya. Crop Protection, 81: 20-29, https://doi.org/10.1016/j.cropro.2015.11.014.

- Nhemachena, C.R., and Kirsten, J., 2017. A historical assessment of sources and uses of wheat varietal innovations in South Africa. South African Journal of Science, 113: 1-8.
- Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., and Hens, L., 2016. Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. Frontiers in Public Health, 4: 148-161, https://www.frontiersin.org/article/10.3389/fpubh.2016.00148.
- Noda, M., Fukui, K., and Uchiyama, S., 2016. Mass Spectrometry. In: Senda T., Maenaka K. (eds), Advanced Methods in Structural Biology, Springer Protocols Handbooks, Springer, Tokyo, https://doi.org/10.1007/978-4-431-56030-2_11.
- Nowrin, F., Yesmin, M.S., and Sultana, N., 2018. Entomopathogenic nematode: an effective bio control agent for insect. Eco-friendly Agricultural Journal, 11(11): 119-125.
- Ntushelo, K., Ledwaba, L.K., Rauwane, M.E., Adebo, O.A., and Njobeh, P.B., 2019. The Mode of Action of Bacillus Species against *Fusarium graminearum*, Tools for Investigation, and Future Prospects. Toxins, 11(10): 606-615, https://doi.org/10.3390/toxins11100606.
- O'Brien, P.A., 2017. Biological control of plant diseases. Australasian Plant Pathology, 46: 293–304.
- O'Connor, S.E., 2015. Engineering of Secondary Metabolism. Annual Review of Genetics, 49: 71–94.
- Orozco, R. A., Molnár, I., Bode, H., and Stock, S. P. (2016). Bioprospecting for secondary metabolites in the entomopathogenic bacterium *Photorhabdus luminescens* subsp. *sonorensis*. Journal of invertebrate pathology, 141: 45-52, https://doi.org/10.1016/j.jip.2016.09.008 .
- Parasuraman, S., R, A., Balamurugan, S., Muralidharan, S., Kumar, K.J., and Vijayan, V., 2014. An Overview of Liquid Chromatography-Mass Spectroscopy Instrumentation. Pharmaceutical Methods, 5(2): 47-55.
- Pardey, P.G., 2011. A strategic look at global wheat production, productivity and R&D developments. Czech Journal of Genetics and Plant Breeding, 47(1): 9-19.

- Park, H.B., and Crawford, J.M., 2015. Lumiquinone A, an α-Aminomalonate-Derived Aminobenzoquinone from *Photorhabdus luminescens*.journal of natural products, 78: 1437-1441.
- Park, H.B., Sampathkumar, P., Perez, C.E., Hallem, E.A., Almo, S.C., and Crawford, J.M., 2017. Stilbene epoxidation and detoxification in a *Photorhabdus luminescens*-nematode symbiosis. Microbiology, 292(16): 6680-6694, https://doi.org/10.1074/jbc.M116.762542.
- Park, Y., Kang, S., Sadekuzzaman, M., Kim, H., Jung, J., Kim, Y., 2017. Identification and bacterial characteristics of *Xenorhabdus hominickii* ANU101 from an entomopathogenic nematode, *Steinernema monticolum*. Journal of Invertebrate Pathology, 144: 74-87.
- Paul, P.A., Bradley, C.A., Madden, L.V., Lana, F.D., Bergstrom, G.C., Dill-Macky, R., Wise, K.A., Esker, P.D., Grybauskas, A., Kirk, W.W., Milus, E., and Ruden, K., 2018. Effects of Pre- and Postanthesis Applications of Demethylation Inhibitor Fungicides on *Fusarium* Head Blight and Deoxynivalenol in spring and Winter Wheat. Plant Disease, 102: 2500-2510.
- Platt, T., Stokwe, N. F., and Malan, A. P., 2018. Potential of Local Entomopathogenic Nematodes for Control of the Vine Mealybug, *Planococcus ficus*. South African Journal for Enology and Viticulture, 39(2): 1-8, https://doi.org/10.21548/39-2-3158.
- Platt, T., Stokwe, N.F., Malan, A.P., 2019. Foliar application of *Steinernema yirgalemense* to control *Planococcus ficus*: assessing adjuvants to improve efficacy. South African Journal for Enology and Viticulture, 40 (1): 1-7.
- Pleadin, J., Vahčić, N., Perši, N., Ševelj, D., Markov, K., & Frece, J., 2013 . *Fusarium* mycotoxins' occurrence in cereals harvested from Croatian fields. Food control, 32(1): 49-54, https://doi.org/10.1016/j.foodcont.2012.12.002 .
- Poinar, G.O.J., 1975. Description and biology of a new insect parasitic rhabditoid *heterorhabditis-bacteriophora* new-genus new-species *rhabditida heterorhabditidae* new family. Nematologica, 21: 463–470.
- Poudel, P.B., and Poudel, M.R., 2020. Heat Stress Effects and Tolerance in Wheat: A Review. Journal of Biology and Today's World, 9(3): 202- 217.
- Powell, A.J, and Vujanovic, V., 2021. Evolution of *Fusarium* Head Blight Management in Wheat: Scientific Perspectives on Biological Control Agents and Crop Genotypes

Protocooperation. Applied Sciences, 11(19): 8951-8960, https://doi.org/10.3390/app11198960.

