Reduction of post-harvest losses in tomato using fungal bio-preservative for smallholder farmers

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DECLARATION

I, undersigned, declare that the work contained in this dissertation is my own work and has not been, previously submitted by me for a degree at another institution.

Signed: _____

Date: _____

DEDICATION

This dissertation is dedicated to my lovely husband *Lesego Modiri Molefe*, my mom *Jeanette Moeng* and to my little brother *Olebogeng Aobakwe Moeng*.

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RESEARCH OUTPUTS AND CONFERENCE ATTENDANCE

1. Training on Basic Microbiological techniques (Sample collection, Medium preparation, Streaking techniques, DNA extraction, Gel electrophoresis, Conventional and qPCR) as an in- house training course at ARC from 19th to 23rd September 2016.
2. L.R. Moeng, O.A. Aiyegoro, R.A. Adeleke & C.C. Bezuidenhout. The reduction of postharvest losses in tomato fruits through the use of fungal bio-preservative. Oral presentation at the International Union of Biochemistry and Molecular Biology (IUBMB) Advanced School, held in Greece, Europe, May 15-19th 2017. Theme: Training about the molecular relationships that occur in food components
3. Bioinformatics (QIIME) training course at ARC - SCW from 11th to 14th September 2017.
4. Programming school (Python and Linux) of the Centre for High Performance Programming (CHPC) at North West University - Potchefstroom Campus from 15th to 19th January 2018.
5. L.R. Moeng, O.A. Aiyegoro, R.A. Adeleke & C.C. Bezuidenhout. The reduction of postharvest losses in tomato fruits through the use of fungal bio-preservative. Poster presentation at the 2018 SASM (South Africa Society for Microbiology) conference, held in Johannesburg, South Africa, April 4-7th, 2018. Theme: Microbes: Livelihood, Economy and Environment.
6. L.R. Moeng, O.A. Aiyegoro, R.A. Adeleke & C.C. Bezuidenhout. The reduction of postharvest losses in tomato fruits through the use of fungal bio-preservative. Oral presentation at the 5th Annual Postgraduate (PDP) conference, held at Roodeplaat, South Africa, August 27-29th, 2018.
7. The work has been published as a report on the project's funder Industrial Development Corporation (IDC) website. It is found on https://www.idc.co.za/images/ARC_Final_report_September_2018.pdf.
8. There is a manuscript that has been submitted for publication to an accredited journal, and the second one that has been drafted.

ABSTRACT

Tomatoes (*Solanum lycopersicum*) are among the important fruits that are widely grown globally. However, they are susceptible to spoilage by fungi due to their high water content and soft endocarp. This spoilage leads to post-harvest losses (PHLs), which make tomato production unprofitable for majority of farmers in developing countries. These PHLs of tomatoes have been estimated to be 42% of annual global harvests. Accordingly, this study aimed to isolate, identify and characterise non-pathogenic, antagonistic fungal strains for the management of PHLs in tomato fruit. The tomato fruit (Cultivar Jasmine) were harvested from the research field of the Agricultural Research Council- Vegetable and Ornamental Plants (ARC- VOP) in Roodeplaat, South Africa. Fungal species were isolated and characterised from the collected tomatoes in order to evaluate their antagonistic properties against known fungal pathogens that cause spoilage in the tomatoes. The fungal colonies were morphologically identified with such identification being further confirmed by phylogenetic analysis of the internal transcribed spacer (ITS) regions 1 and 2, using ITS1 and ITS4 universal primers. The dual culture technique was used to test for the antagonistic ability (percentage growth inhibition (PGI)) of the isolates against eight pathogenic fungi (*Rhizopus stolonifera* ATCC 6227a*, Rhizopus stolonifera* ATCC 6227b, *Geotrichum candidum* ATCC 34614, *Fusarium solani* ATCC 36031, *Fusarium oxysporum, Rhizoctonia solani, Alternaria solani* and *Alternaria alternata*). Those fungal isolates that showed antagonistic properties against the pathogens were further tested for antifungal susceptibility, bile and acid tolerance, in order to test their abilities to serve as probiotics when they are consumed with the tomato fruit. The efficacy of these antagonists to reduce weight loss and spoilage percentage of tomatoes were evaluated in a 15 day storage trial under two storage conditions (8 °C refrigeration and uncontrolled room temperature). A total of 40 pure fungal isolates were identified and then clustered into 17 distinct operational taxonomic units (OTUs) based on 97% sequence similarity. The fungal isolates comprised 10 genera which were identified as *Penicillium, Fusarium*, *Curvularia*, *Alternaria*, *Cladosporium*, *Lecythophora*, *Aureobasidium*, *Byssochlamys*, *Retroconis*, and *Epicoccum*. *Penicillium* and *Fusarium* genera had the highest occurrence of 22.5% each as

compared to *Curvularia* (15.0%), *Alternaria* (12.5%), *Cladosporium* (10.0%), *Lecythophora* (5.0%), *Aureobasidium* (5.0%), *Byssochlamys* (2.5%), *Retroconis* (2.5%), and *Epicoccum* (2.5%). Four fungal isolates (*Byssochlamys spectabilis, Curvularia kusanoi, Epicoccum thailandicum* and *Retroconis fusiformis*) showed high PGI against the growth of tomato fungal pathogens and were selected as antagonists. These antagonists also passed most of the standard criteria used for grading probiotics. Thereafter, during storage the *C. kusanoi* and *E. thailandicum* were the only antagonists that could reduce the weight loss and the spoilage percentage of tomatoes. Hence, from the findings, it was concluded that *C. kusanoi* and *E. thailandicum* showed potential as antagonists to preserve tomatoes during storage and they also possess beneficial (probiotic) properties. They are therefore, promising as bio-preservative agents that could be useful in extending the shelf life of tomatoes at storage and thereby preventing PHLs.

Keywords: tomatoes, fungal antagonist, bio-preservative, pathogens, postharvest loss, probiotics

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
API	Animal Production Institute
ARC	Agricultural Research Council
ATCC	American Type Culture Collection
BLAST	Basic Local Alignment Search Tool
BR	Broad range
BSH	Bile Salt Hydrolase
CFU	Colony Forming Unit
DAFF	Department of Agriculture, Forestry and Fisheries
DNA	Deoxyribonucleic Acid
FAO	Food and Agriculture Organisation
FAOSTAT	Food and Agriculture Organisation Corporate Statistical Database
FAOUN	Food and Agriculture Organisation of the United Nations
GDP	Gross Domestic Product
HCl	Hydrochloric Acid
ITS	Internal Transcribed Spacer
LSDs	Least Significant Differences
LSL	Long Shelf Life
MEGA7	Molecular Evolutionary Genetics Analysis version 7.0
MFC	Minimum Fungicidal Concentration
MIC	Minimum Inhibition Concentration
MT	Million tons
NaOH	Sodium hydroxide
NCBI	National Center for Biotechnology Information
OD	Optical Density
OTU	Operational Taxonomic Units

PBS	Buffered Phosphate Saline
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PepMV	Pepino Mosaic Virus
PHLs	Postharvest losses
PGI	Percentage Growth Inhibition
ROS	Reactive Oxygen Species
rDNA	Ribosomal Deoxyribonucleic Acid
RNA	Ribosomal Nucleic Acid
SAS	Statistical Analysis System
SD	Standard Deviation
ToMarV	Tomato Marchitez Virus
US	United States
USA	United States of America
USD	US dollar
UK	United Kingdom
UV	Ultraviolet
UV-C	Ultraviolet Light
VOP	Vegetable and Ornamental Plants
ZR	Zymo Research

LIST OF SYMBOLS

°C	Degree Celsius
%	Percent
µg	Microgram
µl	Microliter
µm	Micrometre
cm	Centimetre
g	Grams
hr(s)	Hour(s)
ha	Hectare
M	Molar
mbar abs	Millibars (Unit of absolute air pressure)
min	Minute
mg	Milligram
mm	Millimetre
mM	Millimolar
ml	Millilitre
nm	Nanometre
rpm	Revolution per minute
s	Second
v/v	Volume/volume
w/v	Weight/volume

CHAPTER 1: INTRODUCTION

Tomato (*Solanum lycopersicum**)*** is an important crop that belongs to the *Solanaceae* family, together with potato (*Solanum tuberosum*), hot pepper (*Capsicum frutescens)*, pepper (*Capsicum annum)* and eggplant (*Solanum melongena*) (Shah, *et al*., 2013). It ranks first among plants widely grown in the world, and accounts for over 14% of the world's fruit production (FAO, 2011). According to the Food and Agriculture Organisation Corporate Statistical Database (FAOSTAT), China is the leading tomato producer in the world, followed by India, United States, Turkey, Egypt, Iran and Italy with these countries accounting for more than 80% of global tomato production (FAO, 2011). Egypt is the only African country amongst the top ten world's tomato producers (DAFF, 2015). In South Africa, tomatoes are the second most important and popular crop after potato from the *Solanaceae* family and also one of the main plants sold on both local and export markets (DAFF, 2015). Furthermore, the Department of Agriculture Forestry and Fisheries (DAFF) reported that 75% of the tomatoes in South Africa are produced in the northern areas of Limpopo province while the remaining 25% are produced between Onderberg area of Mpumalanga province and the border area of the Eastern Cape province (DAFF, 2015).

Tomato is a popular crop choice for smallholder farmers in South Africa because of high demand for the fresh products as well as the opportunities it presents for industrial processing (Tshiala and Olwoch, 2010). There are approximately 695 tomato producers in both the commercial and emerging sectors (DAFF, 2015). The commercial sector contributes 95% of the total produce while the emerging sector contributes 5% only. Thus, tomato production can serve as a source of income by creating jobs for both rural and peri-urban residents and thereby improve the livelihoods of small-scale producers (DAFF, 2015). After tomato production, the farmers harvest tomatoes and sort them into two classes namely A-grade (marketable) and B-grade (non-marketable) tomatoes according to size and quality (Pienaar, 2014). A-grade tomatoes are medium to large in size with a presentable appearance, having no pest affected or deformation marks, while B-grade tomatoes are characterised by small fruits with pest-affected areas (Parfitt

et al., 2010). Smallholder farming system results in B-grade tomatoes due to certain challenges (Jovanovic *et al*., 2018; Parfitt *et al.*, 2010; Pienaar, 2014).

Most of the smallholder farmers in South Africa are untrained and use traditional management practices for irrigation, fertilisation, pest control, crop management and soil preparation (Pienaar, 2014). Hence, they are the most vulnerable when water resources get depleted or during increased erratic weather events (Ayandiji and Adeniyi, 2011). On the smallholder farms, producers face challenges such as improper harvesting processes, poor farm sanitation, unsuitable harvesting containers and packaging material (Arah *et al*., 2015). During transportation and storage, challenges such as lack of processing factories, an inappropriate transportation system, reduction of quality roads and a lack of reliable market information, leads to the reduction in tomatoes due to spoilage (Pienaar, 2014).

Tomatoes are susceptible to fruit spoilage caused by numerous fungal pathogens (Barkai-Golan and Paster, 2008; Samuel and Orji, 2015; Sanzani *et al.*, 2016; Tournas and Katsoudas, 2005). Examples of fungal induced tomato diseases include Alternaria rot caused by *Alternaria solani* and *Alternaria tenuis,* Phytophthora rot caused by *Phytophthora infestans* and *Phytophthora nicotianae var. parasitica,* Anthracnose ripe rot caused by *Colletotrichum phomoides*, Phoma rot caused by *Phoma destructiva* and Fusarium rot caused by *Fusarium* spp*.* (Wani, 2011). Diseases caused by the above mentioned fungi may be due to large nutrient composition or other factors such as high water content and low pH (Droby *et al*., 1992). These factors may make the produce to become highly susceptible to pathogenic attack. Diseases reduce the yield of tomatoes by up to 25% in industrialised countries and more than 50% in developing countries (Nunes, 2012). However, there are numerous methods for preventing diseases in fruits and one of them is the use of fungicides.

The use of synthetic fungicides has been a primary method for managing the postharvest spoilage of tomatoes (Spadaro and Gullino, 2004). However, there are increasing concerns over fungicide use such as environmental pollution risks, inability to control fungal diseases due to fungicide resistance , and persistence of fungicide residues on the tomato (Ippolito and Nigro, 2000). All those challenges have resulted in the search for safe and effective alternative strategies for the control of plant pathogens (Liu *et al*., 2013). Such strategies include biological control (such as the microbial antagonists) of fungal pathogens in tomatoes using naturally occurring microorganisms (Droby *et al*., 2009). Moreover, this biological control is effective, nontoxic and environmentally friendly alternatives to fungicides (Janisiewicz and Korsten, 2002).

1.1. Problem statement

Microbial spoilage is the main cause of postharvest losses of tomatoes (Deribe *et al*., 2016; Osman, 2015; Suprapta, 2012). The quality of tomato fruit deteriorate after harvesting, thus resulting in reduction of tomato yield. To prevent yield loss, pesticides are used, but their residues on fresh fruits and vegetables have been and will continue to be one of the main concerns of the regulatory agencies (Dukare *et al*., 2018). Human consumption of pesticide residues is toxic to the health of the consumer especially with cumulative effect of prolonged consumption. Therefore, reducing pre- and postharvest use of chemical fungicides by developing alternative management strategies remains a high research priority (Droby *et al*., 2009). Bio-preservation has the potential to be the most suitable method to drastically reduce postharvest tomato losses. Furthermore, bio-preservation is acceptable because it is safe, economical and has minimal side effects as it is developed from indigenous microbial communities. Hence, there is a need to develop a commercially successful postharvest bio-preservative product that is affordable and will be easily available to resource farmers, especially smallholder farmers.

1.2. Aim and objectives

1.2.1. Aim

The present study aimed to investigate the potential utilisation of antagonistic fungal strains in tomato fruit for the management of post-harvest losses.

1.2.2. Objectives

1. To isolate, characterise and identify fungal strains from tomato fruit using cultural and molecular based approaches
2. To test the antagonistic potentials of the isolated fungal strains against selected tomato pathogenic fungi
3. To test the antagonistic isolates obtained above for probiotic properties
4. To evaluate the efficacy of the selected fungal antagonists under different storage conditions

CHAPTER 2: LITERATURE REVIEW

2.1. A brief description of the tomato

Tomato (*Lycopersicon esculentum*) is one of the most important vegetables worldwide. It is a self-pollinated fruit that belongs to the *Solanaceae* family (Arah *et al*., 2015). The family also includes potato (*Solanum tuberosum*), eggplant (*Solanum melongena*), pepper (*Capsicum annum),* tomatillo (*Physalis philadelphica*) andpepper (*Capsicum annuum*, *Capsicum frutescens,* and *Capsicum chinense).* The family also includes plant drugs such as Tobacco *(Nicotiana tabacum)*, deadly nightshade (*Atropa belladonna*), mandrake (*Mandragora officinarum*), jimson weed (*Datura stramonium*) and petunia (*Petunia hybrida*) (Shah *et al.,* 2013). Tomato is widely cultivated in tropical, sub-tropical and temperate climates and is ranked third in the world for vegetable production (FAO, 2011). Tomato is known by different names worldwide, for example, tomate (German, France), tomati (West Africa), tomaatti (Finish), tomat (Indonesia), pomidoro (Italy), kamalis (Malay), jitomate (Spain, Mexico), pomidor (Russia), faan ke'e (China), tomatl (Nahuatl), nyanya (Swahili) and tamatar (Hindi) (Naika *et al*., 2005).

Tomato originated in the South America Andes, in the mountains of Peru (Shnain *et al.*, 2017). It was taken to other parts of the world by the early travellers where it was planted as an ornamental curiosity but not eaten (Arah *et al.*, 2015). By 500 BC it had been moved to Mexico for the purposes of domestication. Tomato was brought to Europe in 1554 by the Spanish conquistadors. It was later cultured in the U.S. in 1710, and introduced from Europe into southern and eastern Asia, Africa and the Middle East. Thereafter, tomato became popular and was exported around the world by 1850 for commercial production (Shnain *et al*., 2017).

2.1.1. Scientific classification of tomato

Kingdom:	Plantae
Sub kingdom:	Tracheobionia

Class: Magnoliopsoda

Sub class: Asterielae

Order: Sultanates

Family: Solanaceae

Genus: *Lycopersicon*

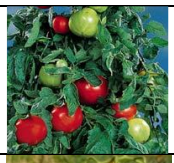
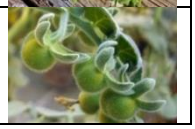
Species: *esculentum*

2.1.2. Different botanical varieties of tomato

There have been numerous changes to the botanical name for tomato. For several years it was known as *Solanum lycopersicum,* which later changed to *Lycopersicon esculentum*(Naika *et al*., 2005). Tomato is a true diploid with 2n = 24 (Stack and Anderson, 1986). The plant is annual with a herbaceous prostrate stem having determinate or indeterminate growth habit (Naika *et al*., 2005). Tomato has three vine types, namely, indeterminate (sprawling, staggered ripening and tall type), semi-determinate (intermediate response and semi-bush type) and determinate (compact, uniform ripening and bush type) (Naika *et al*., 2005). Table 2.1 represents the names of tomato according to diversities.

Table 2.1. Several species of tomato (Berrueto, 2017).

Name	Species variety	Image
garden tomato	*lycopersicum*	
potato-leafed tomato	*grandifolium*	
cherry tomato	*cerasiforme*	

dwarf type tomato	*validum*	
pear tomato	*pyriforme*	
Tiny wild tomato	*pimpinellifolium*	
Hairy tomato	*galapagense*	
Orange tomato	*cheesmaniae*	
Brix (Soluble solids) tomato	*pennelli*	

There are approximately 7 500 tomato varieties which are grown for various purposes (Berrueto, 2017). Tomato varieties can be divided into several categories, based on shape and size. These categories include slicing or globe (also known as round tomatoes), beefsteak (large tomatoes), plum (bred for higher solids) and also grape (smaller variation of a plum tomato) (Berrueto, 2017).

2.1.3. The economic, health and nutritional values of tomatoes

Tomato has become an important cash and industrial crop in many parts of the world. This is not only because of its economic importance but also its nutritional value in the human diet and subsequent importance for human health as a result of the essential nutrients it provides (Ayandiji and Adeniyi, 2011; Yadav *et al*., 2017). It is also a versatile crop that can be classified according to use into two categories as fresh market tomatoes for direct consumption and processing tomatoes which are cultivated for industrial canning and processed foods, respectively (Osman, 2015). Tomato is rich in vitamins A, B, C and E; carbohydrates such as fructose and glucose;

minerals such as phosphorus, sodium, potassium, calcium and magnesium and trace elements such as iron, copper, zinc and dietary fibers (Ayandiji and Adeniyi, 2011; John *et al*., 2016; Yadav *et al*., 2017). It therefore serves as a source of essential nutrients when consumed (Arah *et al*., 2015; Guil-Guerrero and Rebolloso-Fuentes, 2009).

An average size (70 - 150 g weight and 50 - 70 mm diameter) tomato fruit contains energy (18 kcal), protein (0.95 g), fat (0.11 g), carbohydrate (4.01 g), total sugar (2.49 g), niacin (0.731 mg), calcium (11.0 mg), iron (0.68 mg), magnesium (9.0 mg), phosphorus (28.0 mg), potassium (218.0 mg), sodium (11.0 mg), zinc (0.14 mg), thiamin (0.036 mg), riboflavin (0.022 mg), carotene (vitamin A) 320 IU, vitamin B (60.079 mg), vitamin C (16.9 mg), and ascorbic acid (31 mg) per 100 g pulp of fruit (Arah *et al*., 2015; Yadav *et al*., 2017). Tomatoes are ready-to-eat food, and are thus minimally processed (John *et al*., 2016). They are consumed in various ways such as raw in salads and sandwiches, cooked or processed in ketchup, sauces, soup, chutney, pickles, paste, puree, juices, dried powder and whole canned fruits, while it also forms an important ingredient in the cocktail known as a Bloody Mary (Ayandiji and Adeniyi, 2011; Chaudhary, 2014; Yadav *et al.*, 2017).

The deep-red coloration of the ripened tomato is due to the high amount of lycopene, a form of B-carotenoid pigment and a notable antioxidant that is beneficial in reducing the incidence of certain chronic diseases such as prostate cancer, cardiovascular disease and diabetes (Ram *et al*., 2014; Wu and Tanksley, 2010). Tomato juice promotes gastric secretion, acts as a blood purifier and works as an intestinal antiseptic (Chaudhary, 2014). Tomatoes are good sources of vitamin C and vitamin A which are vital in warding off muscular degeneration and improving eyesight. It is also believed to be a powerful blood purifier and clear up urinary tract infections. Tomatoes are high in fibre which aids easy digestion and may assist in weight loss (Arah *et al*., 2015).

Tomatoes have numerous advantages that make them economically important (Naika *et al*., 2005). These advantages include the following: relatively short-duration vegetable crop, short production period, growth as an uncovered field crop and in protected cultivation, easy fitting into different cropping systems, high economic value, and high micronutrient content (Naika *et al*., 2005).