- Qi, P., Balcerzak, M., Rocheleau, H., Leung, W., Wei, Y., Zheng, Y., and Ouellet, T., 2016. Jasmonic acid and abscisic acid play important roles in host–pathogen interaction between *Fusarium graminearum* and wheat during the early stages of *Fusarium* head blight. Physiological and Molecular Plant Pathology, 93: 39-48.
- Qi, T., Guo, J., Peng, H., Liu, P., Kang, Z., and Guo, J., 2019. Host-Induced Gene Silencing: A Powerful Strategy to Control Diseases of Wheat and Barley. International Journal of Molecular Science, 20(1): 196-206, https://doi.org/10.3390/ijms20010206.
- Qiu, H., Zhao, X, Fang, W., Wu, H., Abubakar, Y.S., Lu, G., Zonghua Wang, Z., and Zheng, W., 2019. Spatiotemporal nature of *Fusarium graminearum*-wheat coleoptile interactions. Phytopathology Research, 1(26), https://doi.org/10.1186/s42483-019-0033-7.
- Quinn, L.P., Fernandes-Whaley, M., Roos, C., Bouwman, H., and Kylin, H., 2011. Pesticide use in South Africa: One of the largest importers of pesticides in Africa. In pesticides I the modern world- pesticides use and management, Stoycheva, M.,(ed), Rijeka, Croatia, InTech, 1: 459-507.
- Ray, M., Ray, A., Dash, S., Mishra, A.K., Achary, G., Nayak, S., and Singh, S., 2017. Fungal disease detection in plants: Traditional assays, novel diagnostic techniques and biosensors. Biosensors and Bioelectronics, 87: 708-723, https://doi.org/10.1016/j.bios.2016.09.032.
- Saeidi, M., and Abdoli, M., 2015. Effect of Drought Stress during Grain Filling on Yield and Its Components, Gas Exchange Variables, and Some Physiological Traits of Wheat Cultivars. Journal of Agricultural Science and Technology, 17 (4): 885-898, http://jast.modares.ac.ir/article-23-6741-en.html.
- Sahu, P. K., Ramisetti, N. R., Cecchi, T., Swain, S., Patro, C. S., and Panda, J., 2018. An overview of experimental designs in HPLC method development and validation. Journal of pharmaceutical and biomedical analysis, 147: 590-611, https://doi.org/10.1016/j.jpba.2017.05.006 .
- Sajnaga, E., and Kazimierczak, W., 2020. Evolution and taxonomy of nematode-associated entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus*: an overview. Symbiosis, 80: 1–13. https://doi.org/10.1007/s13199-019-00660-0.

- Scarpino, V., Reyneri, A., Sulyok, M., Krska, R., and Blandino, M. 2015. Effect of fungicide application to control *Fusarium* head blight and 20 *Fusarium* and Alternaria mycotoxins in winter wheat (*Triticum aestivum* L.). World Mycotoxin Journal, 8(4): 499-510.
- Schisler, D.A., Slininger, P.J., Boehm, M.J., and Paul, P.A., 2011. Co-culture of yeast antagonists of *Fusarium* head blight and their effect on disease development in wheat. Plant Pathology Journal, 10: 128-137.
- Shah, F.M., Razaq, M., Ali, A., Han, P., and Chen, J., 2017. Comparative role of neem seed extract, moringa leaf extract and imidacloprid in the management of wheat aphids in relation to yield losses in Pakistan. Plos One, 12(9): e0184639.
- Shahidi, F., and Yeo, J., 2018. Bioactivities of Phenolics by Focusing on Suppression of Chronic Diseases: A Review. International Journal of Molecular Sciences, 19(6): 1573-1586, https://doi.org/10.3390/ijms19061573.
- Shan, S., Ma, H., Li, Y., Gu, X., Jiang, Z., Sun, B., Chen, C., Wei, X., Shen, G., Shapiro-Ilan, D., and Ruan, W., 2020. Metabolites from symbiotic bacteria of entomopathogenic nematodes have antimicrobial effects against *Pythium myriotylum*. European Journal of Plant Pathology, 158: 35–44, https://doi.org/10.1007/s10658-020-020
- Shan, S., Wang, W., Song, C., Wang, M., Sun, B., Li, Y., Fu, Y., Gu, X., Ruan, W., and Rasmann, S., 2019. The symbiotic bacteria *Alcaligenes faecalis* of the entomopathogenic nematodes *Oscheius spp*. exhibit potential biocontrol of plant- and entomopathogenic fungi. Microbial Biotechnology, 12(3): 1751-7915, https://doi.org/10.1111/1751-7915.13365.
- Shapiro-Ilan, D., Hazir, S., and Glazer, I., 2017. Chapter 6 - Basic and Applied Research: Entomopathogenic Nematodes. Microbial Control of Insect and Mite Pests, 91-105, https://doi.org/10.1016/B978-0-12-803527-6.00006-8.
- Sharma, K., Walia, S., Ganguli, S., and Kundu, A., 2016. Analytical characterization of secondary metabolites from indian *Xenorhabdus* species the symbiotic bacteria of entomopatathogenic nematode (*Steinernema* spp.) as antifungal agent. National Academy Science Letters, 39: 175-180, https://doi.org/10.1007/s40009-016-0453-1 .
- Shavit, R., Batyrshina, Z.S., Dotan, N., and Tzin, V., 2018. Cereal aphids differently affect benzoxazinoid levels in durum wheat. PloS one, 13(12): e0208103. https://doi.org/10.1371/journal.pone.0208103.

- Shewry, P.R., 2009. Wheat. Journal of Experimental Botany, 60(6): 1537–1553, https://doi.org/10.1093/jxb/erp058.
- Shewry, P.R., and Hey, S.J., 2015. The contribution of wheat to human diet and health. Food and Energy Security, 4(3): 2048-3694, https://doi.org/10.1002/fes3.64.
- Shi, H., Zeng, H., Yang, X., Zhao, J., Chen, M., and Qiu, D., 2012. An insecticidal protein from *Xenorhabdus ehlersii* triggers prophenoloxidase activation and hemocyte decrease in *Galleria mellonella*. Current Microbiology, 64: 604–610.
- Shude, S.P.N., Yobo, K.S., and Mbili, N.C., 2020. Progress in the management of *Fusarium* head blight of wheat: An overview. South African Journal of Science, 116 (11/12), https://doi.org/10.17159/sajs.2020/7854.
- Simon, J.C, and Peccoud, J., 2018. Rapid evolution of aphid pests in agricultural environments. Current Opinion in Insect Science, 26: 17–24, https://doi.org/10.1016/j.cois.2017.12.009.
- Singh, .RP., Hodson, D.P., Jin, Y., Lagudah, E.S., and Ayliffe, M.A., 2015. Emergence and spread of new races of wheat stem rust fungus: continued threat to food security and prospects of genetic control. Phytopathology, 105: 872- 84, https://doi.org/10.1094/PHYTO-01-15-0030-FI .
- Smigielski, A. J., Akhurst, R. J., and Boemare, E. N. E., 1994. Phase variation in *Xenorhabdus nematophilus* and *Photorhabdus luminescens*: Differences in respiratory activity and membrane energization. Applied and Environmental Microbiology, 60: 120-125.
- Snedecor, G.W., and Cochran, W., 1980. Statistical methods. 7th edition. Iowa State University Press, Ames. 507pp.
- Sosibo, N.Z., Muchaonyerwa, P., Visser, L., Barnard, A., Dube, E., and Tsilo T.J., 2017. Soil fertility constraints and yield gaps of irrigation wheat in South Africa. South African Journal of Science, 113: 1–2.
- South African Department of Agriculture, Forestry and Fisheries (DAFF). Agricultural statistics. Pretoria: DAFF; 2014.
- Starý, P., 1999. Parasitoids and biocontrol of Russian wheat aphid, *Diuraphis noxia* (Kurdj.) expanding in central Europe. Journal of Applied Entomology, 123: 273-280. https://doi.org/10.1046/j.1439-0418.1999.00348.x.

- Stock, S.P., 2019. Partners in crime: symbiont-assisted resource acquisition in *Steinernema* entomopathogenic nematodes. Current Opinion in Insect Science, 32: 22-27. https://doi.org/10.1016/j.cois.2018.10.006.
- Su, Z., Bernardo, A., Tian, B., Chen, H., Wang, S., Ma, H., Cai, S., Liu, D., Zhang, D., Li, T., Trick, H., Amand, P., Yu, J., Zhang, Z., and Bai, G., 2019. A deletion mutation in TaHRC confers Fhb1 resistance to *Fusarium* head blight in wheat. Nature Genetics, 51: 1099–1105.
- Tadesse, W., Bishaw, Z., and Assefa, S., 2019. Wheat production and breeding in Sub-Saharan Africa: Challenges and opportunities in the face of climate change. International Journal of Climate Change Strategies and Management, 11(5): 696-715, https://doi.org/10.1108/IJCCSM-02-2018-0015.
- Tautenhahn, R., Böttcher, C. and Neumann, S., 2008. Highly sensitive feature detection for high resolution LC/MS. BMC Bioinformatics, 9: 504, https://doi.org/10.1186/1471-2105-9-504.
- Thammana, M., 2016. A review on high performance liquid chromatography (HPLC). Research & Reviews: Journal of Pharmaceutical Analysis, 5(2): 22-28.
- Thomson, J.J., 1897. Cathode rays. Philosophical Magazine, 44: 293–316.
- Thomson, J.J., 1911. Rays of positive electricity. Philosophical Magazine, 21: 225–249.
- Tini, F., Beccari, G., Onofri, A., Ciavatta, E., Gardiner, D.M. and Covarelli, L., 2020. Fungicides may have differential efficacies towards the main causal agents of *Fusarium* head blight of wheat. Pest Management Science, 76: 3738-3748, https://doi.org/10.1002/ps.5923.
- Torres, A.M., Palacios, S.A., Yerkovich, N., Palazzini, J.M., Battilani, P., Leslie, J.F., Logrieco, A.F., and Chulze, S.N., 2019. *Fusarium* head blight and mycotoxins in wheat: prevention and control strategies across the food chain.World Mycotoxin Journal, 12(4): 333-355.
- Tulpová, Z.,Toegelová, H., Lapitan, N.L.V., Peairs, F.B., Macas, J., Novák, P., Lukaszewski, A.J., Kopecký, D., Mazáčová, M., Vrána, J., Holušová, K., Leroy, P., Doležel, J., and Šimková, H. 2019. Accessing a Russian Wheat Aphid Resistance Gene in Bread Wheat by Long-Read Technologies. Plant Genome 12: 180065.