2.2. Smallholder agriculture

One of the agricultural pathways towards sustainable food and nutrition security is through the local production of food, where smallholder farmers play a crucial role (Dorward *et al*., 2005; Maliwichi *et al*., 2014; Wiggins and Keats, 2013). The value of smallholder agriculture is being recognised in the developing countries and, hence, governments are implementing programmes in agricultural development that are leading to the empowerment of the smallholder farmers (Aliber and Hall, 2012). A smallholder farmer is categorised as a farmer that owns small plot of land whereby crops are grown mainly to support the family. Depending on the yield produced smallholder farming can range from subsistence to commercial (Raphela, 2014; Shao *et al*., 2004; Thamaga-Chitja and Morojele, 2014). Smallholder farmers play a significantly positive role in poverty alleviation and household food security (Shao *et al*., 2004; Thamaga-Chitja and Morojele, 2014; Wiggins and Keats, 2013). According to Poulton *et al*. (2006), the productivity of smallholders in agriculture contributes to an increase in market profits, encourages a reasonable supply of income and creates both the backward and forward linkages necessary for economic growth (Raphela, 2014; Thamaga-Chitja and Morojele, 2014). According to Van Averbeke and Mohamed (2006), there are three different types of smallholder farmers:

- **Subsistence farmers** – These farmers produce for household consumption with very limited sales. They make up the majority of the small-scale farmers.

- **Emerging smallholder farmers** – These farmers wish to work increasingly towards commercialising their production.
- **Commercial smallholder farmers** – These farmers receive an income from the sale of their produce. They constitute the minority of the small-scale farmers.

2.3. Tomato production

On a global scale, the annual production of fresh tomatoes amounts to approximately 159 million tons with more than a quarter of these 159 million tons grown for the processing industry, thus making tomatoes the world's leading vegetable for processing (Noonari *et al*., 2015). Tomato is cultivated in both the tropics and subtropics of the world and is also cultivated in kitchen gardens, commercial fields under greenhouse and polyhouse conditions and soil-less culture or hydroponic systems (Chaudhary, 2014). Although the root structure of a tomato plant is able to penetrate various soil types up to depths of two metres, the highest percentage of the roots will be found in the top 600 mm of the soil. Tomatoes are grown and produced optimally when the mean temperatures are between 20 °C and 24 °C. When average daily temperature is above 32 °C and the night temperature fails below 21 °C the fruit set is poor (Starke Ayres, 2014). It takes tomato plants three to four months to bear fruits that are ready for harvesting (see figure 2.1). Tomato planting involves different techniques and methods for determinant (generally grown under open-field condition) and indeterminate (normally grown under poly-house condition) varieties (Yadav *et al*., 2017). Moreover, tomato can grow well in soil, organic substrates, soilless mixes, perlite, sand or hydroponics (Shamshiri *et al*., 2018).

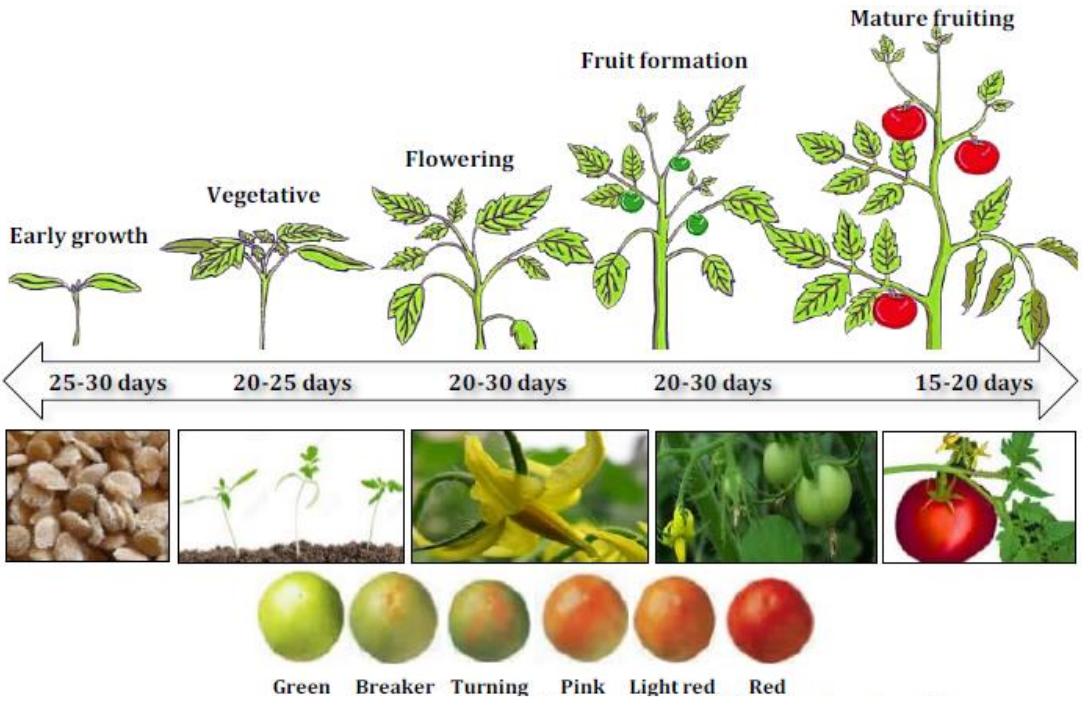


Figure 2.1: The 5 growth stages of tomato, and the different levels of fruit ripeness (Shamshiri *et al.*, 2018).

The following countries are the highest producers, users and exporters of tomato in the world namely China (largest producers and dominates production of processing exports), US and Europe (major users of fresh tomatoes), Mexico (the largest exporter of fresh tomatoes), and Turkey (a major exporter into Europe) (Kahan, 2010; Yadav *et al*., 2017). As illustrated in Table 2.2, the top ten tomato producers are as follows; China, USA, India, Turkey, Egypt, Italy, Iran, Spain, Brazil and Mexico in that order, and these countries account for 80% of the total world tomato production (Chandio *et al*., 2016; FAO, 2011). Egypt is the only African country amongst top ten world tomato producers while South Africa (580 851 tons) ranks as the seventh highest tomato producing country in Africa (FAOSTAT, 2018).

Table 2.2: Average million tons (MT) of top 10 producers of tomatoes around the world (1994-2016)

Rank	Country	Production (MT)
1.	China	33 352 918.78
2.	USA	13 011 761.61
3.	India	10 653 317.83
4.	Turkey	9 744 947.35
5.	Egypt	7 410 736.17
6.	Italy	6 277 585.57
7.	Iran	4 477 189.13
8.	Spain	4 002 324.57
9.	Brazil	3 544 036.96
10.	Mexico	2 880 385.09

Source: (FAOSTAT, 2018)

Agricultural production of tomato in South Africa is dominated by commercial farms (Raphela, 2014; Shao *et al*., 2004; Thamaga-Chitja and Morojele, 2014). South Africa produces tomato in areas such as Trichardt and Onderberg in Mpumalanga province, Pongola and Nkwalini in KwaZulu-Natal province, Western Cape province and Limpopo province (Tshiala and Olwoch, 2010). The established planting periods for tomato production in the Lowveld (frost free areas) are from February to May, in the Middleveld (moderate areas) are from September to December and in the Highveld (cold areas) are from October to November and in the Western Cape are from October to December (ARC-VOPI, 2013; Starke Ayres, 2014). South Africa is one of the few countries in the world that pick their tomato fruit at the mature green to colour break stage (Maboko *et al*., 2009). It would appear that the perception persists in South Africa that a red ripe tomato is over mature, and that the fruit size influences consumer acceptance of, and preference for tomatoes (Maboko *et al*., 2009). However, the fruit size of a tomato rarely indicates the maturity stage as some varieties such as Miramar, Malory, FiveOFive and FA593 are genetically larger in size than others (Maboko *et al*., 2009).

2.4. Tomato production challenges

Tomato production has the potential to improve the livelihood of smallholder farmers in most of the developing countries around the world (Maliwichi *et al*., 2014; Pienaar, 2014). In addition to the health benefits derived from tomatoes and tomato-based foods, the crop may also serve as a source of income for farmers as a result of the numerous uses of tomato (Arah *et al*., 2015). Tomato industry may also increase the foreign export earnings of many African countries, thereby contributing to their gross domestic product (GDP) (Chandio *et al*., 2016). Studies have shown that the full potential of the crop has been under exploited as a result of the many challenges involved in tomato production (Geoffrey *et al*., 2014; Jayne *et al*., 2010).

These challenges include physical infrastructure (poor roads, transport and telecommunications), long production and exacerbating risks, lack of land policy (farmers have no rights to the land they farm), social constraints (the role of women farmers in agricultural production tends to be underestimated), and lack of investment (low output prices, high cost of inputs and limited access to credit make it difficult for smallholder farmers to produce sufficient food efficiently). Other challenges include, environmental constraints (climate change and its related impacts on food production), production constraints (very low average production due to the rain-fed crops and cultivation using unsuitable agricultural practices that increase soil erosion, thereby resulting in low yields), lack of post-harvest processing, inadequate storage facilities and marketing systems (which leads to post-harvest losses of the produce) and pre-harvest losses (Aliber and Hall, 2012b; Arah *et al.,* 2015; Dorward *et al.,* 2005; Ortmann and King, 2007).

To mitigate some of the challenges, quality management practices should be put in place. The quality management starts in the field and continues until the produce reaches the end user (Albrigo, 1978). Understanding and managing the various roles that pre-harvest factors play in

quality are very important in the maximum harvest and post-harvest quality of any crop (Meaza *et al*., 2007). Generally, pre-harvest conditions are known to be important in determining storage performance (Zhao *et al*., 2011). In some instances, their effects may even be greater than the effects of the adjustment of the storage environment. To date, pre-harvest treatment recommendations for fruits and vegetables have been established primarily in order to enhance productivity, and not as diagnostics for good quality, nutritive value and optimum shelf life (Miglioria *et al*., 2017). As a result, the need for the integration of pre- and post-harvest treatment for the improvement of shelf life remains critical.

Post-harvest losses (PHLs) are measured qualitatively and quantitatively along the supply chain, from the beginning of the harvest period until the product is either consumed or used (Hodges *et al*., 2011). The qualitative losses include reduction in nutrient value and change in the colour, taste, and texture of food whereas the quantitative losses refer to the decrease in the volume and weight of food (Buzby and Hyman, 2012). Post-harvest losses result primarily from physiological, physical and environmental factors, namely, high crop perishability, mechanical damage, humidity, rain and excessive exposure to high ambient temperature. It is also caused by inappropriate post-harvest handling, poor infrastructure, poor marketing systems, pests (birds, rodents, insects), disease attack (contamination by spoilage fungus and bacteria), insufficient transport facilities, storage and the processing techniques in relation to the product between the farm and distribution (John *et al*., 2016; World Bank, 2011). The extent of these losses often depends on the relative vulnerability of the product to physical damage (Kitinoja and Kader, 2015).

Total yield of crops are known to reduced due to postharvest diseases, in fact Naureen *et al*. (2009) stated that post-harvest diseases destroy the total yield of crops by 10 to 30% globally. While in developing countries postharvest diseases destroy more than 30% of the yield perishable crops, and much less is recorded in developed countries (Fatima *et al*., 2009; ur Rehman *et al.*,

2007; World Bank, 2011). Post-harvest losses (PHLs) in tomatoes may be as high as 25 to 42% globally (ur Rehman *et al*., 2007). Estimations on PHLs for Africa are often between 20 to 40% (World Bank, 2011). In 2011, PHLs were valued at USD1.6 billion per year in the eastern and southern regions of Africa (World Bank, 2011). Mandiriza-Mukwirimba *et al*. (2016) reported that approximately 61.3% of the farmers in South Africa were not using chemicals to control diseases, compared to 38.7% of farmers who were using such chemicals. The increase in food losses due to PHLs has a negative impact such as low returns to farmers, processors, consumers and traders, as well as the country as a whole ,which is adversely affected in terms of foreign exchange earnings (FAO, 2011).

The post-harvest potential of tomatoes not only depends upon post-harvest handling but may also depend on pre-harvest factors such as cultural practices (nutrient, water supply and harvesting methods), genetic and environmental conditions and also biotic, chemical and hormonal factors (Leonardi *et al*., 2000). Quality management of handling fruits starts in the field and continues until the product reaches the end user (Meaza *et al.,* 2007). Numerous microbial defects (signs and symptoms) of tomatoes are characterised by the type of microorganism responsible for the deterioration in the process of infection which, in the case of fungal invasion follows the development of the fungal penetrating structure (John *et al*., 2016). The susceptibility of tomato to microbial colonisation is due to its differential chemical composition such as a high level of sugar, low pH (4.9-6.5) and its high water activity which favours the growth of microorganisms (John *et al*., 2016). Fungi are the most important and prevalent pathogens, infecting a wide range of fruits and causing destructive and economically important losses in fruits during storage, transportation and marketing (Etebu *et al*., 2013).

2.5. Fungal diseases of tomatoes

In total, there are more than 200 species of fungi that may infect the tomato crop, with diseases often being the limiting factor in tomato production (Agrios, 2004; Suprapta, 2012). The epidemics of a disease depend on complex interactions between host, pathogen and environment as well as cultural practices such as fertilisation and irrigation (Osman, 2015; Aust and Hoyningen Huene, 1986). Plant pathogens use different strategies to survive and spread to new hosts (Osman, 2015). Most pathogens have a life cycle that includes both plants and soil, although they usually need to infect a specific host to increase their population (Abdul-baki, 1996; Berlin, 2005). Fresh vegetable fruits are fairly perishable because their high moisture content renders them vulnerable to microbial diseases as well as to physiological deterioration (Deribe *et al.,* 2016; Naika *et al.*, 2005; Osman, 2015; Peet and Welles, 2005).

A lack of adequate pre-harvest and post-harvest handling factors may lead to diseases such as those caused by certain pests, namely, *Aculops lycorpersici* (causes rusty brown and coarse surface cracking), and *Thrips tabaci* (causes blossom drop and scarring of the fruit) as well as some virus species such as fruit necrosis caused by the Tomato marchitez virus (ToMarV), fruit marbling caused Pepino mosaic virus (PepMV) (Hanssen, 2010). There are also some bacterial diseases such as bacterial speck caused by *Pseudomonas syringae*, bacterial wilt (*Rhizopus solanacearum*), bacterial Spot (*Xanthomonas campestris)* and bacterial canker (*Clavibacter michiganensis)* (Rashid *et al*., 2016). Tomatoes are also affected by the physiological disorders such as blossom end rot which is caused by a shortage in the availability of calcium, and growth cracks caused by the fruit expansion which stretches the epidermis (skin) beyond its capacity, as well as diseases caused by viruses such as the tomato mosaic virus which have been reported on tomato (Arli-Sokmen and Sevik, 2006; Kennelly, 2009).

In addition, many of the smallholder farmers in South Africa encounter attacks of pathogenic fungi because they possess inadequate technical information, in particular relating to crop diseases (Mandiriza-Mukwirimba *et al*., 2016). It has been reported that the highest percentage cause of the PHLs of tomato fruit are associated with different species of soil-borne phytopathogenic fungi (Etebu *et al*., 2013; Fatima *et al*., 2009). These species cause diseases such as early blight (*Alternaria solani*), anthracnose (*Colletotrichum* spp.), *Sclerotium wilt* (*Sclerotium rolfsii*), damping off (*R. solani*), tomato wilt (*Fusarium oxysporum*), *Phoma* rot (*Phoma destructive)*, Fusarium wilt (*Fusarium oxysporum)*, late blight wilting (*Phytophthora capsici),* Septoria leaf spot (*Septoria lycopersici)* and Rhizopus rot (*Rhizopus stolonifer)* (Fatima *et al*., 2009; Ignjatov *et al*., 2012; Kleemann *et al*., 2008; Kumar *et al*., 2008; Osman, 2015).

These pathogens are severe wound pathogens that may infect the fruit in the packing house, and throughout subsequent handling or storage, thereby limiting production and reducing both crop yield and crop quality (Palou *et al*., 2008). Pathogenic microorganisms in tomato are recognised as a source of potential health hazard to both man and animals following ingestion as a result of their production of mycotoxins, which are capable of causing diseases such as respiratory infection, meningitis, gastroenteritis and diarrhoea in man (Beuchat, 2006).

2.6. Current methods to control post-harvest losses

The response of tomatoes during storage and the post-harvest qualities depend to a certain extent on pre-harvest factors such as cultural practices, the use of natural plant extracts, fertilisers, manure, and genetic and environmental conditions (Meaza *et al*., 2007; Pretorius *et al*., 2003). The losses of untreated fruit from fungal decay have been estimated to be as high as 90% during

post-harvest handling and marketing (Albrigo, 1978). Nevertheless, decay in tomato fruits can be controlled by various methods that are explained below.

2.6.1. Physical control

Controlling the storage temperature is the most well-known physical treatment. Such treatment may be applied in the form of a hot water dip, hot water rinsing and brushing, vapour, hot air and curing (Conway *et al*., 2004; Fallik, 2004). The temperature is calculated using an adaptive management framework and the TOMGRO model (Jones *et al*., 1992; Shamshiri *et al*., 2018). During the entire tomato growing season, optimal air temperatures from 18 to 32.2 °C are considered with 50 to 70% humidity (Peet and Welles, 2005; Shamshiri *et al*., 2018). In the green house, the cultivation of tomato temperature is maintained at 17 to 28°C in coastal areas and 17 to 22 °C in inland areas with 85 to 95% humidity (Puyaubert & Baudouin, 2014). During storage, temperature greatly encourages the rate of respiration of fruits and vegetables, and is certainly one of the most important factors in maintaining the post-harvest quality of tomato fruits (Žnidarčič *et al*., 2010). The chilling injury and ripening rate is minimal at 10 to 15 °C temperature and 85 to 95% relative humidity which may extend the postharvest life of fruits (Žnidarčič *et al*., 2010) . Ultraviolet light (UV-C, 254 nm) hormesis has been identified as one of the physical methods which may be used to stimulate positive responses in order to induce resistance to storage diseases and extend the shelf-life of fruits and vegetables (Liu *et al*., 1993). Tomatoes are treated with UV-C doses from 1.3 to 40 KJ/m^2 in order to induce resistance to the various fungal pathogens that lead to spoilage (Buzby and Hyman, 2012; Tang *et al.*, 2015; Vaklounakis, 1991). However, it must be noted that the use of temperature and UV-C lights during storage changes the aroma profile and the taste of fruits after six days of storage (Baloch & Bibi, 2012; de León-Sánchez et al., 2009; Ponce-Valadez et al., 2016).

2.6.2. Chemical control

Strategies such as synthetic fungicides and pesticides applications, resistant-variety cultivation and crop rotation are used to control fungal diseases in crops with pesticide application remaining as the most common control strategy (Gao *et al*., 2017). These strategies are fairly inexpensive, easy to apply and demonstrate both curative and preventive actions against various infections. The azoxystrobin, fludioxonil, and pyrimethanil fungicides were introduced for the post-harvest management of citrus mould (Kanetis *et al*., 2007). These are also chemicals such as sporekill, vinclozolin, copper oxychloride, benomyl and kitazin that are being used against various fungal pathogens that cause spoilage in fruits and vegetables (Amini and Sidovich, 2010; Lee *et al*., 2012; Leroux, 2007; Nel *et al*., 2007; Sahu *et al*., 2013; Stansly *et al*., 2004). However, the intensive use of synthetic pesticides and fungicides may cause pathogen resistance and pesticide residues and release fungicides in the environment (Ma *et al*., 2015; Yang *et al*., 2015). Their use is becoming more restricted because of the concerns of the consumers and the administration about human health (De Curtis *et al*., 2010; Usall *et al*., 2016). Moreover, effective chemical treatments cannot inhibit the growth of some plant diseases and consumers are increasingly demanding pesticide-free food Wang *et al*., 2009).

2.6.3. Biological control

The non-biodegradable nature and the environmental pollution caused by chemical control applications have led to the alternative production of naturally derived substances (Migliori *et al*., 2017). Among these alternatives, biological control using microorganisms with a strong fungal activity such as growth and ecological fitness has been identified (Pal and Gardener, 2006; Shafiq, 2015; Zong *et al*., 2010). There are mechanisms that have been suggested as being liable to the antagonistic activities of biocontrol agents, including competition for nutrients and space, mycoparasitism of the pathogen, emission of antifungal compounds, antibiotics, volatile

metabolites, induction of host resistance, biofilm development and the participation of the reactive oxygen species (ROS) in the defence response (Dukare *et al*., 2018; Liu *et al*., 2013). These biocontrol agents are safe for the environment, they improve crop production and they limit pesticide resistance (Khonglah & Kayang, 2018; Shafiq, 2015). The successful application of these agents, by either spraying, dipping or drenching, occurs during the postharvest period (Di Francesco *et al*., 2016; Liu *et al.*, 2013). The antagonists used to manage postharvest diseases include bacteria and yeast and it is only recently that fungi have been reviewed as well (Liu *et al*., 2013; Lledó *et al*., 2016; Nunes, 2012). Antagonism is a phenomenon whereby a microorganism inhibits the growth or interferes with the development of another microorganism (Liu, *et al*., 2013; Rodrigo *et al*., 2017). Fungal antagonists such as *Debaryomyces hanseniis, Candida guilliermondii, Byssochlamys spectabilis, Trichoderma harzianum, Trochoderma viride, Phythium debaryanum, Gliocladium roseum, Aureobasidium pullulans, Phythophthora cryptogea* and *Cryptococcus laurentii* are among the effective antagonists that have been identified as the best alternatives to monitor postharvest diseases on citrus fruits (Agrios, 2004; Castoria *et al*., 2001; De Curtis *et al*., 2010; Gomathi and Ambikapathy, 2011; Naglot *et al*., 2015; Zong *et al*., 2010).