- Ullah, A., Nadeem, F., Nawaz, A., Siddique, K. H. M., and Farooq, M., 2022. Heat stress effects on the reproductive physiology and yield of wheat. Journal of Agronomy and Crop Science, 208: 1– 17, https://doi.org/10.1111/jac.12572.
- Ullah. I., Khan, A.L., Ali, L., Khan, A.R., Waqas, M., Hussain, J., Lee, I.J., and Shin, J.H., 2015. Benzaldehyde as an insecticidal, antimicrobial, and antioxidant compound produced by *Photorhabdus temperata* M1021. The Journal of Microbiology, 53: 127–133.
- Urban, P.L., 2016. Quantitative mass spectrometry: an overview. Philosophical Transactions of the Royal Society A, 374: 20150382, http://dx.doi.org/10.1098/rsta.2015.03.
- Valletta, A., Iozia, L.M., and Leonelli, F., 2021. Impact of Environmental Factors on Stilbene Biosynthesis. Plants, 10(1): 90- 101, https://doi.org/10.3390/plants10010090.
- Van der Lee, T., Zhang, H., Van Diepeningen, A., and Waalwijk, C., 2015. Biogeography of *Fusarium graminearum* species complex and chemotypes: a review. Food Additives & Contaminants: Part A, 32:453-460.
- Van Lenteren, J.C., Bolckmans, K., Ko¨hl, J., Ravensberg, W.J., and Urbaneja, A., 2017. Biological control using invertebrates and microorganisms: plenty of new opportunities. Biological Control, 63: 39–59, https://doi.org/10.1007/s10526-017-9801-4.
- Van Rijn, P.C.J., and Sabelis, M.W., 2005. Plant-provided food for carnivorous insects: A protective mutualism and its applications. (Eds.) F.L Wäckers, P.C.J van Rijn and J Bruin, 223-266, Cambridge University Press, Cambridge.
- Wachowska, U., and Glowacka, K., 2014. Antagonistic interactions between *Aureobasidium pullulans* and *Fusarium culmorum*, a fungal pathogen of wheat. BioControl, 59: 635-645.
- Wang, H., Dong, H., Qian, H., and Cong, B., 2014. Laboratory assessment of entomopathogenic nematode symbiotic bacteria to control maize pest, *Ostrinia furnacalis*, and fungi diseases, *Bipolaris maydis* and *Curvularia lunata*. Journal of Asia-Pacific Entomology, 17(4): 823-827, https://doi.org/10.1016/j.aspen.2014.08.002.
- Wang, L., Xie, Y., Cui, Y., Xu, J, He, W., Chen, H., and Guo, J., 2015. Conjunctively screening of biocontrol agents (BCAs) against *Fusarium* root rot and *Fusarium* head

blight caused by *Fusarium graminearum*. Microbiological Research, 177: 34-42, https://doi.org/10.1016/j.micres.2015.05.005.

- Wegulo, S., Baenziger, P., Nopsa, J., Bockus, W.W., and Hallen-Adams, H., 2015. Management of *Fusarium* head blight of wheat and barley. Crop Protection, 73: 100-107, https://doi.org/10.1016/j.cropro.2015.02.025 .
- Wollenberg, A. C., Jagdish, T., Slough, G., Hoinville, M. E., and Wollenberg, M. S., 2016. Death becomes them: bacterial community dynamics and stilbene antibiotic production in cadavers of *Galleria mellonella* killed by *Heterorhabditis* and *Photorhabdus* spp. Applied and environmental microbiology, 82(19): 5824-5837, https://doi.org/10.1128/AEM.01211-16 .
- Xiao, J., Chen, L., Pan, F., Deng, Y., Ding, C., Liao, M., Su, X. and Cao, H., 2020. Application method affects pesticide efficiency and effectiveness in wheat fields. Pest Management Science, 76: 1256-1264, https://doi.org/10.1002/ps.5635.
- Xue, A.G., Chen, Y.H., Sant'anna, S.M.R., Voldeng, H.D., Fedak, G., Savard, M.E., Längle, T., Zhang, J.X., and Harman, G.E., 2014. Efficacy of CLO-1 biofungicide in suppressing perithecial production by *Gibberella zeae* on crop residues. Canadian Journal of Plant Pathology, 36(2): 161-169, https://doi.org/10.1080/07060661.2014.881920 .
- Yahya, M., Saeed, N.A., Nadeem, S., Hamed, M., and Shokat, S., 2017. Role of Wheat Varieties and Insecticide Applications against Aphids for Better Wheat Crop Harvest. Pakistan Journal of Zoology, 49(6): 2217-2225, http://dx.doi.org/10.17582/journal.pjz/2017.49.6.2217.2225 .
- Yang, E., Kim, S., Lee, K., and Song, K., 2018. Neuroprotective and Anti Neuroinflammatory Activities of Anthraquinones Isolated from *Photorhabdus temperata* Culture Broth. Journal of Microbiology and Biotechnology, 28(1): 12-21, https://doi.org/10.4014/jmb.1708.08067.
- Yang, X., Qiu, D., Yang, H., and Liu, Z., 2011. Antifungal activity of xenocoumacin 1 from *Xenorhabdus nematophilus* var. *pekingensis* against *Phytophthora infestans*. World Journal of Microbiology and Biotechnology, 27: 523-528, https://doi.org/10.1007/s11274-010-0485-5 .
- Yashin, Y.I., and Yashin, A.Y., 2012. Ya.I. Chapter 10 - Liquid Chromatography. Yolanda Picó (Ed), Chemical Analysis of Food: Techniques and Applications, Academic Press, 285-310, https://doi.org/10.1016/B978-0-12-384862-8.00010-8.

- Yimthin, T., Fukruksa, C., Muangpat, P., Dumidae, A., Wattanachaiyingcharoen, W., and Vitta, A., 2021. A study on *Xenorhabdus* and *Photorhabdus* isolates from Northeastern Thailand: Identification, antibacterial activity, and association with entomopathogenic nematode hosts. PLoS ONE, 16(8): e0255943, https://doi.org/10.1371/.
- Yooyangket T., Muangpat P., Polseela R., Tandhavanant S., Thanwisai A., and Vitta A., 2018. Identification of entomopathogenic nematodes and symbiotic bacteria from Nam Nao National Park in Thailand and larvicidal activity of symbiotic bacteria against *Aedes aegypti* and *Aedes albopictus*. PLoS ONE, 13(4): e0195681, https://doi.org/10.1371/journal.pone.0195681 .
- Zhang, P., Zhang, X., Zhao, Y., Wei, Y., Mu, W., and Liu, F., 2016. Effects of imidacloprid and clothianidin seed treatments on wheat aphids and their natural enemies on winter wheat. Pest Management Science, 72: 1141-1149, https://doi.org/10.1002/ps.4090.
- Zhang, S., Liu, Q., Han, Y., Han, J., Yan, Z., Wang, Y., and Zhang X., 2019. Nematophin, an antimicrobial dipeptide compound from *Xenorhabdus nematophila* YL001 as a potent biopesticide for *Rhizoctonia solani* Control. Frontiers in Microbiology, 10: 1765, https://www.frontiersin.org/article/10.3389/fmicb.2019.01765.
- Zhao, Y., Sui, X., Xu, L., Liu, G., Lu, L., You, M., Xie, C., Li, B., Ni, Z., and Liang, R., 2018. Plant-mediated RNAi of grain aphid CHS1 gene confers common wheat resistance against aphids. Pest Management Science, 74(12): 2754-2760, https://doi.org/10.1002/ps.5062 .

Appendix A

UNISA university of south africa

UNISA-CAES HEALTH RESEARCH ETHICS COMMITTEE

Date: 09/11/2020

Dear Mr Kgosiemang

Decision: Ethics Approval from 05/11/2020 to 31/10/2023

NHREC Registration # : REC-170616-051
REC Reference # : 2020/CAES_HREC/140
Name : Mr JL Kgosiemang
Student #: 65834305

Researcher(s): Mr JL Kgosiemang
kgosiemangj@arc.agric.za

Supervisor (s): Dr T Ramakuwela
ramakuwelat@arc.agric.za; 058-307-3455

Dr S Figlan
figlas@unisa.ac.za; 011-379-3193

Working title of research:

Isolation and identification of entomopathogenic nematode bacterial metabolites for biological control of Fusarium head blight of wheat and wheat aphids

Qualification: MSc Agriculture

Thank you for the application for research ethics clearance by the Unisa-CAES Health Research Ethics Committee for the above mentioned research. Ethics approval is granted for three years, **subject to submission of yearly progress reports. Failure to submit the progress report will lead to withdrawal of the ethics clearance until the report has been submitted.**

The researcher is cautioned to adhere to the Unisa protocols for research during Covid-19.

Due date for progress report: 31 October 2021

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
PO Box 392 UNISA 0003 South Africa
Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150
www.unisa.ac.za

*The **low risk application** was **reviewed** by the UNISA-CAES Health Research Ethics Committee on 05 November 2020 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.*

The proposed research may now commence with the provisions that:

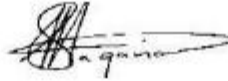
1. The researcher will ensure that the research project adheres to the relevant guidelines set out in the Unisa Covid-19 position statement on research ethics attached.
2. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.
3. Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study should be communicated in writing to the Committee.
4. The researcher(s) will conduct the study according to the methods and procedures set out in the approved application.
5. Any changes that can affect the study-related risks for the research participants, particularly in terms of assurances made with regards to the protection of participants' privacy and the confidentiality of the data, should be reported to the Committee in writing, accompanied by a progress report.
6. The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study. Adherence to the following South African legislation is important, if applicable: Protection of Personal Information Act, no 4 of 2013; Children's act no 38 of 2005 and the National Health Act, no 61 of 2003.
7. Only de-identified research data may be used for secondary research purposes in future on condition that the research objectives are similar to those of the original research. Secondary use of identifiable human research data require additional ethics clearance.
8. No field work activities may continue after the expiry date. Submission of a completed research ethics progress report will constitute an application for renewal of Ethics Research Committee approval.

Note:

*The reference number **2020/CAES_HREC/140** should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.*

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
PO Box 392 UNISA 0003 South Africa
Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150
www.unisa.ac.za

Yours sincerely,



Prof MA Antwi
Chair of UNISA-CAES Health REC
E-mail: antwima@unisa.ac.za
Tel: (011) 670-9391

Prof SR Magano
Acting Executive Dean : CAES
E-mail: magansr@unisa.ac.za
Tel: (011) 471-3649

Appendix B

Artificial feed for great wax moth (*galleria mellonella*)

Dry diet ingredients (neonates feed)

Wheat flour (445g), dry yeast powder (111g), honey (250ml), glycerol (222.5g), and wheat bran (222.5g).

Method

Mix dry ingredients first (wheat flour, wheat bran and yeast powder), then add liquid components (honey and glycerol). After mixing well, pour off the dry diet mixture into glass jars for larval feeding.

Wet diet ingredients (instar larvae)

Wheat flour (130g), wheat bran (130g), soy milk powder (130g), dry yeast powder (97.5g), beeswax (26g), honey (195 ml) and glycerol (195 ml).

Method

Mix dry ingredients first (wheat flour, wheat bran, yeast powder, soy milk power and beeswax), then add liquid components (honey and glycerol). After mixing well, pour off the wet diet mixture into glass jars for larval feeding.