CHAPTER 3: MATERIALS AND METHODS

3.1. Sample collection and storage

Fresh and ripe tomatoes of cultivar 'Jasmine' were collected on the 25th January 2016 using the twist and rotate hand method from the research field of the Agriculture Research Council - Vegetable and Ornamental Plants (ARC-VOP) (lat. 25°59"S, 28°35"E, 1200-m altitude), Roodeplaat, Pretoria. The collected tomato samples were properly screened for selection. These samples were processed immediately.

3.2. Fungal isolation and morphological identification

Fungi were isolated from the collected tomato fruit. Tomatoes were cut using a sterile blade. One gram of cut tomato fruit was homogenised in a laboratory blender (BagMixer® 400, Interscience, France) and used for serial ten-fold dilution with sterile distilled water. The dilutions were inoculated on potato dextrose agar (PDA) medium using the spread plate method and incubated at 25 °C for 5 days. Distinct fungal growth mass was sub-cultured on sterile PDA plates following Fusaro (1972) method and incubated for 5 days at 25 °C to obtain pure cultures. These pure fungal isolates were then examined and identified microscopically through slide culture and wet mount techniques following the standardised methodology of Chinedu and Emmanuel (2014) and Yadav and Singh (2016). The wet mount technique was done using Lactophenol blue solution (Sigma Aldrich, Johannesburg, South Africa). All the fungal isolates were stored on PDA at 4 °C prior to use.

3.3. Molecular-based identification of fungal isolates

3.3.1. DNA extraction

All fungal isolates were cultured as previously described in section 3.2. Fungal genomic DNA (gDNA) was extracted from the isolates using the ZR Fungal/Bacterial DNA MiniPrep™ extraction

kit (Zymo Research (Pty) Ltd, United States) following manufacturer's protocol. The extracted gDNA was quantified using a Qubit™ dsDNA broad range (BR) assay kit on a Qubit 2.0 Fluorometer (ThermoFisher Scientific, Edenvale, South Africa). Thereafter, the DNA integrity was ascertained on 1% (w/v) agarose gel after electrophoresis at 80 volts for 60 minutes.

3.3.2. Amplification of the fungal genomic DNA

The identity of the fungal isolates was confirmed by ITS rDNA sequencing. The partial gene sequences of 5.8S-ITS region (1 and 2) were amplified in a thermocycler (Bio-Rad Model T100^{TM}, USA) as described by Al-Najada and Gherbawy (2015) using universal primers (10 µm) of ITS1 (5'-TCCGTAGGTGAACCTTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Adeleke *et al.*, 2010; White *et al*., 1990). Polymerase chain reaction (PCR) was performed under the following conditions: initial denaturation at 94 °C for 30 s, 35 cycles of denaturing at 94 °C for 30 s, annealing at 55 °C for 1 min, extension at 68 °C for 3 min, final extension at 68 °C for 5 min. The amplified DNA was electrophoresed, and detected under ultra violet (UV) light using an EZ Image Analysis System (Bio-Rad, USA). The amplified products were sequenced using Sanger's Dideoxy method on an Applied Biosystems 3730XL sequencer (Biolink, New Delhi, India) at Stellenbosch University, South Africa.

3.3.3. ITS data analysis

The ITS rDNA isolate sequences were compared to relevant fungal sequences from GenBank database (National Centre for Biotechnology Information, USA) (www.ncbi.nlm.nih.gov/blast/), using the Basic Local Alignment Search Tool (BLAST) and the highest matching sequences were downloaded (Naglot *et al.,* 2015). The isolate sequences were aligned with reference sequences from GenBank using the CLUSTALW2 program of the BioEdit software (Thompson *et al.,*1994).

Phylogenetic trees were used to demonstrate the evolutionary relationship between the genotypes obtained in this study and the GenBank (Shukla *et al.,* 2010; El-katatny and Emam, 2012). A neighbour-joining method was used to deduce the evolutionary history of the tree (Saitou and Nei, 1987). The branch length of 3.08517791 of the best possible tree was then shown. The associated taxa were clustered together in the bootstrap test (1000 replicates) into branches that showed the percentage of the replicate trees (Felsenstein, 1985). The evolutionary distances using the number of base substitutions per site were calculated using the Jukes-Cantor method (Jukes and Cantor, 1969). MEGA7 software (Kumar *et al.,* 2016) was used for the evolutionary analysis containing 52 nucleotide sequences. The 1^{st}+2^{nd}+3^{rd}+noncoding positions were included. For each sequence pair all ambiguous positions were eliminated. A total of 537 positions were identified in the final dataset. The tree was drawn to scale, with branch distances in the same units as those of the evolutionary distances used to deduce the phylogenetic tree.

3.4. *In vitro* antagonistic assay of fungal isolates

The dual culture plate technique was used to study the effect of fungal isolates against selected fungal pathogens (Chérif and Benhamou, 1990). Fungal isolates and pathogenic fungi (*Rhizopus stolonifera* ATCC 6227a*, Rhizopus stolonifera* ATCC 6227b, *Geotrichum candidum* ATCC 34614 and *Fusarium solani* ATCC 36031, *Fusarium oxysporum, Rhizoctonia solani, Alternaria solani and Alternaria alternata*) were cultured separately on PDA, incubated as previously described in section 3.2 in preparation for the dual culture technique. The actively developing margins of all the fungal isolates and pathogen species were cut to 5 mm of agar blocks, and were inoculated approximately 3 cm away from each other on the PDA. The fungal isolates and the pathogenic fungi were cultured separately on PDA as control plates and incubated as previously described in section 3.2 simultaneously. The experiment was conducted in triplicate for each set. The

distance of fungal growth was calculated from the point of inoculation to the colony margin on the treated dishes in the direction of the pathogens (Naglot *et al.,* 2015). Percentage growth inhibition was calculated according to Živković et al. (2010) as shown in equation 3.1.

$$\mathrm{PGI} = \frac{\mathrm{A}-\mathrm{B}}{\mathrm{A}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.1)$$

Where:

PGI represents the percentage growth inhibition (%),

A represents the distance (measured in mm) from the point of inoculation to the colony margin on the control plate and;

B represents the distance (measured in mm) of the fungal growth from the point of inoculation to the colony margin on the treated dishes in the direction of the antagonist.

3.5. Evaluation of antagonistic fungal isolates for beneficial properties

The antifungal resistance, pH and bile salt variability were used to screen for probiotic properties on the identified antagonists. Antagonists were cultured on PDA, incubated at 25 °C for 5 days prior to probiotic testing.

3.5.1. Tolerance to low pH

Isolates were cultured on Potato Dextrose Broth (PDB) and optical density (OD) was adjusted to 0.6 using the McFarland Standard 1 (Hardy diognostics, Santa Maria, CA). The OD was measured at 600 nm on a V1100D UV spectrophotometer (Labex (Pty) Ltd, Edenvale, South Africa). The suspension was concentrated by centrifugation at 61 000 rpm for 10 min, washed with phosphate buffered saline (PBS, 10 mM phosphate, pH 7.4), and re-suspended in 3 ml of the same buffered solution adjusted to pH 2.0, 2.5, 3.0 and 7.4 with 1M NaOH and 2M HCl.

Suspensions were incubated for 3 hours, and the aliquots then inoculated (1/10, v/v) on PDB and incubated. After 24 hours the samples were serially diluted with sterile distilled water, and inoculated onto PDA and incubated as previously described in section 3.2 to determine the quantity in colony forming unit per milliliter (cfu/mL) (García-Hernández *et al.,* 2012). The test was performed in triplicate according to a completely randomised design. The survival percentage (S) was calculated using equation 3.2:

$$S = \frac{[(\text{cfu/mL})_{\text{PDB+inoculum pH x}} \times 100]}{(\text{cfu/mL})_{\text{PDB+inoculum pH 7.4}}} \quad \text{..... (3.2)}$$

Where:

S represents the survival percentage,

$(\text{cfu/ml})_{\text{PDB+inoculum pH x}}$ represents the cfu/mL in PDB at the respective pH (x) and;

$(\text{cfu/ml})_{\text{PDB+inoculum pH 7.4}}$ represents the cfu/mL in PDB at pH 7.4.

3.5.2. Bile salt tolerance

Isolates were cultured on PDB and optical density (OD) was adjusted to 0.6 using the McFarland Standard 1 (Hardy diognostics, Santa Maria, CA). The OD was measured at 600 nm on a V1100D UV spectrophotometer (Labex (Pty) Ltd, Edenvale, South Africa).. The suspensions were serially diluted, cultivated on PDA containing 1, 2 and 3% (w/v) of bile salt (Ox-Gall, Oxoid, UK) and incubated as previously described on section 3.2. The number of colonies in each millimeter of treatment was determined (García-Hernández *et al*., 2012). The assay was performed in triplicates and the survival percentage (S) was calculated applying the formula in equation 3.3:

$$S = \frac{[(\text{cfu/mL})_{\text{PDA+salt}} \times 100]}{(\text{cfu/mL})_{\text{PDA}}} \quad \text{..... (3.3)}$$

Where:

S represents the survival percentage,

$(cfu/ml)_{PDA+Salt}$ represents the cfu/mL on PDA with bile salt and;

$(cfu/ml)_{PDA}$ represents cfu/mL on PDA without bile salt.

3.5.3. Antifungal susceptibility

Well diffusion method was used to assess the susceptibility of various fungal isolates to known antifungals (Boyer, 1976; Kefi *et al*., 2015). Fungi were cultured in a conical flasks (150 ml) containing 50 ml of PDB and incubated in an Orbital platform shaking incubator, at 133 rpm (Scientific engineering (Pty) Ltd, Stormill, South Africa) at 25 °C for 5 days. Thereafter, 100 µl aliquots of prepared fungal cultures were spread on the PDA plates. The antifungal discs were placed at the centre of the agar and incubated as previously described in section 3.2. The antifungal discs used included 20 µg Amphotericin B (Amp B), 10 µg Clotrimazole (Clotri), 25 µg Fluconazole (Fluco), 1 µg Flucytosine (Flucy), 10 µg Ketoconazole (Keto), 10 µg Mecillinam (Meci), 10 µg Nystatin (Nysta) and 10 µg Penicillin G (Peni G) obtained from Mast discsTM (Mast diagnostics, Merseyside, United Kingdom). Inhibition zone created around the antifungal discs was measured to determine the sensitivity of fungal isolates. The diameter of inhibition zone was measured using a ruler and it reflected antifungal susceptibility. Inhibition zones around the discs greater than 8 mm implied susceptibility of the respective antagonist to the antifungal agent, whereas inhibition zones less than 8 mm implied resistance (Bhalodia and Shukla, 2011). The experiment was conducted in triplicate, and results were recorded as average of the three readings (Makete, *et al.,* 2017).

3.6. Effect of antagonists as bio-preservative agents during storage

3.6.1. Preparation of fungal antagonists

The fungal antagonists were inoculated on PDA and incubated at 25 °C for seven days. Fungal spores were obtained by flooding the surface of the culture with sterile distilled water containing 0.05% (v/v) Tween-80 (Zhu *et al.,* 2010). The spores were suspended in 50 ml of 10% glycerol and freeze dried using a single gauge vacuum freeze dryer (Air and vacuum technologies, Johannesburg) following the protocol of Nakasone *et al*. (2004) with modification as specified below. The solution was lyophilised by a 24 hours process of freezing (at a rate of -1 °C per minute to -40 °C), vacuuming (10 mbar abs) and drying (-30 °C for 7 hrs, adjusted to -10 °C, then increased to +30 °C). Thereafter, the viable spores were enumerated using a hemocytometer (ThermoFisher Scientific, Edenvale, South Africa) (Supplementary Table S1). The freeze dried fungal antagonists were stored at 4 °C prior to use.

3.6.2. Tomato fruit (cultivar 'Jasmine') used for storage

Five-week-old tomato seedlings (cultivar 'Jasmine') were transplanted to 16 000 plants/ha in a sandy loam soil and cultivation practices were followed as described by Maboko and Du Plooy (2018). Tomato plants were cultured on the field from December 2016 to April 2017 at ARC-VOP. During the growing season, the mean temperatures were 33 °C day and 12 °C night. The plants were foliar sprayed with Copper-count N, Sporekill®, Benomyl, Bravo and Ridmol according to manufacturer's recommendations, to control powdery mildew, blight and leaf spot diseases.

Ripened tomatoes were harvested at 100 days after transplantation, and were immediately transported to the ARC-AP for storage. Tomatoes were selected based on size, free of physical

injuries and/or spoilage and washed in a 2% (v/v) sodium hypochlorite solution for 2 min, rinsed with tap water, and air-dried prior to use (Zhu *et al.,* 2010).

3.6.3. Test for efficacy of fungal antagonists as biopreservative agents

The storage trial was designed using the experimental designs of Bhagwat and Datar (2014) with modifications as described. One hundred and eighty ripe tomatoes were randomly distributed into six sets of 30. Each set was further divided into three equal sets of 10 fruits. The distribution of the fruits was done on both the ambient storage conditions, namely, the 8 °C refrigeration and the uncontrolled room temperature (Reddy *et al.,* 2000). The treatment sets were as follows with each fungal antagonist and the fungicide being diluted with distilled water to 1% concentrations. The negative control comprised tomatoes stored without any treatment and the positive control comprised tomatoes sprayed with Sporekill® (Hygrotech) fungicide and stored for 15 days (Žnidarčič *et al*., 2010). Tomatoes stored at two different conditions were assessed for weight loss and spoilage percentage.

Weight loss

Weight loss of tomatoes was assessed over a 15 day period, and were weighed non-destructively at 5 days interval. The weight loss was performed in triplicates and the difference between the initial and the final fruit weights were calculated by the standard method in equation 3.4 according to Fagundes *et al.* (2015).

$$W = \frac{M - Mx}{M} \times 100 \quad \text{.....(3.4)}$$

Where:

W represents weight loss percentage,

M represents the weight of tomatoes at the beginning of storage and;

Mx represents the weight of tomatoes during x interval.

Spoilage percentage

The total number of spoilt tomatoes, were visually counted at 5 days interval. The difference between the initial and the final fruit spoilage was considered during each storage interval and calculated as percentages by the standard method according to Fagundes *et al*. (2015). The spoilage percentage was calculated by the standard method in equation 3.5.

$$R = \frac{Mx}{M} \times 100 \quad \text{.....(3.5)}$$

Where:

R represents spoilage percentage,

Mx represents the number of spoiled tomatoes during x interval and;

M represents the total number of tomatoes per treatment.

3.7. Statistical analysis

The experimental data was subjected to one way analysis of variance (ANOVA). The Shapiro-Wilk's test was performed on the standardised residuals to test for deviations from normality (Shapiro and Wilk, 1965). In cases where a significant deviation from normality was observed due to skewness, the outliers were removed until significant deviation was either normal or symmetrically distributed (Glass *et al*., 1972). The student's t-least significant differences (t-LSDs) were calculated at a 5% significance level ($P < 0.05$) to compare the means of the significant source effects (Snedecor and Cochran, 1956). The above analysis was performed using SAS version 9.3 statistical software (SAS, 1999) and Genstat Release 18.

CHAPTER 4: RESULTS

4.1. Fungi morphology

A total of 40 fungal colonies were obtained from tomatoes and their morphological characteristics are presented in Table 4.1. Most of the isolated colonies were aerial, and the fully grown colonies were dark in colour (Figure 4.1). For some of these isolates, centre of the colonies are darker in colour (black, grey and green), but lighter on the edges (white, yellow and light grey) (Figure 4.1B). In addition, different colonies produced different types of spores and mycelia (Figure 4.1C and D).

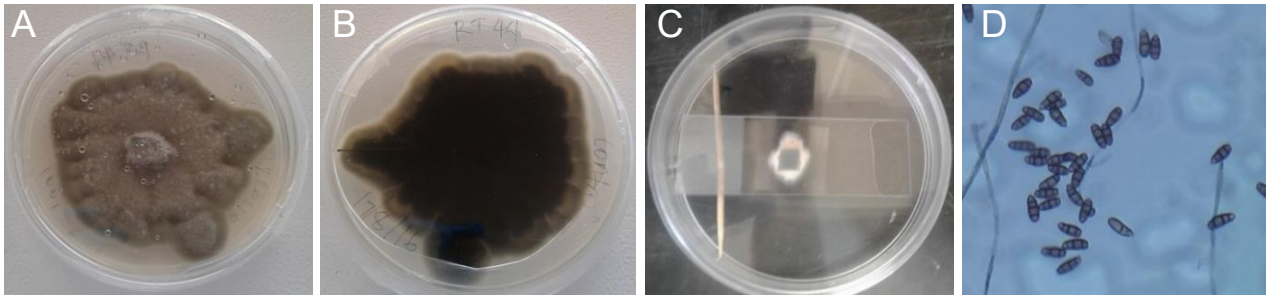


Figure 4.1: Morphological characteristics of (A) Topside and (B) bottom side of *Penicillium griseofulvum* as cultured on PDA medium, (C) *Epicoccum thailandicum* cultured on PDA medium using culture slide technique, (D) Ascomycetes of Curvularia sp. under a light microscope.

Table 4.1: Cultural, morphological and microscopic characteristics of fungal colonies from tomato fruit

Fungal Isolates	Pigmentation		Mycelial Growth			Hyphae	Mould/ Yeast
	Color	Hyaline/ Dematiaceous	Circular/ Irregular	Smooth/ Rough	Aerial/ Vegetative	Aseptate/ Septate	
RT7	Green	Dematiaceous	Irregular	Smooth	Aerial	Septate	Mould
RT8 = RT18 = RT6 = RT24A	Pink- Cream white	Hyaline	Circular	Rough	Aerial	Septate	Mould
RT9	Grey	Dematiaceous	Irregular	Smooth	Aerial	Septate	Mould
RT10A	Olive green	Dematiaceous	Circular	Rough	Aerial	Septate	Mould
RT11 = RT5	Orange	Dematiaceous	Irregular	Rough	Aerial	Septate	Mould
RT15 =RT12 = RT17 = RT21 = RT32	Dark brown	Dematiaceous	Irregular	Smooth	Aerial	Aseptate	Mould
RT19 = RT13 = RT16 = RT4	Purple	Hyaline	Circular	Rough	Aerial	Septate	Mould
RT20 = RT29 = RT33 = RT36	Black-olive green	Dematiaceous	Circular	Rough	Aerial	Septate	Mould
RT24B	Pale orange-white	Hyaline	Circular	Smooth	Aerial	Septate	Mould
RT26	Brown	Dematiaceous	Irregular	Rough	Aerial	Aseptate	Mould
RT27= RT10B	Black	Dematiaceous	Irregular	Smooth	Aerial	Septate	Mould
RT31 =RT25 = RT34	Grayish green	Dematiaceous	Irregular	Rough	Aerial	Septate	Mould
RT35	Black	Dematiaceous	Circular	Rough	Aerial	Septate	Mould
RT37 = RT38 = RT39 = RT42	Olive green	Dematiaceous	Irregular	Smooth	Aerial	Septate	Mould
RT41 =RT1 = RT2	White	Hyaline	Circular	Smooth	Aerial	Septate	Mould
RT43 = RT40	Dark Grey	Dematiaceous	Circular	Rough	Aerial	Septate	Mould
RT44	Grayish white	Hyaline	Circular	Smooth	Aerial	Septate	Mould

4.2. Molecular identification

Seventeen distinct operational taxonomic units (OTUs) were clustered based on 97% sequence similarity from the identified 40 fungal colonies (Table 4.2; Supplementary Figure S1). Of the 17 OTUs, 10 genera were obtained (Table 4.3). The obtained genera were *Penicillium, Fusarium, Curvularia, Alternaria, Cladosporium, Lecythophora, Aureobasidium, Byssochlamys, Retroconis,* and *Epicoccum*, with *Penicillium* and *Fusarium* having the highest occurrence of 22.5% each (Table 4.2; Table 4.3).