Appendix C

UNIVERSITY OF SOUTH AFRICA COVID-19 GUIDELINES: IMPLICATIONS OF ALERT LEVELS FOR RESEARCHERS AND POSTGRADUATE STUDENTS

Issued by: Prof T Meyiwa, Vice-Principal: Research, Postgraduate Studies, Innovation and Commercialisation

Date: 26 June 2020

Version 2.0

This guideline document is complementary to the Unisa Covid-19 position statement on research ethics, dated 8 April 2020, and the Unisa Covid-19 guidelines for ERCs, dated 28 April 2020. The document aims to provide ERCs, researchers and postgraduate students with practical guidance on the implications of the alert levels on research to limit the transmission of the coronavirus and reduce the risk for both the researcher and the research participants.

Unisa supports the continuation of research activities, where possible, and researchers are advised to follow national regulations and adhere to the restrictions imposed by the various alert levels. ERCs, researchers and postgraduate supervisors must consider the potential risks of harm to research participants, communities, researchers, postgraduate students and the university relating to the collection of data during this time.

1. **The potential risks of harm to the researcher/postgraduate student/members of research teams relating to Covid-19**

   1.1 Researchers, postgraduate students, and members of research teams might become infected with Covid-19 when:
      1.1.1 They come in contact with an asymptomatic/symptomatic person.
      1.1.2 They are handling objects contaminated by the virus.
      1.1.3 They are entering a high-risk/hotspot Covid-19 area.

   1.2 The aforementioned actions pose a risk of transmitting Covid-19 to their families and subsequently to the community.

   1.3 They could be fined or arrested for violating lockdown laws or appropriate lockdown level restrictions, such as:
      - visiting participants when social contact is restricted, and
      - travelling between provinces without the necessary permits.

   1.4 Researchers, postgraduate students, and members of research teams that are above 60, and have co-morbidities, could put their health in jeopardy when they collect data by means of face-to-face activities.

2. **The potential risks of harm to the University**

   Potential reputational harm to the University relating to risks of real or perceived non-adherence to governmental directives by staff, postgraduate students and members of research teams, as a result of:

2.1 Claims that the participants contracted Covid-19 due to their interaction with researchers or members of a research team.
2.2 Claims that researchers, postgraduate students, or members of research teams contracted the virus during the conduct of the study.

3. **The implications of the alert levels for researchers**:

3.1 The government is following a risk-adjusted approach based on the following criteria:
   3.1.1 The level of the infection rate;
   3.1.2 The rate of transmission;
   3.1.3 The capacity of health facilities;
   3.1.4 The extent of public health intervention;
   3.1.5 The economic and social impact of continued restrictions.
3.2 The restrictions for the alert levels are set out below. The current Covid-19 context is dynamic, and the implications below could change in tandem with adjustments to governmental directives.
3.3 Consider whether your data collection methods can be adapted considering the national restrictions.
3.4 Should researchers choose to adjust their data collection methods (for instance to electronic interviews), these amendments must be scientifically justifiable.
3.5 Requests for amendments must be submitted for approval by the relevant ERC.
3.6 In principle, any adjustments to data collection methods should limit the risks to researchers of breaking national Covid-19 regulations or putting the researcher at risk of contracting or spreading the virus.
3.7 Postgraduate students must consult their supervisors in relation to possible changes to data collection methods.
3.8 Issues of connectivity, online accessibility and data costs should be considered when switching to online means of data collection.

Alert levels	*Implications for researchers and postgraduate students*
Alert level 5: Drastic measures to contain the spread of the virus and save lives. Higher Education under total lockdown with only essential services allowed on campus.	○ No research activities e.g., laboratory work or contact with human participants. ○ Only critical clinical research as part of patient care or treatment and vaccine trial research or laboratory work linked to Covid-19 research. ○ Telephone and/or online platform interaction with human participants. ○ Online quantitative research, e.g., surveys.
Alert level 4: Extreme precautions to limit community transmission and outbreaks while allowing some limited activity. Higher Education under total lockdown with only essential services allowed on campus.	○ Essential laboratory work with a Unisa permit. ○ No research that requires physical human participant interactions in close proximity e.g. face-to-face interviews; focus groups or human sample collection (excluding clinical research as permitted by national guidelines and relevant Unisa permit). ○ Telephone and/or online platform interaction with human participants. ○ Online quantitative research e.g., surveys.

Alert level 3: Restrictions on many activities including workplaces and socially, to address a high risk of transmission. Maximum of 33% of students return to campus. Controlled return of students: ○ Final year students ○ Practical/clinical training ○ Laboratory work	○ Essential laboratory work with a permit conditional to level 3 restrictions and relevant Unisa permit. ○ Access to university and research facilities only if you are a final year student conditional to level 3 restrictions and relevant Unisa permit. ○ No research that requires physical human participant interactions nearby, e.g., face-to-face interviews, focus groups, or human sample collection (excluding clinical research as permitted by national guidelines and relevant Unisa permit). ○ Telephone and/or online platform interaction with human participants. ○ Online quantitative research, e.g., surveys. ○ No research is to be conducted in homes, communities, restricted government facilities for the aged.
<u>Advanced alert level 3:</u> 33% of the student population will be allowed to return to campuses which include: ○ All groups that have already returned. ○ Students in the final year of their programmes. ○ Student in years of study that require clinical training. ○ Postgraduate students who require laboratory equipment and other technical equipment.	○ Essential laboratory work with a Unisa permit. ○ Telephone and/or online platform interaction with human participants. ○ Online quantitative research, e.g., surveys. ○ Limited research that requires physical human participant interaction in close proximity conducted under strict conditions, in pre-arranged public space and adhering to strict safety conditions. ○ *Postgraduate students that need to proceed with data collection methods involving physical human interaction with participants must inform their supervisors and notify the ERC by completing an amendment form, signed by the supervisor and student – the safety precautions must be clearly described (Refer to the "toolkit" in section 4 below).* ○ *All other researchers that want to proceed with data collection methods involving physical human interaction with participants must notify the ERC by completing an amendment form, signed by the researcher – the safety precautions must be clearly described (Refer to the "toolkit" in section 4 below).*
Alert level 2: Physical distancing and restrictions on leisure and social activities to prevent a resurgence. Maximum of 66% of the student population may return to campus. Controlled return of students: ○ Final year students ○ Practical/clinical training ○ Laboratory work ○ First-year undergraduate students	○ *Essential laboratory work with a Unisa permit.* ○ *Telephone and/or online platform interaction with human participants.* ○ *Online quantitative research e.g., surveys.* ○ *Limited research that requires physical human participant interaction in close proximity conducted under strict conditions, in pre-arranged public space and adhering to strict safety conditions.* ○ *Postgraduate students that need to proceed with data collection methods involving physical human interaction with participants must inform their supervisors and notify the ERC by completing an amendment form, signed by the supervisor and student – the safety precautions must be clearly described (Refer to the "toolkit" in section 4 below).*

<table>
<tr><td></td><td>

- *All other researchers that want to proceed with data collection methods involving physical human interaction with participants must notify the ERC by completing an amendment form, signed by the researcher – the safety precautions must be clearly described (Refer to the "toolkit" in section 4 below).*

</td></tr>
<tr><td>

Alert level 1: Most normal activities can resume with certain restrictions, precautions, and health guidelines to be followed at all times.

100% of the student population returns with:

- Physical distancing
- Health protocols
- International students return

Age and co-morbidity of staff and students managed

</td><td>

- Essential laboratory work with a Unisa permit.
- Telephone and/or online platform interaction with human participants.
- Online quantitative research, e.g., surveys.
- Research that requires physical human participant interaction in close proximity conducted under strict conditions in homes, communities, restricted government facilities, schools, facilities for the aged (Refer to the "toolkit" in section 4 below).
- *Postgraduate students that need to proceed with data collection methods involving physical human interaction with participants must inform their supervisors and notify the ERC by completing an amendment form, signed by the supervisor and student – the safety precautions must be clearly described (Refer to the "toolkit" in section 4 below).*
- *All other researchers that want to proceed with data collection methods involving physical human interaction with participants must notify the ERC by completing an amendment form, signed by the researcher – the safety precautions must be clearly described (Refer to the "toolkit" in section 4 below).*

</td></tr>
</table>

4. **Strict safety and protocol guidelines with human participant contact (alert 1 and 2) – also referred to as the "toolkit":**

The following guidelines are recommended to ensure the safety of the researcher(s) and participant(s):

4.1 Do not proceed with the intended contact data collection visit or meeting if the researcher and/or participant is feeling unwell.

4.2 Telephonic pre-screening before the visit is advised, as well as keeping a register of participants that were involved in face-to-face data collection activities.

4.3 The researcher and members of the research team must also be screened before any human participant contact. Keep evidence of the screening data signed by a witness.

4.4 Useful Covid-19 guidance is provided on the Department of Health WhatsApp group +27 60 012 3456.

4.5 When the visit can go ahead, please be mindful of the following procedures:

- 4.5.1 Wearing an appropriate cloth mask. Do not touch your face and advise the participants to do the same.
- 4.5.2 The researcher has to ensure that the research team and participants have masks and sanitizer.

4.5.3 In specific contexts, it will be essential to handout sanitiser and sealed cloth masks to the participants.

4.5.4 If possible, do a pre-screening by measuring the participants' temperature (including those of the researcher) and ask questions that were not included in the telephonic pre-screening.

4.5.5 Keep a physical distance of 2 meters;

4.5.6 Sanitize hands with 70% alcohol-based sanitizer or wash with soap and water for at least 40 seconds before commencing any activities.

4.5.7 Sanitize all surfaces before commencing activities and again before leaving.

4.5.8 Avoid the exchange of paper between participants and researchers, unless the use of paper is ethically or scientifically justified.

4.5.9 Use disposable gloves with the handling of hard copies of documents, put it in a paper envelope, and store it away. Researcher and participants to remove the gloves or sanitize your hands since the novel coronavirus can reside on paper for up to 3 days.

4.5.10 Store documents for a minimum of 3 days before taking them out.

4.5.11 The risk of contagion during the use and exchange of pens, digital devices, smartphones, and tablets must be considered and mitigated. Please refer to the links below, how to clean these devices.

4.5.12 No food may be shared. Pre-packed, sanitized items such as chips or water could be handed out if necessary.

Resources:

https://www.cdc.gov/coronavirus/2019-ncov/community/disinfecting-building-facility.html

https://www.ehs.washington.edu/covid-19-health-and-safety-resources

Acknowledgment:

The guideline document was informed, with permission from Prof Minrie Greeff, Emeritus Professor: Africa Unit for Transdisciplinary Health Research, by:

- M. Greeff (21 May 2020), Implications of Alert Levels for Researchers and Postgraduate students during the Covid-19 pandemic. Guideline document.
- M. Greeff (11 June 2020), Implications of Alert Levels for Researchers and Postgraduate students during the Covid-19 pandemic. Webinar hosted by AUTHeR, North West University.