Table 4.2: Molecular identification of fungal isolates

OTU No:	Fungal isolates	Molecular identification	Percentage similarity	Accession numbers
OTU1	RT7	*Penicillium citreosulfuratum*	99	MG975610
OTU2	RT8 =RT24A = RT18 = RT6	*Fusarium verticillioides*	99	MG975609
OTU3	RT9	*Penicillium thomii*	98	MG975612
OTU4	RT10A	*Byssochlamys spectabilis*	99	MG975613
OTU5	RT11= RT5	*Lecythophora sp.*	98	MG975615
OTU6	RT15 = RT32 = RT21 = RT17 = RT12	*Curvularia kusanoi*	99	MG975620
OTU7	RT19 = RT4 = RT13 = RT16	*Fusarium oxysporum*	99	MG975622
OTU8	RT20= RT36 = RT33 = RT29	*Alternaria tenuissima*	99	MG975636
OTU9	RT24B	*Epicoccum thailandicum*	97	MG975626
OTU10	RT26	*Retroconis fusiformis*	99	MG975628
OTU11	RT27 = RT10B	*Aureobasidium pullulans*	99	MG975614
OTU12	RT31 = RT34 = RT25	*Penicillium crustosum*	99	MG975627
OTU13	RT35	*Alternaria brassicicola*	86	MG975635
OTU14	RT37 = RT42 = RT39 = RT38	*Cladosporium sp.*	99	MG975639
OTU15	RT41 = RT2 = RT1	*Penicillium sp.*	99	MG975641
OTU16	RT43 = RT40	*Curvularia sp.*	99	MG975643
OTU17	RT44	*Penicillium griseofulvum*	92	MG975644

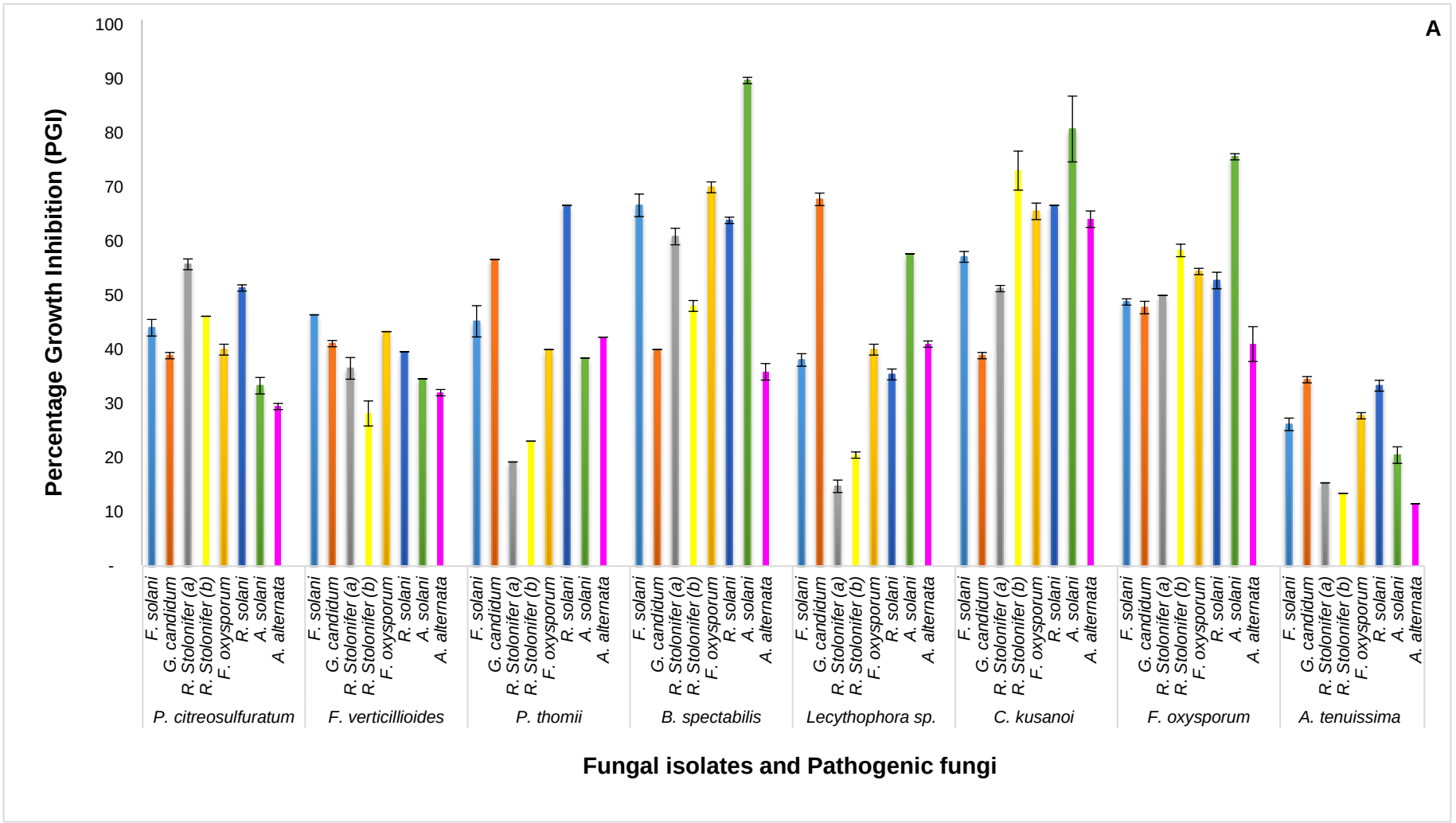
= ; Identical

Table 4.3: Prevalence of fungal isolates

Fungal genera	Number of isolates	% Frequency
Penicillium	9	22.5
Fusarium	9	22.5
Curvularia	6	15.0
Alternaria	5	12.5
Cladosporium	4	10.0
Lecythophora	2	5.0
Aureobasidium	2	5.0
Byssochlamys	1	2.5
Retroconis	1	2.5
Epicoccum	1	2.5
Total	**40**	**100**

4.3. Antagonistic effects of fungal isolates against selected fungal pathogens

Antagonism activities of the 17 fungal species against fungal pathogens showed variabilities in the percentage of inhibition, but only *Byssochlamys spectabilis, Curvularia kusanoi, Epicoccum thailandicum* and *Retroconis fusiformis* were showed more inhibition percentage (Figure 4.3A and B; Supplementary Figure S2; Supplementary Figure S3). Amongst the 4 fungal species, *Epicoccum thailandicum* was found to be the most antagonistic, whereas *Alternaria tenuissima* was found to be the least antagonistic (Figure 4.3A and B; Supplementary Figure S3).


A
Percentage Growth Inhibition (PGI)
100
90
80
70
60
50
40
30
20
10
-
F. solani
G. candidum
R. Stolonifer (a)
R. Stolonifer (b)
F. oxysporum
R. solani
A. solani
A. alternata
P. citreosulfuratum
F. verticillioides
P. thomii
B. spectabilis
Lecythophora sp.
C. kusanoi
F. oxysporum
A. tenuissima
Fungal isolates and Pathogenic fungi

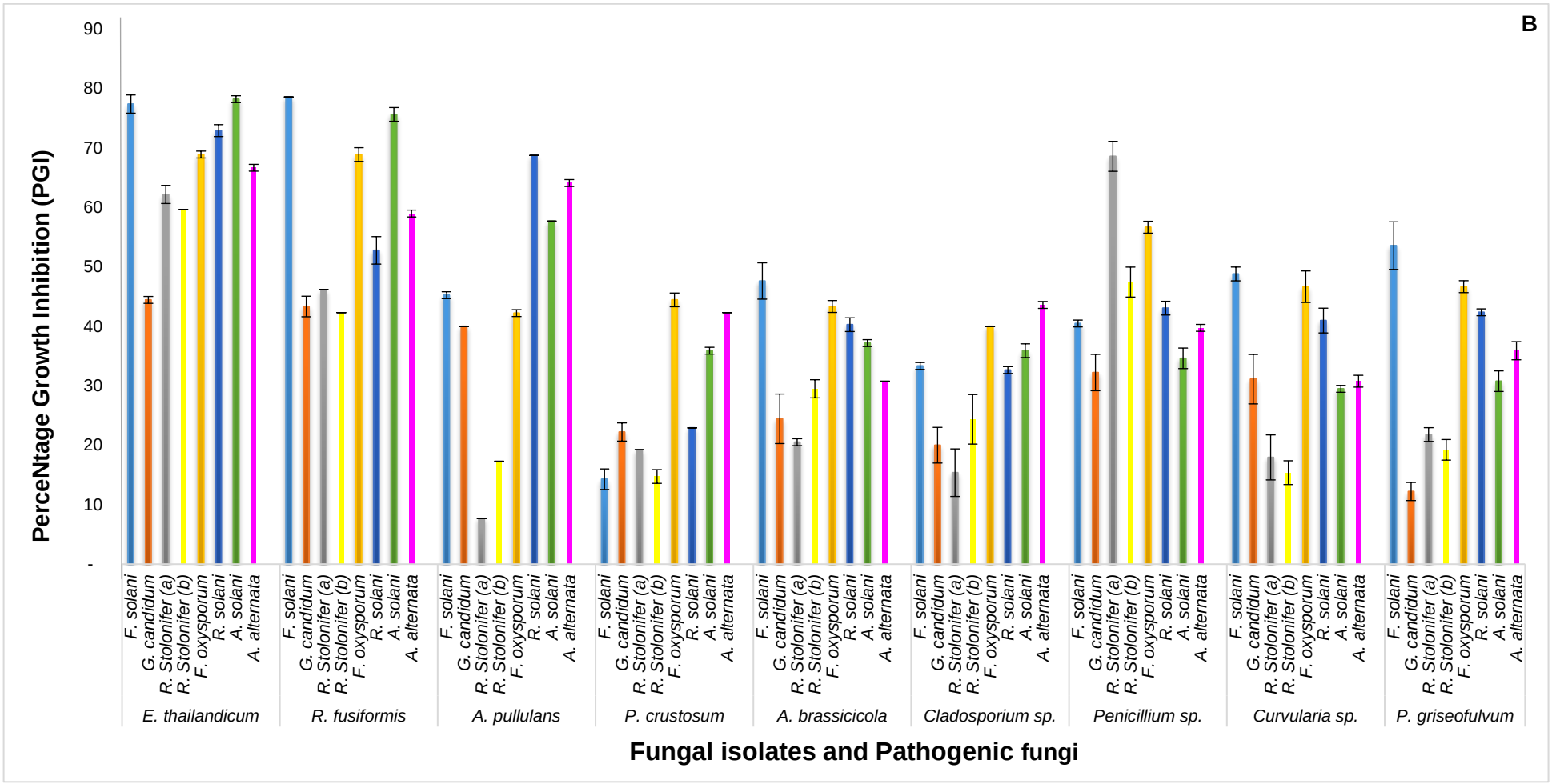


Figure 4.3 (A) and (B): The antagonistic ability of fungal isolates against pathogenic fungi

4.4. Beneficial (probiotic potential) assay

The potential abilities of the identified isolates to survive under the conditions of transit through the gastrointestinal tract are as presented in Table 4.4, Figure 4.4 and 4.5.

4.4.1. Tolerance to bile salt

The ability of the 4 fungal antagonists to resist inhibitory factors in the gastrointestinal tract such as bile salt concentrations was evaluated. There was a vast difference in the viable counts of all the isolates in the presence of 1, 2 and 3% bile salt. *B. spectabilis* and *R. fusiformis* species survived in the presence of 1, 2 and 3% bile salt, while the viability of *C. kusanoi* and *E. thailandicum* species decreased significantly (P > 0.05) (Figure 4.4). The results indicate that *B. spectabilis* and *R. fusiformis* were tolerant to bile salt than *C. kusanoi* and *E. thailandicum.*

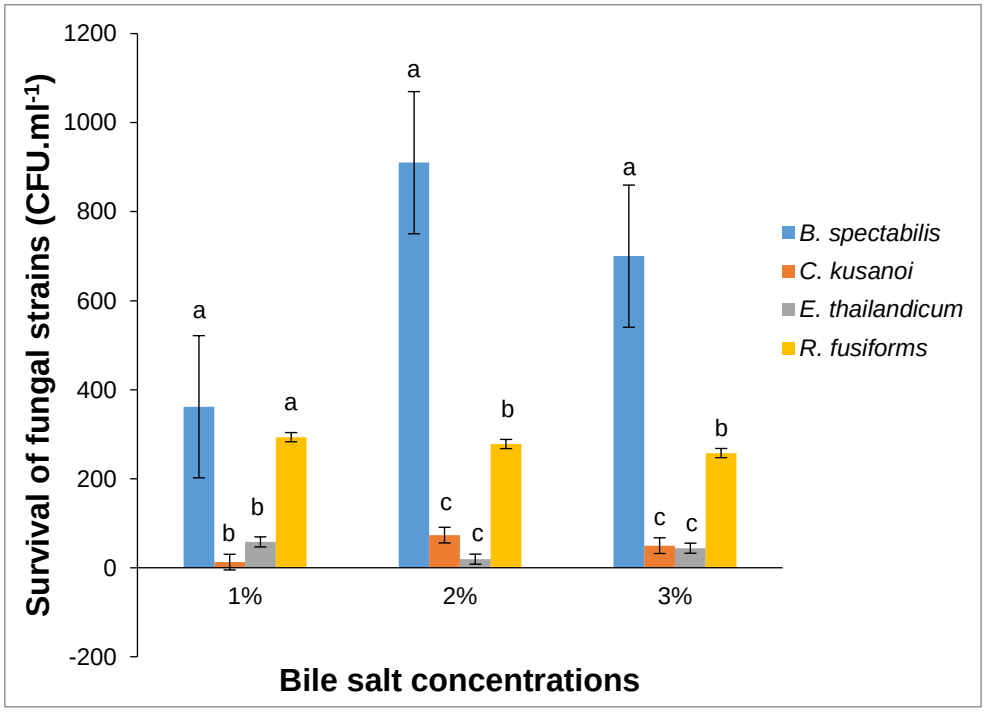


Figure 4.4: Adaptation of fungal isolates to different bile salt concentrations. Values are Mean±SD (n = 3). Similar letters on the bars represents no significance (P > 0.05), whereas different letters represents significant difference (P < 0.05)

4.4.2. pH tolerance

The effect of different acidic conditions on the viability of *C. kusanoi*, *E. thailandicum, B. spectabilis* and *R. fusiformis* is shown in Figure 4.5. In this study, *E. thailandicum* was resistant to all the different acidic concentrations with various survival percentages. *R. fusiformis* was susceptible to pH 2.0 and 2.5, and resistant to pH 3.0 and 7.4. *B. spectabilis* was susceptible to pH 2.5, 3.0 and 7.4 and showed resistance when exposed to pH 2.0. Lastly, C. *kusanoi* was susceptible to all the different acidic concentrations with various survival percentages. The results indicate that *E. thailandicum* and *R. fusiformis* were more acid tolerant than *C. kusanoi* and *B. spectabilis.*

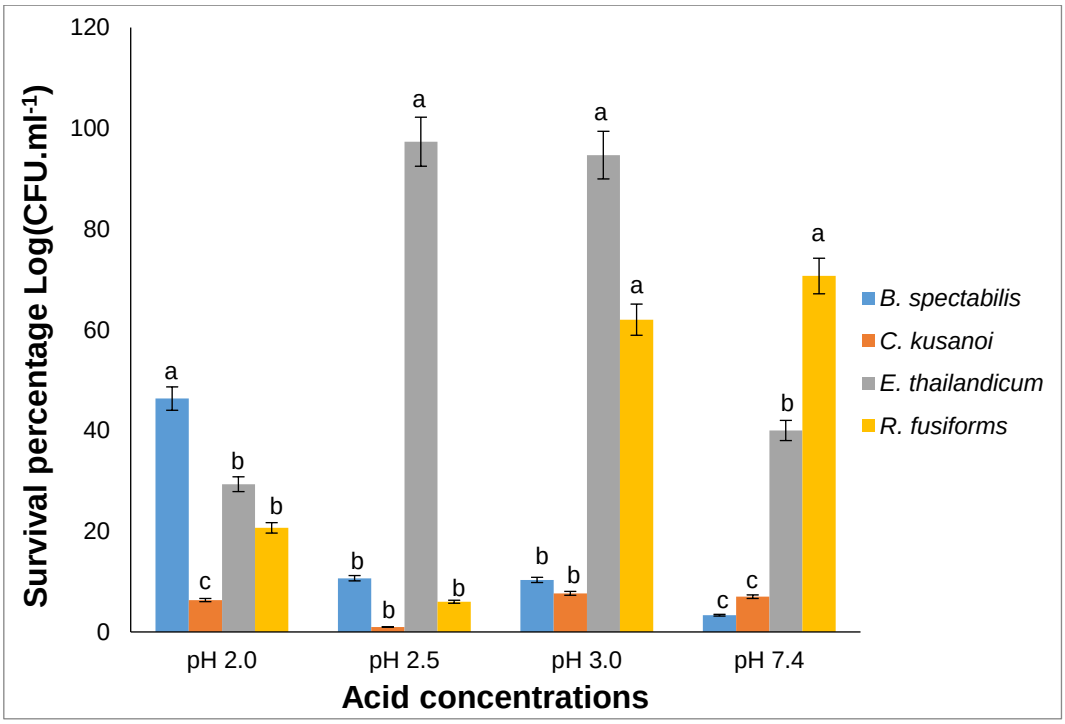


Figure 4.5: Adaptation of fungal isolates to various pH. Values are Mean±SD (n = 3). Similar letters on the bars represents no significance (P > 0.05), whereas different letters represents significant difference (P < 0.05)

4.4.3. Antifungal susceptibility tests

For this section, *C. kusanoi*, *E. thailandicum, B. spectabilis* and *R. fusiformis* were assayed for their susceptibility to eight antifungals, using the disc diffusion method. *R. fusiformis* was resistant to majority of the antifungal drugs as compared to *B. spectabilis*, *C. kusanoi* and *E. thailandicum*. *R.*

fusiformis was resistant to the seven antifungals and susceptible only to Amphotericin B (Amp B). *E. thailandicum* was resistant to six antifungals and susceptible to Clotrimazole (Clotri) and Mecillinam (Meci) whereas, *C. kusanoi* was also resistant to six antifungals and susceptible to Amphotericin B (Amp B) and Mecillinam (Meci). Lastly, *B. spectabilis* was resistant to five antifungals and susceptible to Amphotericin B (Amp B), Clotrimazole (Clotri) and Nystatin (Nysta) (Table 4.4). All the four fungal antagonists were resistant to Fluconazole (Fluco), Flucytosine (Flucy), Ketoconazole (Keto) and Penicillin G (Peni G) antifungal drugs with various zones of inhibition.

Table 4.4: Antifungal activity of fungal isolates of tomato.

Fungal isolates	Antibiotic drugs							
	Zone of inhibition in mm							
	Amp. B	Clotri.	Fluco.	Flucy.	Keto.	Meci	Nysta.	Peni G
B. spectabilis	9.3 ± 1.5 s	8.7 ± 0.6 s	0.0 ± 0.0 r	0.0 ± 0.0 r	4.3 ± 0.6 r	0.0 ± 0.0 r	17.0 ± 2.0 s	0.0 ± 0.0 r
C. kusanoi	10 ± 0.0 s	4.0 ± 1.0 r	4.7 ± 0.6 r	0.0 ± 0.0 r	0.0 ± 0.0 r	9.0 ± 1.0 s	4.7 ± 0.6 r	6.7 ± 0.6 r
E. thailandicum	0.0 ± 0.0 r	10.3 ± 0.6 s	0.0 ± 0.0 r	0.0 ± 0.0 r	0.0 ± 0.0 r	8.7 ± 0.6 s	0.0 ± 0.0 r	5.7 ± 0.6 r
R. fusiforms	10 ± 0.0 s	3.3 ± 0.6 r	4.3 ± 0.6 r	0.0 ± 0.0 r	0.0 ± 0.0 r	0.0 ± 0.0 r	6.0 ± 0.0 r	4.7 ± 0.6 r

Amp B = Amphotericin B, Clotri = Clotrimazole, Fluco = Fluconazole, Flucy = Flucytosine, Keto = Ketoconazole, Meci = Mecillinam, Nysta = Nystatin, Peni G = Penicillin G, r=resistant (< 8 mm), i = intermediate (= 8 mm) and s = susceptible (> 8 mm). Values are means of triplicate determinations with standard deviations (Mean ± SD).

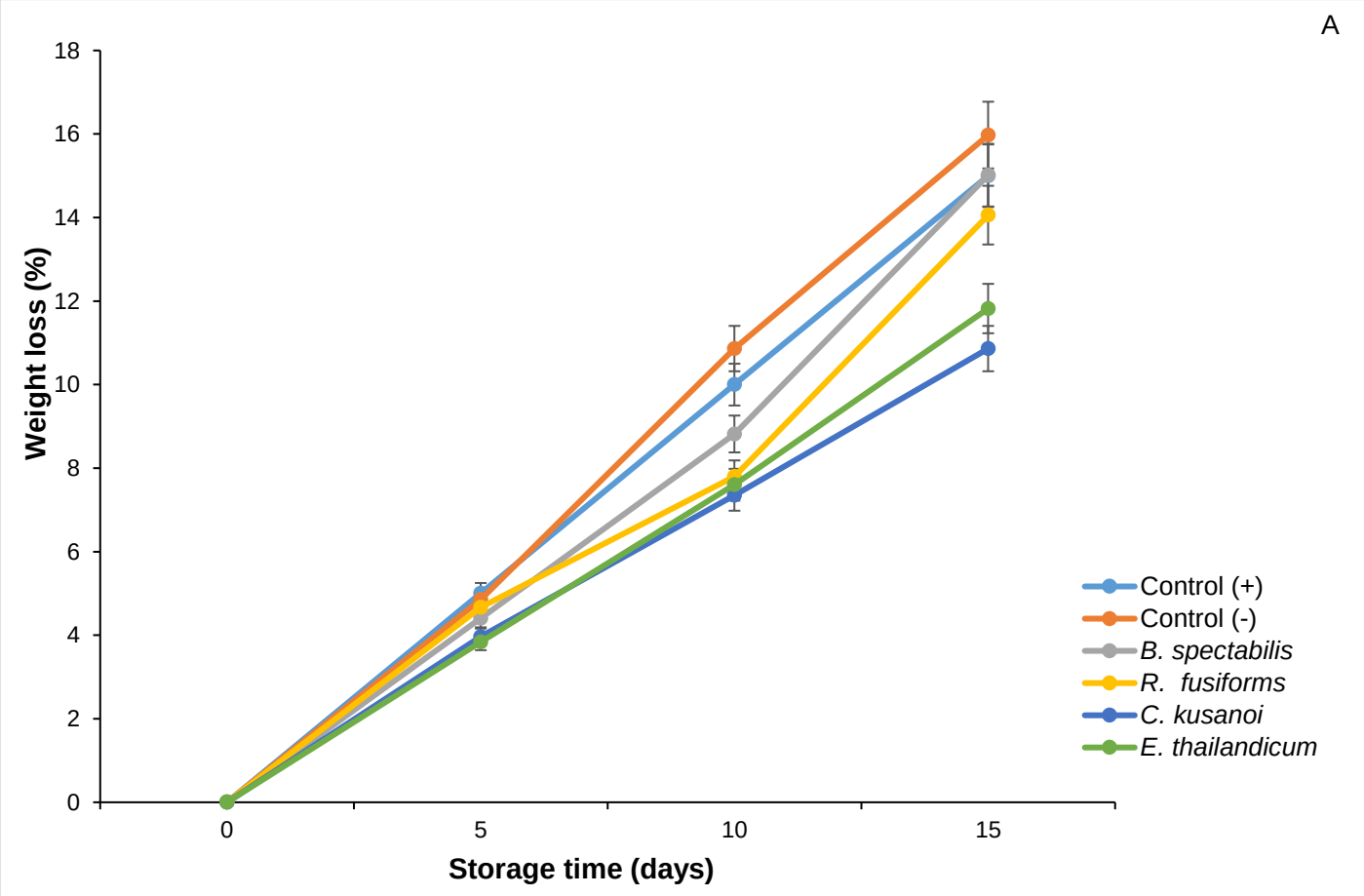
4.5. Effect of antagonists as bio-preservative agents during storage

4.5.1. Weight loss

Tomatoes stored at a temperature of 8 °C and uncontrolled room temperature showed significant weight loss ($P < 0.05$) in all treatments (Figure 4.6A and B; Supplementary Table S2). On the 5th day of storage at 8 °C, tomatoes treated with *R. fusiforms* resulted in more weight loss as compared to the 10th day and thereafter, increased again on the 15th day. On the 15th day at the same storage condition, *C. kusanoi* and *E. thailandicum* treatments resulted in less weight loss (10.86% and 11.82% respectively), as compared to other treatments (above 14%) (Figure 4.6A). Similarly, on the 10th day at 8 °C refrigeration, tomatoes treated with *C. kusanoi, E. thailandicum* and *R. fusiforms* exhibited a significantly ($P < 0.05$) lower weight loss than *B. spectabilis* and both controls (Figure

4.6A), whereas at uncontrolled room temperature storage (Figure 4.6B) the same effect was observed with *C. kusanoi* treatment losing the least weight.

During the uncontrolled room temperature storage, the positive control treatment lost less weight as compared to 8 °C refrigeration against other treatment. On the other hand, by the 15th day of storage the negative control tomatoes had the maximum of 22.36% weight loss (Figure 4.6B). Thus, at both storage conditions (8 °C refrigeration and uncontrolled room temperature), weight loss increased throughout the 15 days storage period; however, it was higher for uncontrolled room temperature storage as compared to 8 °C refrigeration with weight loss of 18.21% and 13.41% respectively (Figure 4.6A and B; Supplementary Table S2).



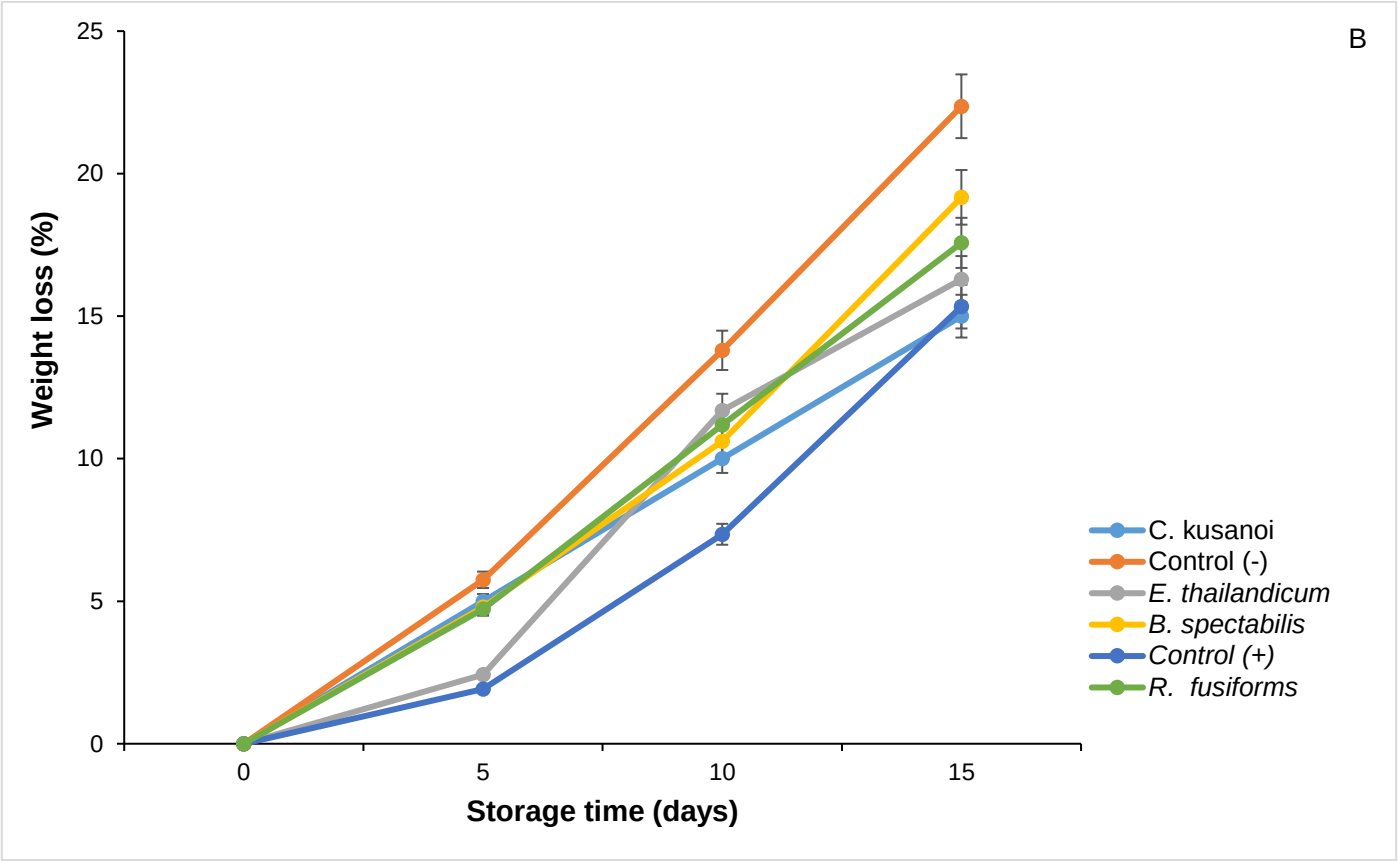


Figure 4.6: Effect of postharvest spraying with different treatments on weight loss of tomato fruit stored at (A) 8 °C refrigeration and (B) uncontrolled room temperature storage. Negative control (-) are untreated tomatoes, whereas positive control (+) represents tomatoes treated with Sporekill® fungicide.

4.5.2. Spoilage percentage

Any damage on the skin of tomatoes, for example shrinking, softening and infection were considered as a sign of spoilage (Bhagwat and Datar, 2014). The maximum spoilage percentage was 15.97% and 22.36% at 8 °C and uncontrolled room temperature respectively, whereas the minimum spoilage percentage was 10.86% and 15.34% respectively (Table 4.5 and Supplementary Table S3). Tomatoes treated with *C. kusanoi,* and *E. thailandicum* reduced more spoilage of tomatoes as compared to other treatments (Table 4.5). On the 15th day, at both storage temperatures, negative control, *R. fusiforms* and *B. spectabilis* showed an increase in spoilage (46.67% and 60%; 40% and 53.33%; 30% and 50% respectively), but the positive control, *E. thailandicum* and *C. kusanoi* (20% and 23.33%; 20% and 36.67%; 20% and 26.67% respectively) were still at moderate state (Table

4.5 and Supplementary Table S3). In addition natural spoilage in tomatoes treated with *B. spectabilis* and *R. fusiforms* were much higher at both storage conditions after 15 days of storage. The percentage spoilage with *C. kusanoi* and *E. thailandicum* treatments was much less on the 10th and 15th days in comparison to the negative control, and treatments with *B. spectabilis, R. fusiforms.* On the 15th day of storage at 8 °C and uncontrolled room temperatures, tomato spoilage resulted in an average of 30% and 41.67% respectfully. These results indicate that *C. kusanoi* and *E. thailandicum* are able to inhibit spoilage of tomato fruit caused by different fungal pathogen.

Table 4.5: Effect of antagonist application on tomato spoilage during storage

Storage conditions	Days	Spoilage percentage					
		B. spectabilis	***C. kusanoi***	***E. thailandicum***	***R. fusiforms***	**Control (-)**	**Control (+)**
Controlled (8°C) temperature	5	6.67	3.33	3.33	6.67	13.33	0.00
	10	16.67	6.67	10.00	20.00	26.67	6.67
	15	30.00	20.00	20.00	40.00	46.67	20.00
Uncontrolled (Room) temperature	5	23.33	16.67	10.00	30.00	40.00	3.33
	10	33.33	23.33	23.33	46.67	53.33	10.00
	15	50.00	26.67	36.67	53.33	60.00	23.33

Values are means of triplicate determinations with standard deviations (Mean ± SD) (n = 30 tomatoes). Negative control (-) are untreated tomatoes, whereas positive control (+) represents tomatoes treated with Sporekill® fungicide.

CHAPTER 5: DISCUSSION

Fungal contamination is one of the major causes of spoilage in food, leading to severe health problems in human beings, ranging from immune suppression to death in severe cases. Thus, food safety remains a major global concern when addressing the prevailing health problems especially in developing countries. The pursuit of natural methods for food preservation has gained huge attention recently because of the consumers' demand for chemical-free, safe, and minimally processed food with extended shelf life.

Amongst the identified 17 representative OTUs from the 40 fungal isolates, *Curvularia kusanoi, Epicoccum thailandicum, Byssochlamys spectabilis,* and *Retroconis fusiformis,* showed the highest antagonistic properties and may be used as bio-preservatives against various pathogens (Figure 4.3A and B; Supplementary Figure S3). *C. kusanoi* was found to inhibit seven pathogens (Supplementary Figure S3). This is probably due to its production of metabolites which possess antibiotic properties such as curvularin and αβ-dehydrocurvularin (Manamgoda *et al.*, 2015; Robeson and Strobel, 1981; Tan *et al*., 2018). According to a study conducted by Xie *et al.* (2009), curvularin and αβ-dehydrocurvularin appeared to be the two main antifungal metabolites that inhibit the growth of pathogens when bioassayed against gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* as well as fungi *Sclerotinia sclerotiorum* and *Saccharomycetes cerevisiae*. In addition, Ghisalberti *et al.* (1993) posited that *Curvularia* are able to act as inhibitors in delaying the cell division of pathogens which may impede fungal diseases in tomatoes. The inhibitory potential of *E. thailandicum* was similar to that of *C. kusanoi,* which also inhibited seven pathogens (Figure 4.3A and B; Supplementary Figure S3). A study by Fávaro *et al.* (2012) indicated that *E. thailandicum* produces coloured pigments that are used as antibacterial and antifungal agents in soil. *E. thailandicum* together with *R. fusiformis* belong to a heterogeneous group of Ascomycota (anamorphic fungi) called Deuteromycota (mitosporic fungi. These antagonists are widely distributed and commonly isolated from air, soil and food stuff (de Hoog and van der Vegte, 1989; Thambugala *et al.,* 2017).

R. fusiformis has the ability to antagonise *F. solani, R. solani, A. solani, F. oxysporum* and *A. alternata* (Figure 4.3A and B; Supplementary Figure S3). *R. fusiformis* has been isolated from soil

and identified as an antagonistic fungus against parasitic fungal pathogens (Mungía-Pérez *et al*., 2011; Nonaka *et al*., 2012). *R. fusiformis* (anamorph *Hyalodendron fusiforme*) is known to produce hyalodendrin, which is an antibiotic produced by hyalodendron, and also anti-fungal products such as hyalodendrosides A and B (Bills *et al*., 2000; de Hoog and van der Vegte, 1989; Reddy and Bilgrami, 1971). *B. spectabilis* was able to antagonise *F. solani, R. solani, A. solani, F. oxysporum* and *R. stolonifer* (Figure 4.3A and B; Supplementary Figure S3). The antagonistic ability of *B. spectabilis* is probably due to the production of viriditoxin, which has antibiotic properties (Samson *et al.,* 2009; Noh *et al.,* 2017; Ansari, 2017; Houbraken *et al*., 2008). *B. spectabilis* is described as a strong heat-resistant environmental mould that is commonly found in composts, soils and food products (Houbraken *et al.*, 2008; Mioso *et al.,* 2015). These results are in accordance with the findings of the study by Rodrigo *et al.* (2017) in which the antagonistic capabilities of *B. spectabilis* (anamorph *P.variotii*) was assessed against *B. mediterranea*, *F. moniliforme,* and *P. cinnamomi*. Their study also highlighted that *B. spectabilis* or its bioactive compounds had antagonistic effects towards these phytopathogens. The literature search revealed that biotechnological potential of *B. spectabilis*, *C. kusanoi*, *E. thailandicum* and *R. fusiformis* as well as their interaction with food are unknown and there have been no studies on their antagonistic interaction with tomato fruit.

Although 13 of the fungal isolates identified in this study were not antagonists of the tested fungal pathogens, few of them have been reported as fungal antagonists. For instance, *A. pullulans* is considered to have biotechnological significance as well as an effective biocontrol agent, but could be pathogenic to fruits (Castoria *et al.,* 2001b; Chi *et al.,* 2009; Ippolito and Nigro, 2000; Schena *et al*., 2003; Zalar *et al.*, 2008). Furthermore, their biocontrol capabilities are compromised by their mycotoxins production such as alternariol, methyl ester, altenuene, altertoxin, and tenuazonic acid (Lee *et al.,* 2015; Logrieco *et al.*, 2009). In spite of the potential antagonistic capabilities of the two most dominant species, *Penicillium* and *Fusarium* with frequency percentage of 22.5% each (Table 4.3) no such results were recorded in the present study.

Penicillium species such as *P. citreosulfuratum, P. thomii, P. crustosum* and *P. griseofulvum,* were identified in this study, which extended across OTU1, 2, 11 and 17 respectively (Table 4.2;

Supplementary Figure S1). Several studies have reported that *Penicillium* species have the potential to produce antifungal antibiotics such as, griseofulvin, extrolites and rastin A, as well as enzymes such as β-glucanase, cyclopenin, penitrem A, cyclopenol, roquefortine C and terrestric acids, which are associated with food spoilage (Barkai-Golan and Paster, 2008; Frisvad *et al*., 2006; Refai *et al*., 2015; Visagie *et al*., 2014). On the other hand the *Fusarium* species which were identified as *F. verticillioides* and *F. oxysporum*, spanned through OTU7 and 6 respectively (Table 4.2; Supplementary Figure S1). The *Fusarium* species identified were explained to produce fusaric acid, fumonisins and fusaproliferin toxins, which colonises, blocks and break down the xylem of the host plant, leading subsequently to wilting, cortical rot, yellowing and the eventual death of the plant (Hassan *et al.*, 2014; Oren *et al.,* 2003; Ramaiah and Garampalli, 2015; Sánchez-Rangel *et al.*, 2012; Selim and El-Gammal, 2015). Comparable results were obtained by Thiyam and Sharma (2013) and Ezikanyi (2016), who isolated and identified several fungal pathogens that cause postharvest disease and the deterioration of tomatoes in Asia and Nigeria respectively. They reported that *Aspergillus*, *Penicillium,* and *Fusarium* had been identified as the dominant microorganisms in spoiled tomatoes as compared to other pathogenic fungi. Furthermore, they explained that strains of some of these fungi are known to produce toxins that may adversely affect human health by causing severe food poisoning.

Other potential pathogenic species identified included *Alternaria* with 12.5% frequency, spanning through OTU12 and 13, which represent *A. tenuissima* and *A. brassicicola* respectively. Studies by Ogórek *et al.* (2012) and Ostry (2008) reported that *Alternaria* species are pathogenic fungi that are able to produce toxins such as AAL-toxins (*A. alternata f. sp. lycopersici* toxins) which cause blight infections in tomatoes. Al-Najada and Gherbawy (2015) explained that, *A. alternata* and *A. tenuissima* were isolated from tomatoes in Saudi Arabia and identified using their macromorphological and micromorphological characteristics, which were in accordance with this study. In addition, studies conducted by Rahimloo and Ghosta (2015) and Siciliano *et al.* (2017) identified *A. tenuissima* as saprophytic pathogens of tomato which causes postharvest losses.

Other representative OTUs identified in this study included *Byssochlamys, Lecythophora, Epicoccum, Retroconis, Cladosporium, Curvularia and Aureobasidium* represented by OTU3, 5, 8, 9, 15, 16 and 4 with the least occurrences. These fungal species have been reported in previous studies and are known to inhabit different environments such as fruit cell tissues, food products, air, soil, and water (Alcock *et al*., 2015; Ansari *et al*., 2017; Chi *et al*., 2009; Collemare *et al*., 2014; Damm *et al*., 2010; Rixin *et al*., 2015; Tan *et al*., 2018). Among these, *Cladosporium* is a biotrophic fungus that is pathogenic to various plants (Crous *et al*., 2007; Ogórek *et al*., 2012). The *Cladosporium* species are also known to produce secondary metabolites that cause leaf mould of tomato (Bensch *et al*., 2010; Collemare *et al*., 2014) while the *Lecythophora* species are known to produce high activities of different plant cell wall degrading enzymes. They are mostly isolated primarily from soil and decomposing wood (Lim *et al*., 2013).

The fungal antagonists may easily be washed off the surface of fruits or killed when cooked due to high temperatures (Bhagwat and Datar, 2014). This section of the study was conducted to test the selected fungal antagonists in order to ascertain whether they could be commensals in the case of consumption. The effects of probiotic microorganisms have been widely documented in various studies (Makete *et al*., 2017; Pennacchia *et al.*, 2008; Song *et al*., 2012). Probiotics are defined as viable microorganisms that have health benefits, such as improving the intestinal microbial balance, when ingested in adequate amounts (Rolfe, 2000; Sanders, 2008; Sarao and Arora, 2017). For the probiotic to survive and subsequently colonise the gastrointestinal tract, it must tolerate the first biological barriers after ingestion, namely, stomach acid and intestinal bile salts, and hence, the major factor in selecting a probiotic strain is the assessment of its resistance to low pH and bile tolerance (Gueimonde and Salminen, 2006; Pennacchia *et al*., 2008; Verón *et al*., 2017).