Additional resources consulted:

Watermeyer, J, Knight, J & Small, M, WITS non-medical REC guidelines for research during Covid-19.

South African Government, Webpage - Corona information and updates, https://www.gov.za/Coronavirus

Appendix D

NEMATODES as potential biofungicide for Fusarium head blight

Julius Leumo Kgosiemang,
ARC-Small Grain,
Bethlehem

Dr Tshimangadzo Ramakuwela,
ARC-Small Grain,
Bethlehem

The underlying principle of integrated pest management (IPM) is based on minimising the use of chemical pesticides as these can be a threat to ecosystems and an expense to the economy.

An alternative strategy that is eco-friendly and economically sound is biological control. By definition, biological control is the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be (Eilenberg *et al.*, 2001).

One such biological agent is entomopathogenic nematodes (EPNs) from the genera *Steinernema* and *Heterorhabditis*, which are lethal to a number of agriculturally important insect pests. EPNs have gained attention due to their many attributes as effective biological control agents and have been commercialised in several continents. In contrast to their obvious potential, the efficacy of EPN application is often challenged by a number of biotic and abiotic factors.

In nature, *Steinernema* spp. and *Heterorhabditis* spp. are symbiotically associated with insect-killing bacteria in the genera *Xenorhabdus* and *Photorhabdus*, respectively. The infective juvenile (IJ) is a non-feeding stage in the life cycle of EPNs and is the only infectious stage. These IJs search their host insect, infect and release the bacterial symbiont into the haemolymph of the insect. The bacterial symbiont kills the host and assists in establishing and maintaining favourable conditions for nematode development and reproduction by producing secondary metabolites that overcome the host insect's immune system.

These metabolites inhibit the growth of various fungal and bacterial competitors, thereby 'protecting' the cadaver and resident EPNs. A number of secondary metabolites are produced, including lipases, phospholipases, proteases and peptides, which are understood to produce novel natural compounds with diverse biological activities. Metabolites of *Photorhabdus* and *Xenorhabdus* bacteria have demonstrated inhibitory effects against various fungal plant pathogens without phytotoxicity when applied to various plant species in the glasshouse. However, efficacy of metabolites produced by symbiotic bacteria of EPNs against *Fusarium graminearum*, the causative agent of Fusarium head blight (FHB) in small grains, has not been evaluated.

Management of FHB

FHB is a significant floral disease of cereals and poses serious health hazards by contaminating the grain with harmful mycotoxins. FHB development is mainly influenced by air, temperature and atmospheric humidity during flowering and early stages of seedling development. Worldwide, the disease is responsible for losses amounting to billions of dollars annually – with most of these losses in wheat and barley. In order to control FHB, chemical pesticides (triazoles and imidazoles) have been used for years.

Although chemical pesticide application forms a critical part of an integrated FHB management programme, they may also destroy natural antagonists of plant pathogens and facilitate resistance development to chemical pesticides. To date, there are no effective FHB control strategies available for producers despite its significance, underscoring the importance of research to address the challenges posed by this pathogen.

Recently, secondary metabolites produced by bacterial symbionts of indigenous South African EPN isolates (curated by the ARC-Small Grain) were evaluated for antifungal activity against *F. graminearum* group II. Antifungal activity was determined in two ways: firstly by comparing mycelial growth inhibition on treated versus untreated solid medium, and secondly by comparing spore germination percentage in treated versus untreated liquid medium. Antifungal activity varied significantly among isolates using the solid media technique.

The highest mycelial growth inhibition rate (96,25%) was achieved with a *Xenorhabdus* isolate, *S. beitlechemi* SGI 197, crude treatment after seven days incubation (**Figure 1**). For spore germination, isolates from the genus *Xenorhabdus* displayed higher inhibition of *Fusarium* spore germination compared to isolates of *Photorhabdus*. All *Xenorhabdus* isolates resulted in over 83% inhibition of *Fusarium* spore germination, while that of *Photorhabdus* isolates remained below 15% when compared to the control.

These results confirm that secondary metabolites from EPN bacterial symbionts are potential biofungicide candidates for control of FHB. Although field efficacy against FHB is still not studied, the significant inhibition of growth and spore germination suggests that application of secondary metabolites at flowering stage may provide protection of plants against infection or spread of *F. graminearum*. This research continues at ARC-Small Grain through funding by the Agricultural Research Council and the National Research Foundation, under grant number TTK180425324117. ●

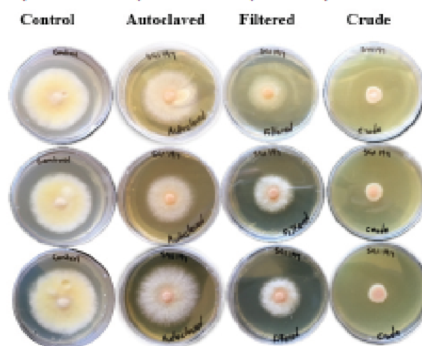


Figure 1: ***Fusarium graminearum*** **mycelia growth challenged by different EPN bacterial metabolite treatments of** ***Steinernema beitlechemi*** **SGI 197 compared to control.**

Contact Dr Tshimangadzo Ramakuwela at 058 307 3400 or ramakuwelat@arc.agric.za for more information.

Reference

Eilenberg, J, Hajek, AE & Lomer, C. 2001. Suggestions for unifying the terminology in biological control. *BioControl* 46, 387-400.