Bile tolerance along with pH tolerance are crucial for the ability of the isolates to survive and colonise the small intestine (Klayraung and Okonogi, 2009). Bile salts are surface-active chemicals which are

produced in the liver from the catabolism of cholesterol (Soomro and Masud, 2012). A study by Makete *et al*. (2017), explained that bile salt in the gastrointestinal system may range from 0.5 to 2.5%. Tolerance to bile salts is considered to be the main prerequisite for the growth, colonisation, and metabolic activity of fungi in the human gut (Shukla *et al*., 2010). In this study it was observed that *B. spectabilis* and *R. fusiformis* survived and tolerated the various bile salt (1, 2 and 3%) concentrations significantly ($P < 0.05$) as compared to *E. thailandicum* and *C. kusanoi* (Figure 4.4). According to the study by Ruiz *et al.,* (2013), the *B. spectabilis* and *R. fusiformis* may possess essential resistance mechanisms that allow them to survive in different bile salt concentrations. The difference in the level of bile tolerance of the strains in this study was probably due to the differences in their ability to grow due to *in vivo* conditions in the laboratory such as temperature, various pHs and growth atmospheres (Begley *et al.*, 2005; Makete *et al*., 2017; Usman and Hosono, 1999). These conditions may increase the susceptibility of the fungal antagonists, thus, affecting the bile (Dunne *et al.*, 2001; Kheadr, 2006; Shukla *et al*., 2010). Thus, the tolerance of *B. spectabilis* and *R. fusiformis* to bile salts may play a role in maintaining the equilibrium of the gut microflora.

Other beneficial aspects of probiotic strains may be expected only when they are able to survive the stressful conditions of the stomach digestive system with a pH between 1.5 and 3.0 and colonise the human gut (Sarao and Arora, 2017). Although the stomach pH may be as low as 1.0, in most of *in vitro* assays, a pH 3.0 was preferred (Aymerich *et al*., 2005). In this study *E. thailandicum* was found to be resistant to all the acidic concentrations, while C. *kusanoi* was found to be the most susceptible (Figure 4.5). The study by Shukla *et al.* (2010) explained that the *E. thailandicum* and *R. fusiformis* were more tolerant to acidic conditions than other isolated fungi due to a high cytoplasmic pH that allows an increase in stability under acidic conditions. (Sarao & Arora, 2017; Verón, Di Risio, Isla, & Torres, 2017) also explained that bile and acid tolerance are strain independent thus, isolates susceptible to pH2 may still be exploited

A key requirement for probiotic strains is that they should not carry transferable antibiotic resistance genes (Aymerich *et al.*, 2005). The overwhelming use of antibiotics over the past years in both

animals and humans has played a significant role in the widespread emergence of antibiotic resistant microorganisms (Ashraf and Shah, 2011). In general, antimicrobial activity is known to be the result of the presence of a mixture of protein and acidic-like substances (lactic acid mainly), that are acting directly or through the decrease in the pH of foods (Guglielmotti *et al*., 2007; Marcó *et al*., 2014; Verón *et al*., 2017; Vinderola *et al*., 2008).

In this study the four fungal antagonists (*C. kusanoi*, *E. thailandicum, B. spectabilis* and *R. fusiformis*) were found to be resistant to the inhibitors of the cell wall synthesis of Fluconazole (Fluco), Flucytosine (Flucy), Ketoconazole (Keto) and Penicillin G (Peni G) antifungal drugs various zones of inhibition (Table 4.4). The differences in the degree of inhibition with different antifungals have been attributed to various modes of action on the cell components such as the cell wall, protein and DNA synthesis, and RNA polymerase (Neu, 1992). This inhibitory activity may have been eliminated after the cultures were neutralised using NaOH, which could have resulted in the production of organic acids. The four antagonists may also be able to reduce the intracellular concentration of the fluconazole, flucytosine, ketoconazole antifungals and thereby resist transpeptidase inhibition exerted by the antifungals (Ghannoum, 1996; Kanafani and Perfect, 2007; Nguyen and Yu, 1998). On the other hand, the penicillin G antibitotic resistance may be because these antagonists are able to hydrolise the antibiotic by producing β-lactamases enzymes (Al-Ahmad *et al.*, 1999; Makete *et al*., 2017; Yocum *et al.,* 1980). The results observed in this study correlated with reports by Temitope and Oluchi (2015) who studied the antifungal activity of *Lactobacillus plantarum* and *Lactobacillus fermentum* on *Aspergillus niger*, *Rhizopus stolonifer* and *Mucor sp*. using the agar well diffusion technique which indicated that the antifungal activity exhibited by *Lactobacillus plantarum* and *Lactobacillus fermentum* are highly effective in eliminating the spoilage fungi affecting tomatoes. Thus, these four antagonists may be consumed and may not have any effect on either the host or consumer.

According to Bhagwat and Datar (2014) and Dukare *et al.* (2018), the quality of fresh tomatoes is characterised by any kind of damage on the skin such as size (shrinking), softening, mould growth, softening, shininess and loss of marketability during storage. This study evaluated the efficacy of the four fungal antagonists (*C. kusanoi*, *E. thailandicum, B. spectabilis* and *R. fusiformis*) in preserving

tomatoes under two different storage conditions. Factors such as weight loss and the spoilage percentages were calculated every five days. These factors have been used previously for evaluating tomato preservation during storage (Bhagwat and Datar, 2014; Conesa *et al*., 2014; Moneruzzaman *et al*., 2009; Pila *et al*., 2010; Rahman *et al*., 2010).

The difference in the storage temperatures significantly affected the disease incidence and spoilage of tomatoes. For example, storage at 8 °C refrigeration preserved the freshness of tomatoes better than that of tomatoes stored at uncontrolled room temperature (Table 4.5). This may be attributed to the fact that tomato fruit exhibit significant damages (such as ripening, softening and high susceptibility to postharvest fungal pathogens) when they are exposed to temperatures above 13 °C (Maul *et al*., 2000; Moretti *et al*., 2010). Refrigeration leads to delayed ripening as a result of metabolic activities such as ethylene production (Ponce-Valadez *et al*., 2016). This trend was expected because, at high temperature, the ripening rate (CO_2 production) increases, thereby, reducing the time for which the fruit may be stored (de León-Sánchez *et al*., 2009; Guillén *et al*., 2006). This is in accordance with the study of Ponce-Valadez *et al*. (2016) and Renard *et al*. (2013), where they investigated the effect of tomato weight loss after 15 days refrigeration at 12.5 °C and 20 °C respectively, and concluded that weight loss and firmness decreases for tomatoes stored at higher temperature after two to four days of storage as compared to tomatoes stored at a lower temperature.

The weight loss of tomatoes was lesser when stored at 8 °C in comparison to the weight loss of tomatoes stored at uncontrolled room temperature although it increased gradually during the 15 day storage period (Figure 4.6A and B; Supplementary Figure S2). The water content of the fruits is very high during harvesting and reduces during storage or as the fruits ripens (Baloch and Bibi, 2012; Fagundes *et al.*, 2015; Silva *et al.*, 2017). This loss of water content is caused by high relative humidity (moisture present in the ambient air), which tends to be a crucial determinate of storage life and the quality of tomatoes (Arah *et al*., 2015; Baloch and Bibi, 2012; Migliori *et al*., 2017; Silva *et al*., 2017). During uncontrolled temperature, the atmosphere had a high relative humidity, which

resulted in shrinkage caused evapotranspiration and the moisture condensation on the surface of the fruits which stimulated mould and fungal growth (Arah *et al*., 2015; Fagundes *et al*., 2013).

The study then highlighted the differences in the spoilage percentage of tomatoes when sprayed with the four fungal antagonists during the 15 days of storage (Table 4.5; Supplementary Figure S3). This spoilage is due to the call wall of tomatoes' carbohydrate metabolism degradation, which increases the fruits' susceptibility to fungal pathogen infections (Pila *et al*., 2010). The study by Arah *et al*. (2015) further explained that the spoilage percentage on tomatoes stored at uncontrolled room temperature is due to the heat stored inside the fruits, which increases tomato's deterioration rate. Similar results were obtained by Moneruzzaman *et al*. (2009), when they treated and stored tomatoes for 15 days at different storage conditions. They reported that the spoilage of tomatoes increased rapidly with increased temperature and relative humidity.

The results of this study also revealed that treating tomatoes with *C. kusanoi* and *E. thailandicum* may extend the shelf life of tomatoes by reducing the weight loss and spoilage percentages during storage. These antagonists occupied tomato's fruit surface, by rapidly growing within 24 hours and were, thus, able to outcompete the other microorganisms for nutrients and function exclusion (Liu *et al*., 2013). *C. kusanoi* is known to secrete αβ-dehydrocurvularin whereas, *E. thailandicum* secretes flavipin compounds, that firmly attach to the hyphae of numerous pathogens which degrade the pathogens' cell wall (jiang *et al*., 2009; Wisniewski *et al*., 1991). These antagonists have not been identified or studied as bio-preservatives hence, this research represents the first of its kind in South African tomato industry.

The use of *C. kusanoi* and *E. thailandicum* as antagonists has several advantages. Their preparations are non-toxic, easily washed off and do not remain as a residue (Bhagwat and Datar, 2014). The Sporekill® (Poly dimethyl ammonium chloride) fungicide which was used as a control is already marketed, commercially available and is also effective. However, its preparation is a lengthy process and several acids and chemicals are required (Fourie and Halleen, 2006; Mtasa *et al*., 2014; Nel *et al*., 2007; Whitaker *et al*., 2004). In addition, Kowalski and van Staden (1998) mentioned that

Sporekill® does not eliminate all the contaminations during low temperature storage. On the other hand, the *C. kusanoi* and *E. thailandicum* do not involve such problems and their effectiveness is comparable to that of Sporekill®, while their formulations were very effective in maintaining tomatoes under ambient storage conditions. *C. kusanoi* and *E. thailandicum* may be used as bioaerosols or agricultural sprays during post-harvest to ensure food safety.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusion

Tomato (*Lycopersicon esculentum*) is an important crop that is widely grown in the world. However, the quality of tomato fruit deteriorate after harvesting, thus resulting in reduction of tomatoes. Currently, chemical fungicides are primarily used in reducing pre- and postharvest losses. The use of these fungicides have detrimental effects on tomatoes as well as the consumers. Therefore, reducing their use remains a high research priority.

6.1.1. Isolation, characterisation and identification of fungal strains

This objective was conducted to isolate and identify fungal isolates from tomato fruit, using colony identification, wet mount and Sanger sequencing techniques. A total of 17 distinct OTUs which comprised of 10 genera such as *Penicillium, Fusarium*, *Curvularia*, *Alternaria*, *Cladosporium*, *Lecythophora*, *Aureobasidium*, *Byssochlamys*, *Retroconis*, and *Epicoccum* were successfully identified. These isolates comprised of various fungal isolates known to be pathogenic to tomato fruit, whereas some were isolates rarely obtained from tomato fruit.

6.1.2. Antagonistic potentials of the isolated fungal strains

This objective was conducted to investigate the potential utilisation of antagonistic fungal strains in tomato fruit for the management of post-harvest losses, using a dual culture technique. From the findings, the growth of most fungal pathogens tested were inhibited by *Curvularia kusanoi, Epicoccum thailandicum, Byssochlamys spectabilis,* and *Retroconis fusiformis* isolates, and thereby were regarded as antagonists.

6.1.3. Probiotic (beneficial) properties of antagonistic isolates

To investigate the additional possible benefit of the fungal antagonists, they were subjected to typical probiotic tests such as antifungal susceptibility test, pH and acid tolerance. These antagonists passed most of the standard criteria investigated in this study for grading probiotics, therefore, they are of acceptable standard to be used as preservatives of tomatoes.

6.1.4. Evaluating efficacy of the selected fungal antagonists under different storage conditions

The ability of the 4 antagonists to preserve tomatoes from fungal spoilage during storage was evaluated by calculating the weight loss and spoilage percentages. Out of the 4 antagonists it was observed that treatment with *C. kusanoi* and *E. thailandicum* antagonists in single cultures is effective in controlling the spoilage of tomatoes during ambient storage conditions.

Tomatoes, the fruit of interest in the present study, have high water content and nutrient composition, hence they are vulnerable to spoilage. This study has shown that there are potential environmentally-friendly solutions to PHLs. In addition, the culture-based approach was used to conduct different tests on the fungal isolates obtained in the present study and this may enable the mass-production of the fungal isolates for their use as bio-preservative agents. In conclusion, the potential application of postharvest treatment with formulations of *C. kusanoi* and *E. thailandicum* may be useful because of their perceived safety for the environment and consumers. These antagonists may be used for the production of bio-preservatives that may assist smallholder famers in reducing spoilage of tomato fruit during storage.

6.2. Recommendations

The present study identified a range of areas that needs attention in terms of further research and production. The findings of this study suggests that:

- The combination of the identified antagonists should be tested depending on the pathogen target.
- The identified antagonists were evaluated on purchased pathogens, it is recommended to test them against pathogens isolated from tomatoes in this study as well.

- Their dosage and method of application that could improve the shelf life of tomato fruit should be researched.
- The insight of the optimisation of culture method and the stability of antagonistic fungi for large scale production (powder, pellets, bags etc.) should be implemented as direct food bio-preservatives.

BIBLIOGRAPHY

Adeleke, R., Cloete, E., & Khasa, D. (2010). Isolation and identification of iron ore-solubilising fungus. *South African Journal of Science*, *106*(9–10), 1–6.

Agrios, G. N. (2004). *Plant pathology: 5th edition*. *Molecular plant pathology*.

Al-Ahmad, A., Daschner, F. D., & Kümmerer, K. (1999). Biodegradability of cefotiam, ciprofloxacin, meropenem, penicillin G, and sulfamethoxazole and inhibition of waste water bacteria. *Archives of Environmental Contamination and Toxicology*, *37*, 158–163.

Al-Najada, A. R., & Gherbawy, Y. A. (2015). Molecular identification of spoilage fungi isolated from fruit and vegetables and their control with chitosan. *Food Biotechnology*, *29*(2), 166–184.

Albrigo, L. G. (1978). Occurrence and identification of preharvest fruit blemishes in Florida citrus groves. *Proceedings of the Florida State Horticultural Society*, *91*, 78–81.

Alcock, A., Elmer, P., Marsden, R., et al. (2015). Inhibition of Botrytis cinerea by Epirodin: A secondary metabolite from New Zealand isolates of Epicoccum nigrum. *Journal of Phytopathology*, 841–852.

Aliber, M., & Hall, R. (2012a). Support for smallholder farmers in South Africa: Challenges of scale and strategy. *Development Southern Africa*, *29*(4), 548–562. https://doi.org/10.1080/0376835X.2012.715441

Aliber, M., & Hall, R. (2012b). Support for smallholder farmers in South Africa: Challenges of scale and strategy. *Development Southern Africa*, *29*(4), 548–562.

American Type Culture Collection (ATCC). (2011). Preservation and recovery of filamentous fungi. *The Essentials of Life Science Research*, 1–4. Retrieved from www.atcc.org

Amini, J., & Sidovich, D. F. (2010). The effects of fungicides on Fusarium oxysporum f.sp. lycopersici associated with Fusarium wilt of tomato. *Journal of Plant Protection Research*, *50*(2), 172–178.

Ansari, L., Sirchi, R. V., Bonjar, G. H. S., et al. (2017). Screening of antagonistic activity in different Streptomyces species against Paecilomyces variotii and verification of some of the physiological properties of the antagonists. *International Journal of Farming and Allied Sciences*, *6*(6), 135–142.

Arah, I. K., Amaglo, H., Kumah, E. K., et al. (2015). Preharvest and postharvest factors affecting the quality and shelf life of harvested tomatoes : A mini review. *Hindawi Publishing Corporation International Journal of Agronomy*, 1–6.

Arah, I. K., Kumah, E. K., Anku, E. K., et al. (2015). An overview of post-harvest losses in tomato production in Africa: Causes and possible prevention strategies. *Journal of Biology, Agriculture and Healthcare*, *5*(16), 78–89.

ARC-VOPI. (2013). Production Guideline for Summer Vegetables. *Agricultural Research Council*, 1–44.

Arli-Sokmen, M., & Sevik, M. (2006). Viruses infecting field-grown tomatoes in Samsun province, Turkey. *Archives of Phytopathology and Plant Protection*, *39*(4), 283–288.

Ashraf, R., & Shah, N. P. (2011). Review article: Antibiotic resistance of probiotic organisms and safety of probiotic dairy products. *International Food Research Journal*, *18*(3), 837–853.

Ayandiji, A. O. R., & Adeniyi, O. D. (2011). Determinant post harvest losses among tomato farmers in Imeko-Afon local government area of Ogun state, Nigeria. *Global Journal of Science Frontier Research*, *11*(5), 23–28.

Aymerich, T., Martín, B., Garriga, M., et al. (2005). Safety properties and molecular strain typing of lactic acid bacteria from slightly fermented sausages. *Journal of Applied Microbiology*, *100*, 40–49.

Baloch, M. K., & Bibi, F. (2012). Effect of harvesting and storage conditions on the post harvest quality and shelf life of mango (Mangifera indica L.) fruit. *South African Journal of Botany*, *83*, 109–116.

Barkai-Golan, R., & Paster, N. (2008). *Mycotoxins in fruits and vegetables*. *Academic Press*.

Begley, M., Gahan, C. G. M., & Hill, C. (2005). The interaction between bacteria and bile. *FEMS Microbiology Reviews*, *29*(4), 625–651.

Bensch, K., Groenewald, J. Z., Dijksterhuis, J., et al. (2010). Species and ecological diversity within the Cladosporium cladosporioides complex (Davidiellaceae, Capnodiales). *Studies in Mycology*, *67*, 1–94.

Berrueto, F. G. A. (2017). Tomato suspension agreement : An analysis of U.S. fresh tomato market. *The University of Arizona.*, 1–61.

Beuchat, L. R. (2006). Vectors and conditions for preharvest contamination of fruits and vegetables with pathogens capable of causing enteric diseases. *British Food Journal*, *108*(1), 38–53.

Bhagwat, M. K., & Datar, A. G. (2014). Enhancement of shelf life of tomatoes by postharvest herbal treatment, during storage under ambient conditions. *Archives of Phytopathology and Plant Protection*, *47*(10), 1262–1273.

Bhalodia, N., & Shukla, V. (2011). Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal plant. *Journal of Advanced Pharmaceutical Technology & Research*, *2*(2), 104. https://doi.org/10.4103/2231-4040.82956

Bills, G., Dombrowski, A., Morris, S. A., et al. (2000). Hyalodendrosides A and B, antifungal triterpenoid glycosides from a lignicolous hyphomycete, Hyalodendron species. *Journal of Natural Products*, *63*(1), 90–94.

Boyer, J. M. (1976). Impregnated disk method for antifungal antibiotic testing. *Antimicrobial, Agents and Chemotheraphy*, *9*(6), 1070–1071.

Buzby, J. C., & Hyman, J. (2012). Total and per capita value of food loss in the United States - Comments. *Food Policy*, *37*, 561–570.

Castoria, R., De Curtis, F., Lima, G., et al. (2001a). Aureobasidium pullulans (LS-30) an antagonist of postharvest pathogens of fruits: Study on its modes of action. *Postharvest Biology and Technology*, *22*, 7–17.

Castoria, R., De Curtis, F., Lima, G., et al. (2001b). Aureobasidium pullulans (LS-30) an antagonist of postharvest pathogens of fruits: Study on its modes of action. *Postharvest Biology and Technology*, *22*(1), 7–17. https://doi.org/10.1016/S0925-5214(00)00186-1

Chandio, A. A., Yuansheng, J., & Magsi, H. (2016). Agricultural sub-sectors performance: An analysis of sector-wise share in agriculture GDP of Pakistan. *International Journal of Economics and Finance*, *8*(2), 156–162.

Chaudhary, S. . (2014). Effect of biofertilizers and organic manures on growth, yield and quality of tomato (Lycopersicon esculentum Mill.) c.v. Arka vikas. *Department of Horticulture Institute of Agricultural Sciences Banaras Hindu University Varanasi -221005 India*, 1–78.

Chérif, M., & Benhamou, N. (1990). Cytochemical aspects of chitin breakdown during the parasitic action of a Trichoderma sp. on Fusarium oxysporum f. sp. radicis-lycopersici. *Phytopathology*.

Chi, Z., Wang, F., Chi, Z., et al. (2009). Bioproducts from Aureobasidium pullulans, a biotechnologically important yeast. *Applied Microbiology and Biotechnology*, *82*, 793–804.

Chinedu, S. M., & Emmanuel, E. (2014). Isolation of microorganisms associated with deterioration of tomato (Lycopersicon esculentum) and pawpaw (Carica papaya) fruits. *International Journal of Current Microbiology and Applied Sciences*, *3*(5), 501–512.

Collemare, J., Griffiths, S., Iida, Y., et al. (2014). Secondary metabolism and biotrophic lifestyle in the tomato pathogen Cladosporium fulvum. *PLoS ONE*, *9*(1), 1–11.

Conesa, M. A., Galmés, J., Ochogavía, J. M., et al. (2014). The postharvest tomato fruit quality of long shelf-life Mediterranean landraces is substantially influenced by irrigation regimes. *Postharvest Biology and Technology*, *93*, 114–121.

Conway, W. S., Leverentz, B., Janisiewicz, W. J., et al. (2004). Integrating heat treatment, biocontrol and sodium bicarbonate to reduce postharvest decay of apple caused by Colletotrichum acutatum and Penicillium expansum. *Postharvest Biology and Technology*, *34*(1), 11–20.

Crous, P. W., Braun, U., Schubert, K., et al. (2007). Delimiting Cladosporium from morphologically similar genera. *Studies in Mycology*, *58*, 33–56.

DAFF. (2015). *A profile of the South African tomato market value chain*. https://doi.org/10.1007/s13398-014-0173-7.2

Damm, U., Fourie, P. H., & Crous, P. W. (2010). Coniochaeta (Lecythophora), collophora gen. nov. and phaeomoniella species associated with wood necroses of prunus trees. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, *24*, 60–80.

De Curtis, F., Lima, G., Vitullo, D., et al. (2010). Biocontrol of Rhizoctonia solani and Sclerotium rolfsii on tomato by delivering antagonistic bacteria through a drip irrigation system. *Crop Protection*, *29*, 663–670.

De Gannes, V., Eudoxie, G., & Hickey, W. J. (2013). Insights into fungal communities in composts revealed by 454-pyrosequencing: Implications for human health and safety. *Frontiers in Microbiology*, *4*, 1–9.

de Hoog, G. S., & van der Vegte, W. H. B. (1989). Retroconis, a new genus of ascomycetous hyphomycetes. *Studies in Mycology*, *31*, 99–105.

de León-Sánchez, F. D., Pelayo-Zaldívar, C., Rivera-Cabrera, F., et al. (2009). Effect of refrigerated storage on aroma and alcohol dehydrogenase activity in tomato fruit. *Postharvest Biology and Technology*, *54*, 93–100.

Deribe, H., Beyene, B., & Beyene, B. (2016). Review on pre and post-harvest management on quality tomato (Lycopersicon esculentum Mill.) production. *Food Science and Quality Management*, *54*, 72–79.

Di Francesco, A., Martini, C., & Mari, M. (2016). Biological control of postharvest diseases by microbial antagonists: how many mechanisms of action? *European Journal of Plant Pathology*, *145*(4), 711–717. https://doi.org/10.1007/s10658-016-0867-0

Dorward, A., Kydd, J., & Poulton, C. (2005). Beyond liberalisation: "Developmental coordination" policies for African smallholder agriculture. *IDS Bulletin*, *36*(2), 80–85.

Droby, S., Chalutz, E., Wilson, C. L., et al. (1992). Biological Control of Postharvest Diseases : A Promising Alternative to the Use of Synthetic Fungicides.

Droby, S., Wisniewski, M., Macarisin, D., et al. (2009). Twenty years of postharvest biocontrol research: Is it time for a new paradigm? *Postharvest Biology and Technology*, *52*(2), 137–145.

Dukare, A. S., Paul, S., Nambi, V. E., et al. (2018). Exploitation of microbial antagonists for the control of postharvest diseases of fruits: A review. *Critical Reviews in Food Science and Nutrition*, 1–16.

Dunne, C., O'Mahony, L., Murphy, L., et al. (2001). In vitro selection criteria for probiotic bacteria of human origin : Correlation with in vivo findings 1 – 4. *The American Journal of Clinical Nutrition*, *73*, 386S–392S.

El-katatny, M. H., & Emam, A. S. (2012). Control of postharvest tomato rot by spore suspension and antifungal metabolites of Trichoderma harzianum. *Journal of Microbiology, Biotechnology and Food Sciences*, *1*(6), 1505–1528.

Etebu, E., Nwauzoma, A. B., & Bawo, D. D. S. (2013). Postharvest spoilage of tomato (Lycopersicon esculentum Mill.) and control strategies in Nigeria. *Journal of Biology, Agriculture and Healthcare*, *3*(10), 51–61.

Ezikanyi, N. D. (2016). Isolation and identification of fungi associated with postharvest decay of

Lycopersicum esculentum M. sold in Abakaliki , Nigeria. *IOSR Journal of Agriculture and Veterinary Science*, *9*(7), 87–89.

Fagundes, C., Moraes, K., Pérez-Gago, M. B., et al. (2015). Effect of active modified atmosphere and cold storage on the postharvest quality of cherry tomatoes. *Postharvest Biology and Technology*, *109*, 73–81.

Fagundes, C., Pérez-Gago, M. B., Monteiro, A. R., et al. (2013). Antifungal activity of food additives in vitro and as ingredients of hydroxypropyl methylcellulose-lipid edible coatings against Botrytis cinerea and Alternaria alternata on cherry tomato fruit. *International Journal of Food Microbiology*, *166*(3), 391–398.

Fallik, E. (2004). Prestorage hot water treatments (immersion, rinsing and brushing). *Postharvest Biology and Technology*, *32*, 125–134.

FAO. (2011). *Global food losses and food waste - Extent, causes and prevention. Rome*.

FAOSTAT. (2018). *Global yeild quantities*. *FAO*. Retrieved from http://www.fao.org/faostat/en/#search/ 2018 production tomato crops data

Fatima, N., Batool, H., Sultana, V., et al. (2009). Prevalance of post-harvest rot of vegetables and fruits in Karachi, Pakistan. *Pakistan Journal of Botany*, *41*(6), 3185–3190.

Fávaro, L. C. de L., Sebastianes, F. L. de S., & Araújo, W. L. (2012). Epicoccum nigrum P16, a sugarcane endophyte, produces antifungal compounds and induces root growth. *PLoS ONE*, *7*(6), 1–11.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, *39*(4), 783–791.

Fourie, P. H., & Halleen, F. (2006). Chemical and biological protection of grapevine propagation material from trunk disease pathogens. *European Journal of Plant Pathology*, *116*, 255–265.

Frisvad, J. ., Thrane, U., Samson, R. ., et al. (2006). *Advances in Food Mycology: Understanding the fungi producing important mycotoxins: Important mycotoxins and the fungi which produce them*. *Journal of Chemical Information and Modeling* (Vol. 53). https://doi.org/10.1017/CBO9781107415324.004

Fusaro, R. M. (1972). Inoculation technique for fungus cultures. *Applied Microbiology*, *23*(1), 174–176.

Gao, Z., Zhang, B., Liu, H., et al. (2017). Identification of endophytic Bacillus velezensis ZSY-1 strain and antifungal activity of its volatile compounds against Alternaria solani and Botrytis cinerea. *Biological Control*, *105*, 27–39.

García-Hernández, Y., Rodríguez, Z., Brandão, L. R., et al. (2012). Identification and in vitro screening of avian yeasts for use as probiotic. *Research in Veterinary Science*, *93*(2), 798–802. https://doi.org/10.1016/j.rvsc.2011.09.005

Geoffrey, S. K., Hillary, N. K., Antony, K. M., et al. (2014). Challenges and strategies to improve tomato competitiveness along the tomato value chain in Kenya. *International Journal of Business and Management*, *9*(9), 205–212.

Ghannoum, M. a. (1996). Is antifungal susceptibility testing useful in guiding fluconazole therapy? *Clinical Infectious Diseases*, *22 Suppl 2*, S161-5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8722845

Ghisalberti, E. L., Hockless, D. C. R., Rowland, C. Y., et al. (1993). Structural study of curvularin, a cell division inhibitor. *Australian Journal of Chemistry*, *46*, 571–575.

Glass, G. V., Peckham, P. D., & Sanders, J. R. (1972). Consequences of Failure to Meet Assumptions Underlying the Fixed Effects Analyses of Variance and Covariance. *Review of Educational Research*, *42*(3), 237–288.

Gomathi, S., & Ambikapathy, V. (2011). Antagonistic Activity of Fungi Against Pythium debaryanum (Hesse) Isolated From Chilli Field Soil. *Advances in Applied Science Research*, *2*(4), 291–297.

Gueimonde, M., & Salminen, S. (2006). New methods for selecting and evaluating probiotics. *Digestive and Liver Disease*, *38*(Suppl.2), S242–S247.

Guglielmotti, D. M., Marcó, M. B., Golowczyc, M., et al. (2007). Probiotic potential of Lactobacillus delbrueckii strains and their phage resistant mutants. *International Dairy Journal*, *17*, 916–925.

Guil-Guerrero, J. L., & Rebolloso-Fuentes, M. M. (2009). Nutrient composition and antioxidant activity of eight tomato (Lycopersicon esculentum) varieties. *Journal of Food Composition and Analysis*, *22*, 123–129.

Guillén, F., Castillo, S., Zapata, P. J., et al. (2006). Efficacy of 1-MCP treatment in tomato fruit 2.

Effect of cultivar and ripening stage at harvest. *Postharvest Biology and Technology*, *42*, 235–242.

Hanssen, I. M. (2010). Pepino mosaic virus : an endemic pathogen of tomato crops. *Wageningen University*, 7–170.

Hassan, M. M., Gaber, A., & El-Hallous, E. I. (2014). Molecular and morphological characterization of Trichoderma harzianum from different Egyptian soils. *Wulfenia Journal*, 1–17.

Hodges, R. J., Buzby, J. C., & Bennett, B. (2011). Postharvest losses and waste in developed and less developed countries: opportunities to improve resource use. *The Journal of Agricultural Science*, *149*(S1), 37–45. https://doi.org/10.1017/S0021859610000936

Houbraken, J., Varga, J., Rico-Munoz, E., et al. (2008). Sexual reproduction as the cause of heat resistance in the food spoilage fungus Byssochlamys spectabilis (anamorph Paecilomyces variotii). *Applied and Environmental Microbiology*, *74*(5), 1613–1619.

Ignjatov, M., Milosevic, D., Nikolic, Z., et al. (2012). Fusarium oxysporum as causal agent of tomato wilt and fruit rot. *Journal Pesticides and Phytomedicine*, *27*(1), 25–31.

Ippolito, A., & Nigro, F. (2000). Impact of preharvest application of biological control agents on postharvest diseases of fresh fruits and vegetables. *Crop Protection*, *19*(8–10), 715–723.

Janisiewicz, W. J., & Korsten, L. (2002). Biological control of postharvest diseases of fruits. *Annual Review of Phytopathology*, *40*(1), 411–441. https://doi.org/10.1146/annurev.phyto.40.120401.130158

Jayne, T. S., Mather, D., & Mghenyi, E. (2010). Principal challenges confronting smallholder agriculture in Sub-saharan Africa. *World Development*, *38*(10), 1384–1398.

jiang, F., Zheng, X., & Chen, J. (2009). Microarray analysis of gene expression profile induced by the biocontrol yeast Cryptococcus laurentii in cherry tomato fruit. *Gene*, *430*, 12–16.

John, W., Anyanwu, N., & Ogunmodi, O. (2016). Determination of pathogenic effect of fungi on fresh healthy tomatoes in Jos North local government area, Plateau state, Nigeria. *Annual Research & Review in Biology*, *9*(5), 1–8.

Jones, J. B., Jones, J. P., Stall, R. E., et al. (1992). Compendium of Tomato Diseases. *Journal of Plant, Diseases and Protection*, *99*(4), 444–445.

Jovanovic, N., Musvoto, C., De Clercq, W., et al. (2018). A comparative analysis of yield gaps and

water productivity on smallholder farms in Ethiopia, South Africa and Tunisia. *Irrigation and Drainage*, (January), 1–19.

Jukes, T. H., & Cantor, C. R. (1969). Evolution of protein molecules. *Mammalian Protein Metabolism*, 21–132.

Kahan, D. (2010). Farm management extension guide: Farm business analysis using benchmarking. *Food and Agricultural Organization of the United Nations, Rome*, 1–158.

Kanafani, Z. A., & Perfect, J. R. (2007). Resistance to antifungal agents: Mechanisms and clinical impact. *Clinical Infectious Diseases*, *46*, 120–128.

Kanetis, L., Förster, H., & Adaskaveg, J. E. (2007). Comparative efficacy of the new postharvest fungicides azoxystrobin, fludioxonil, and pyrimethanil for managing citrus green mold. *Plant Disease*, *91*(11), 1502–1511.

Kefi, A., Slimene, I. Ben, Karkouch, I., et al. (2015). Characterization of endophytic Bacillus strains from tomato plants (Lycopersicon esculentum) displaying antifungal activity against Botrytis cinerea Pers. *World Journal of Microbiology and Biotechnology*, 1–11.

Kennelly, M. (2009). Tomato leaf and fruit diseases and disorders. *Plant Pathology*, 1–6.

Kheadr, E. E. (2006). Impact of acid and oxgall on antibiotic susceptibility of probiotic Lactobacilli. *African Journal of Agricultural Research*, *1*(5), 172–181.

Khonglah, D., & Kayang, H. (2018). Antagonism of indegenous fungal isolates against Botrytis cineria the causal of gray mold disease of tomato (Solanum lycopersicum L.). *International Journal of Current Research in Life Sciences*, *7*(1), 806–812.

Kitinoja, L., & Kader, A. A. (2015). Measuring postharvest losses of fresh fruits and vegetables in developing countries, (September), 1–26. https://doi.org/10.13140/RG.2.1.3921.6402

Klayraung, S., & Okonogi, S. (2009). Antibacterial and antioxidant activities of acid and bile resistant strains of Lactobacillus fermentum isolated from Miang. *Brazilian Journal of Microbiology*, *40*, 757–766.

Kleemann, R., Zadelaar, S., & Kooistra, T. (2008). Cytokines and atherosclerosis: A comprehensive review of studies in mice. *Cardiovascular Research*, *79*(3), 360–376.

Kowalski, B., & van Staden, J. (1998). Cold treatment, as part of the process, improves explant decontamination. *Plant Growth Regulation*, *26*, 203–205.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, *33*(7), 1870–1874. https://doi.org/10.1093/molbev/msw054

Kumar, V., Haldar, S., Pandey, K. K., et al. (2008). Cultural, morphological, pathogenic and molecular variability amongst tomato isolates of Alternaria solani in India. *World Journal of Microbiology and Biotechnology*, *24*(7), 1003–1009.

Lee, H. B., Patriarca, A., & Magan, N. (2015). Alternaria in food: Ecophysiology, mycotoxin production and toxicology. *Mycobiology*, *43*(2), 93–106. https://doi.org/10.5941/MYCO.2015.43.2.93

Lee, J., Huh, N., Hong, J. H., et al. (2012). The antagonistic properties of Trichoderma spp. inhabiting woods for potential biological control of wood-damaging fungi. *Holzforschung*, *66*, 883–887.

Leonardi, C., Guichard, S., & Bertin, N. (2000). High vapour pressure deficit influennces growth, transpiration and quality of tomato fruits. *Scientia Horticulturae*, *84*, 285–296.

Leroux, P. (2007). *Chemical control of botrytis and its resistance to chemical fungicides*. *Botrytis: Biology, Pathology and Control*.

Lim, Y., Kim, K., & Park, U. (2013). Isolation of Lecythophora sp . YP363 , a secretor of various thermostable plant cell wall-degrading enzymes with high activity. *African Journal of Microbiology Research*, *7*(27), 3517–3525.

Liu, J., Stevens, C., Khan, V. A., et al. (1993). Application of ultraviolet-C light on storage rots and ripening of tomatoes. *Journal of Food Protection*, *56*(10), 868–872.

Liu, J., Sui, Y., Wisniewski, M., et al. (2013). Review: Utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit. *International Journal of Food Microbiology*, *167*, 153–160.

Lledó, S., Rodrigo, S., Poblaciones, M. J., et al. (2016). Biomass yield, nutritive value and accumulation of minerals in Trifolium subterraneum L. as affected by fungal endophytes. *University of Extremadura*, 1–37.

Logrieco, A., Moretti, A., & Solfrizzo, M. (2009). Alternaria toxins and plant diseases: an overview of origin, occurrence and risks. *World Mycotoxin Journal*, *2*(2), 129–140.

Ma, X., Wang, X., Cheng, J., et al. (2015). Microencapsulation of Bacillus subtilis B99-2 and its

biocontrol efficiency against Rhizoctonia solani in tomato. *Biological Control*, *90*, 34–41.

Maboko, M. M., & Du Plooy, C. P. (2018). Response of field-grown indeterminate tomato to plant density and stem pruning on yield. *International Journal of Vegetable Science*, 1–10.

Maboko, M. M., Du Plooy, C. P., & Bertling, I. (2009). Comparative performance of tomato cultivars in soilless vs. in-soil production systems. *Acta Horticulturae 843, ISHS*, 319–326.

Makete, G., Aiyegoro, O. A., & Thantsha, M. S. (2017). Isolation, identification and screening of potential probiotic Bacteria in milk from South African Saanen goats. *Probiotics and Antimicrobial Proteins*, *9*(3), 246–254.

Maliwichi, L. L., Pfumayaramba, T. K., & Katlego, T. (2014). An analysis of constraints that affect smallholder farmers in the production of tomatoes in Ga-Mphahlele, LepelleNkumbi municipality, Limpopo province, South africa. *Journal of Human Ecology*, *47*(3), 269–274.

Manamgoda, D. S., Rossman, A. Y., Castlebury, L. A., et al. (2015). A taxonomic and phylogenetic re-appraisal of the genus Curvularia (Pleosporaceae): human and plant pathogens. *Phytotaxa*, *212*(3), 175–198.

Mandiriza-Mukwirimba, G., Kritzinger, Q., & Aveling, T. (2016). A survey of brassica vegetable smallholder farmers in the Gauteng and Limpopo provinces of South Africa. *Journal of Agriculture and Rural Development in the Tropics and Subtropics*, *117*(1), 35–44.

Marcó, B. M., Zacarías, M. F., Vinderola, G., et al. (2014). Biological and probiotic characterisation of spontaneous phage-resistant mutants of Lactobacillus plantarum. *International Dairy Journal*, *39*, 64–70.

Maul, F., Sargent, S. A., Sims, C. A., et al. (2000). Tomato flavor and aroma quality as affected by storage temperature. *JFS: Sensory and Nutritive Qualities of Food*, *65*(7), 1228–1237.

Meaza, M., Seyoum, T., & Woldetsadik, K. (2007). Effects of preharvest treatments on yield and chemical composition of tomato. *African Crop Science Journal*, *15*(3), 149–159.

Migliori, C. A., Salvati, L., Di Cesare, L. F., et al. (2017). Effects of preharvest applications of natural antimicrobial products on tomato fruit decay and quality during long-term storage. *Scientia Horticulturae*, *222*, 193–202.

Mioso, R., Marante, T. F. J., & de Laguna, I. H. B. (2015). The chemical diversity of the Ascomycete fungus Paecilomyces variotii. *Applied Biochemistry and Biotechnology*, *177*(4),

781–791.

Moneruzzaman, K. M., Hossain, A. B. M. S., Sani, W., et al. (2009). Effect of harvesting and storage conditions on the post harvest quality of tomato (Lycopersicon esculentum Mill) cv. Roma VF. *Australian Journal of Crop Science*, *3*(2), 113–121.

Moretti, C. L., Mattos, L. M., Calbo, A. G., et al. (2010). Climate changes and potential impacts on postharvest quality of fruit and vegetable crops: A review. *Food Research International*, *43*, 1824–1832.

Mtasa, T., Mangoma, N., & Dhlamini, Z. (2014). Sensitivity of some pathogen isolates to fungicides commonly used in vegetable greenhouses in Zimbabwe. *Zimbabwe Journal of Science & Technology*, *9*, 81–89.

Mungía-Pérez, R., Díaz-Cabrera, E., Martínez-Montiel, N., et al. (2011). Fungal from a mexican diversity in soil samples region with endemic dermatomycoses. *Micología Aplicada International*, *23*(1), 11–19.

Naglot, A., Goswami, S., Rahman, I., et al. (2015). Antagonistic potential of native Trichoderma viride strain against potent tea fungal pathogens in north east India. *Plant Pathology Journal*, *31*(3), 278–289.

Naika, S., de Jeude, J. van L., de Goffau, M., et al. (2005). *Agrodock 17- Cultivation of tomato: Production, processing and marketing*.

Nakasone, K. K., Peterson, S. W., & Jong, S. C. (2004). Preservation and distribution of fungal cultures. In *Preservation and Distribution of Fungal Cultures* (pp. 37–49).

Naureen, Z., Price, A. H., Hafeez, F. Y., et al. (2009). *Identification of rice blast disease-suppressing bacterial strains from the rhizosphere of rice grown in Pakistan*. *Crop Protection*.

Nel, B., Steinberg, C., Labuschagne, N., et al. (2007). *Evaluation of fungicides and sterilants for potential application in the management of Fusarium wilt of banana*. *Crop Protection*.

Neu, H. C. (1992). The crisis in Antibiotic resistance. *Science*, *257*, 1064–1073.

Nguyen, M. H., & Yu, C. Y. (1998). Voriconazole against fluconazole-susceptible and resistant candida isolates: in-vitro efficacy compared with that of itraconazole and ketoconazole. *Journal of Antimicrobial Chemotherapy*, *42*, 253–256.

Noh, T. H., Sen, L., Hong, J., et al. (2017). Antibacterial activities of viriditoxin congeners and

synthetic analogues against fish pathogens. *Bioorganic and Medicinal Chemistry Letters*, *27*(22), 4970–4974.

Nonaka, K., Miyazaki, H., Iwatsuki, M., et al. (2012). Staphylotrichum boninense, a new hyphomycete (Chaetomiaceae) from soils in the Bonin islands, Japan. *Mycoscience*, *53*(4), 312–318.

Noonari, S., Memon, M. I. N., Solangi, S. U., et al. (2015). Economic implications of tomato production in Naushahro feroze district of Sindh Pakistan. *International Journal of Agricultural Extension and Rural Development Studies*, *2*(2), 19–28.

Nunes, C. A. (2012). Biological control of postharvest diseases of fruit. *European Journal of Plant Pathology*, *133*, 181–196.

Ogórek, R., Lejman, A., Pusz, W., et al. (2012). Characteristics and taxonomy of Cladosporium fungi. *Mikologia Lekarska*, *19*(2), 80–85.

Oren, L., Ezrati, S., Cohen, D., et al. (2003). Early events in the Fusarium verticillioides - Maize interaction characterized by using a green fluorescent protein-expressing transgenic isolate. *Applied and Environmental Microbiology*, *69*(3), 1695–1701.

Ortmann, G. F., & King, R. P. (2007). Agricultural cooperatives II: Can they facilitate access of small-scale farmers in South Africa to input and product markets? *Agrekon*, *46*(2), 219–244.

Osman, A. O. A. (2015). Antifungal evaluation of some plants extracts and fungicide against (Fusarium oxysporum f.sp. Lycopersici) causal agent wilt of tomato. *Sudan University of Science and Technology College of Graduate Studies*, 1–73.

Ostry, V. (2008). Alternaria mycotoxins: An overview of chemical characterization, producers, toxicity, analysis and occurrence in foodstuffs. *World Mycotoxin Journal*, *1*(2), 175–188.

Pal, K. K., & Gardener, B. M. (2006). Biological control of plant pathogens. *The Plant Health Instructor*, 1–25. https://doi.org/10.1094/PHI-A-2006-1117-02.Biological

Palou, L., Smilanick, J. L., & Droby, S. (2008). Alternatives to conventional fungicides for the control of citrus postharvest green and blue moulds. *Stewart Postharvest Review*, *2*(2), 1–16.

Parfitt, J., Barthel, M., & Macnaughton, S. (2010). Food waste within food supply chains: quantification and potential for change to 2050. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*, 3065–3081.

Peet, M. M., & Welles, G. (2005). Greenhouse tomato production. *Centre for Agriculture and Bioscience International*, 257–304.

Pennacchia, C., Blaiotta, G., Pepe, O., et al. (2008). Isolation of Saccharomyces cerevisiae strains from different food matrices and their preliminary selection for a potential use as probiotics. *Journal of Applied Microbiology*, *105*, 1919–1928.

Pienaar, C. J. (2014). Increased water productivity in irrigated tomato production in the smallholder farming community of Giyani. *Stellenbosch University*, 1–157.

Pila, N., Gol, N. B., & Rao, T. V. R. (2010). Effect of post harvest treatments on physicochemical characteristics and shelf Life of tomato (Lycopersicon esculentum Mill.) fruits during storage. *American-Eurasian Journal Of Agricultural & Environmental Sciences*, *9*(5), 470–479.

Ponce-Valadez, M., Escalona-Buendía, H. B., Villa-Hernández, J. M., et al. (2016). Effect of refrigerated storage (12.5°C) on tomato (Solanum lycopersicum) fruit flavor: A biochemical and sensory analysis. *Postharvest Biology and Technology*, *111*, 6–14.

Poulton, C., Kydd, J., Wiggins, S., et al. (2006). State intervention for food price stabilisation in Africa: Can it work? *Food Policy*, *31*, 342–356.

Pretorius, J. C., Magama, S., & Zietsman, P. C. (2003). Growth inhibition of plant pathogenic bacteria and fungi by extracts from selected South African plant species. *South African Journal of Botany*, *69*(2), 186–192.

Puyaubert, J., & Baudouin, E. (2014). New clues for a cold case: Nitric oxide response to low temperature. *Plant, Cell and Environment*, *37*, 2623–2630.

Rahimloo, T., & Ghosta, Y. (2015). The occurrence of Alternaria species on cabbage in Iran. *Zemdirbyste-Agriculture*, *102*(3), 343–350.

Rahman, M. M., Islam, M. N., Wazed, M. A., et al. (2010). Determination of post harvest losses and shelf Life of tomato as influenced by different types of polythene at refrigerated condition (10°C). *International Journal of Sustainable Crop Production*, *5*(4), 62–65.

Ram, R. B. R., Prakash, J., & Meena, M. L. (2014). Growth, flowering, fruiting and quality of tomato (Lycopersicon esculentum Mill.) as influenced plant bio regulators. *International Jornal of Plant Science*, *9*(1), 67–71.

Ramaiah, A. K., & Garampalli, R. K. H. (2015). In vitro antifungal activity of some plant extracts

against Fusarium oxysporum f. sp. lycopersici. *Asian Journal of Plant Science and Research*, *5*(1), 22–27.

Raphela, M. G. M. (2014). Smallholder farmers in Ekurhuleni: The challenges and constraints of access to agricultural markets. *Stellenbosch University*, 1–89.

Rashid, T. S., Sijam, K., Awla, H. K., et al. (2016). Pathogenicity assay and molecular identification of fungi and bacteria associated with diseases of tomato in Malaysia. *American Journal of Plant Sciences*, *7*, 949–957.

Reddy, S. M., & Bilgrami, K. S. (1971). A new species of Hyalodendron from India. *Current Science Association*, *40*(24), 667–668.

Reddy, B. M. V., Belkacemi, K., Corcuff, R., et al. (2000). Effect of pre-harvest chitosan sprays on post-harvest infection by Botrytis cinerea quality of strawberry fruit. *Postharvest Biology and Technology*, *20*, 39–51.

Refai, M., El-Yazid, H. A., & Tawakkol, W. (2015). Monograph on the genus Penicillium: A guide for historical, classification and identification of penicilli, their industrial applications and detrimental effects. *Cairo University*, 1–157.

Renard, C. M. G. C., Ginies, C., Gouble, B., et al. (2013). Home conservation strategies for tomato (Solanum lycopersicum): Storage temperature vs. duration-Is there a compromise for better aroma preservation? *Food Chemistry*, *139*, 825–836.

Rixin, H., Mao, T., & Ren, C. L. (2015). Taxonomic identities and phylogenetic relationships of fungal species isolated from tree shrew hair samplesfile:///F:/Articles/identification/2014, Manamgoda, The genus Bipolaris... curvularia kusanoi.pdf. *International Journal of Microbiology Research and Reviews ISSN 2329-9800*, *4*(7), 161–163.

Robeson, D. J., & Strobel, G. A. (1981). α/β- Dehydrocurvularin and Curvularin from Alternaria cinerariae. *Notizen*, *36e*, 1081–1083.

Rodrigo, S., Santamaria, O., Halecker, S., et al. (2017). Antagonism between Byssochlamys spectabilis (anamorph Paecilomyces variotii) and plant pathogens: Involvement of the bioactive compounds produced by the endophyte. *Annals of Applied Biology*, 1–13.

Rolfe, R. D. (2000). The role of probiotic cultures in the control of gastrointestinal health. *The Journal of Nutrition*, 396S–402S.

Ruiz, L., Margolles, A., & Sánchez, B. (2013). Bile resistance mechanisms in Lactobacillus and Bifidobacterium. *Frontiers in Microbiology*, *4*, 1–8.

Sahu, D. K., Khare, C. P., Singh, H. K., et al. (2013). Evaluation of Newer Fungicide for Management of Early Blight of Tomato in Chhattisgarh. *The Bioscan*, *8*(4), 1255–1259.

Saitou, N., & Nei, M. (1987). The neighbor-joining method. a new method for reconstructing phylogenetic trees.

Samson, R. A., Houbraken, J., Varga, J., et al. (2009). Polyphasic taxonomy of the heat resistant ascomycete genus Byssochlamys and its Paecilomyces anamorphs. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, *22*, 14–27.

Samuel, O., & Orji, M. U. (2015). Fungi associated with the spoilage of post-harvest tomato fruits sold in major markets in Awka , Nigeria. *Universal Journal of Microbiology Research*, *3*(2), 11–16.

Sánchez-Rangel, D., Sánchez-Nieto, S., & Plasencia, J. (2012). Fumonisin B1, a toxin produced by Fusarium verticillioides, modulates maize β-1,3-glucanase activities involved in defense response. *Planta*, *235*, 965–978.

Sanders, M. E. (2008). Probiotics: Definition, sources, selection and uses. *Clinical Infectious Diseases*, *46*, S58–S61.

Sanzani, S. M., Reverberi, M., & Geisen, R. (2016). Mycotoxins in harvested fruits and vegetables: Insights in producing fungi, biological role, conducive conditions, and tools to manage postharvest contamination. *Postharvest Biology and Technology*, *122*, 95–105.

Sarao, L. K., & Arora, M. (2017). Probiotics, prebiotics, and microencapsulation: A review. *Critical Reviews in Food Science and Nutrition*, *57*(2), 344–371.

SAS. (1999). SAS / STAT Software : Changes and Enhancements, Release 8.2. *SAS Institute Inc, SAS Campus Drive, Cary, North Carolina 27513*, 1–347.

Schena, L., Nigro, F., Pentimone, I., et al. (2003). Control of postharvest rots of sweet cherries and table grapes with endophytic isolates of Aureobasidium pullulans. *Postharvest Biology and Technology*, *30*(3), 209–220.

Selim, M. E., & El-Gammal, N. A. (2015). Role of fusaric acid mycotoxin in pathogensis process of tomato wilt disease caused by Fusarium oxysporum. *Journal of Bioprocessing &*

Biotechniques, *5*(10), 1–5.

Shafiq, S. A. (2015). Antagonistic activity of probiotic and sea weed extract against vegetative growth for some fungi and zearalenone production. *World Journal of Pharmaceutical Research*, *4*(1), 1577–1585.

Shah, V. V., Shah, N. D., & Patrekar, P. V. (2013). Medicinal plants from solanaceae family. *Research Journal of Pharmacy and Technology*, *6*(2), 143–151.

Shamshiri, R. R., Jones, J. W., Thorp, K. R., et al. (2018). A review of optimum temperature, humidity, and vapor pressure deficit for microclimate evaluation and control in greenhouse cultivation of tomato: A review. *International Agrophysics*, *32*, 287–302.

Shao, X., Clark, B., Konovalchuk, V., et al. (2004). Identifying problems facing smallholder South african farmers through participatory rural appraisals - Case studies with smallholder farmers. *Association for International Agricultural and Extension Education 20th Annual Conference*, 588–597.

Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika Trust*, *52*(3), 591–611.

Shnain, R. S., Prasad, V. M., & Saravanan, S. (2017). Effect of zinc and boron on growth of tomato (Solanum esculentum Mill.) c.v. Heem sohna, under protected cultivation. *The Allahabad Farmer*, *LXXIII*(1), 46–49.

Shukla, G., Sharma, G., & Goyal, N. (2010). Probiotic characterization of Lactobacilli and Yeast strains isolated from whey beverage and therapeutic potential of Lactobacillus yoghurt in Murine giardiasis. *American Journal of Biomedical Sciences*, *2*(3), 248–261.

Siciliano, I., Gilardi, G., Ortu, G., et al. (2017). Identification and characterization of Alternaria species causing leaf spot on cabbage, cauliflower, wild and cultivated rocket by using molecular and morphological features and mycotoxin production. *European Journal of Plant Pathology*, *149*(2), 401–413.

Silva, G. M. C., Silva, W. B., Medeiros, D. B., et al. (2017). The chitosan affects severely the carbon metabolism in mango (Mangifera indica L. cv. Palmer) fruit during storage. *Food Chemistry*, *237*, 372–378.

Snedecor, G. W., & Cochran, W. G. (1956). Statistical methods. 6th ed. *Ames, Iowa: Iowa State*

University Press, *19*, 1–593.

Song, D., Ibrahim, S., & Hayek, S. (2012). Recent application of probiotics in food and agricultural science: Chapter 1. *Probiotics*, 3–36.

Soomro, A. H., & Masud, T. (2012). Probiotic characteristics of Lactobacillus spp. isolated from fermented milk product Dahi. *Food Science and Technology Research*, *18*(1), 91–98.

Spadaro, D., & Gullino, M. L. (2004). State of the art and future prospects of the biological control of postharvest fruit diseases. *International Journal of Food Microbiology*, *91*, 185–194.

Stansly, P. A., Sánchez, P. A., Rodríguez, J. M., et al. (2004). Prospects for biological control of Bemisia tabaci (Homoptera, Aleyrodidae) in greenhouse tomatoes of southern Spain. *Crop Protection*, *23*, 701–712.

Starke Ayres. (2014). *Tomato production guideline*. *Starke Ayres Member of The Plennegy Group*.

Suprapta, D. N. (2012). Potential of microbial antagonists as biocontrol agents against plant fungal pathogens. *ISSAAS Journal*, *18*(2), 1–8.

Tan, Y. P., Crous, P. W., & Shivas, R. G. (2018). Cryptic species of Curvularia in the culture collection of the Queensland Plant Pathology Herbarium. *MycoKeys*, *35*, 1–25.

Tang, J., Liu, Y., Li, H., et al. (2015). Combining an antagonistic yeast with harpin treatment to control postharvest decay of kiwifruit. *Biological Control*, *89*, 61–67. https://doi.org/10.1016/j.biocontrol.2015.04.025

Temitope, F. P., & Oluchi, E. U. (2015). Studies of the antifungal activity Lactobacillus plantarum and Lactobacillus fermentum on spoilage fungi of tomato fruit. *Journal of Microbiology Research*, *5*(3), 95–100.

Thamaga-Chitja, J. M., & Morojele, P. (2014). The context of smallholder farming in South africa: Towards a livelihood asset building framework. *Journal of Human Ecology*, *45*(2), 147–155.

Thambugala, K. M., Wanasinghe, D. N., Phillips, A. J. L., et al. (2017). Mycosphere notes 1-50: Grass (Poaceae) inhabiting Dothideomycetes. *Mycosphere*, *8*(4), 697–796.

Thiyam, B., & Sharma, G. D. (2013). Isolation and identification of fungi associated with local fruits of Barak valley, Assam. *Current World Environment*, *8*(2), 319–322.

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap

penalties and weight matrix choice. *Nucleic Acids Research*, *22*(22), 4673–4680.

Tournas, V. H., & Katsoudas, E. (2005). Mould and yeast flora in fresh berries, grapes and citrus fruits. *International Journal of Food Microbiology*, *105*, 11–17.

Tshiala, M. F., & Olwoch, J. M. (2010). Impact of climate variability on tomato production in Limpopo province, South Africa. *African Journal of Agricultural Research*, *5*(21), 2945–2951.

ur Rehman, M., Khan, N., & Jan, I. (2007). Post harvest losses in tomato crop (A case of Peshawar valley). *Sarhad Journal of Agriculture*, *23*(4).

Usall, J., Ippolito, A., Sisquella, M., et al. (2016). Physical treatments to control postharvest diseases of fresh fruits and vegetables. *Postharvest Biology and Technology*, *122*, 30–40.

Usman, & Hosono, A. (1999). Bile tolerance, taurocholate deconjugation, and binding of cholesterol by Lactobacillus gasseri Strains. *Journal of Dairy Science*, *82*, 243–248.

Vaklounakis, D. J. (1991). Control of early blight og greenhouse tomato, caused by Alternaria solani, by inhibiting sporulation with ultraviolet-absorbing vinyl film. *The American Phytopathological Society*, 795–797.

van Averbeke, W., & Mohamed, S. S. (2006). Smallholder farming styles and development policy in South africa: The case of Dzindi irrigation scheme. *Agrekon*, *45*(2), 136–151.

Verón, H. E., Di Risio, H. D., Isla, M. I., et al. (2017). Isolation and selection of potential probiotic lactic acid bacteria from Opuntia ficus-indica fruits that grow in Northwest Argentina. *LWT - Food Science and Technology*, *84*, 231–240.

Vinderola, G., Capellini, B., Villarreal, F., et al. (2008). Usefulness of a set of simple in vitro tests for the screening and identification of probiotic candidate strains for dairy use. *LWT - Food Science and Technology*, *41*, 1678–1688.

Visagie, C. M., Houbraken, J., Frisvad, J. C., et al. (2014). Identification and nomenclature of the genus Penicillium. *Studies in Mycology*, *78*, 343–371.

Wang, Y.-T., Liu, R.-L., Huang, S.-W., et al. (2009). Effects of potassium application on flavor compounds of cherry tomato fruits. *Journal of Plant Nutrition*, *32*(9), 1451–1468.

Wani, A. H. (2011). An overview of the fungal rot of tomato. *Mycopathologica, 9*(1), 33–38.

Whitaker, C., Berjak, P., Kolberg, H., et al. (2004). Responses to various manipulations, and storage potential, of seeds of the unique desert gymnosperm, Welwitschia mirabilis Hook. fil.

South African Journal of Botany, *70*(4), 622–630.

White, T. J., Bruns, T., Lee, S., et al. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications*.

Wiggins, S., & Keats, S. (2013). Smallholder agriculture's contribution to better nutrition. *Overseas Development Institute*, 1–114.

Wisniewski, M., Biles, C., Droby, S., et al. (1991). Mode of action of the postharvest biocontrol yeast, Pichia guilliermondii. I. Characterization of attachment to Botrytis cinerea. *Physiological and Molecular Plant Pathology*, *39*, 245–258.

World Bank. (2011). Missing Food :The Case of postharvest Grain Losses in Sub-Saharan African. *The World Bank*, *60371–AFR*(60371), 116. https://doi.org/Report No. 60371-AFR

Wu, F., & Tanksley, S. D. (2010). Chromosomal evolution in the plant family Solanaceae. *BMC Genomics*, *11*(182), 1–11.

Xie, L. W., Ouyang, Y. C., Zou, K., et al. (2009). Isolation and difference in anti-Staphylococcus aureus bioactivity of curvularin derivates from fungus Eupenicillium sp. *Applied Biochemistry and Biotechnology*, *159*(1), 284–293.

Yadav, S., Ameta, K. D., Sharma, S. K., et al. (2017). Effect of spacing and training on vegetative growth characteristics and yield of tomato (Solanum lycopersicum L .) grown in polyhouse. *International Journal of Current Microbiology and Applied Sciences*, *6*(5), 1969–1976.

Yadav, S. M., & Singh, V. (2016). Cultural, morphological and pathogenic variability of different tomato isolates of Alternaria solani in India.

Yang, R., Fan, X., Cai, X., et al. (2015). The inhibitory mechanisms by mixtures of two endophytic bacterial strains isolated from Ginkgo biloba against pepper phytophthora blight. *Biological Control*, *85*, 59–67.

Yocum, R. R., Rasmussen, J. R., & Strominger, J. L. (1980). The mechanism of action of Penicillin. *Journal of Biological Chemistry*, *255*(9), 3977–3986. Retrieved from https://davisplus.fadavis.com/3976/meddeck/pdf/penicilling.pdf

Zalar, P., Gostinčar, C., de Hoog, G. S., et al. (2008). Redefinition of Aureobasidium pullulans and its varieties. *Studies in Mycology*, *61*, 21–38.

Zhao, Y., Wang, R., Tu, K., et al. (2011). Efficacy of preharvest spraying with Pichia guilliermondii on postharvest decay and quality of cherry tomato fruit during storage. *African Journal of Biotechnology*, *10*(47), 9613–9622. https://doi.org/10.5897/AJB11.870

Zhu, Z., Zhang, Z., Qin, G., et al. (2010). Effects of brassinosteroids on postharvest disease and senescence of jujube fruit in storage. *Postharvest Biology and Technology*, *56*(1), 50–55. https://doi.org/10.1016/j.postharvbio.2009.11.014

Živković, S., Stojanović, S., Ivanović, Ž., et al. (2010). Screening of antagonistic activity of microorganisms against Colletotrichum acutatum and Colletotrichum gloeosporioides. *Archives of Biological Sciences (Belgrade)*, *62*(3), 611–623.

Žnidarčič, D., Ban, D., Oplanić, M., et al. (2010). Influence of postharvest temperatures on physicochemical quality of tomatoes (Lycopersicon esculentum Mill.). *Journal of Food, Agriculture and Environment*, *8*(1), 21–25.

Zong, Y., Liu, J., Li, B., et al. (2010). Effects of yeast antagonists in combination with hot water treatment on postharvest diseases of tomato fruit. *Biological Control*, *54*, 316–321.

SUPPLEMENTARY INFORMATION

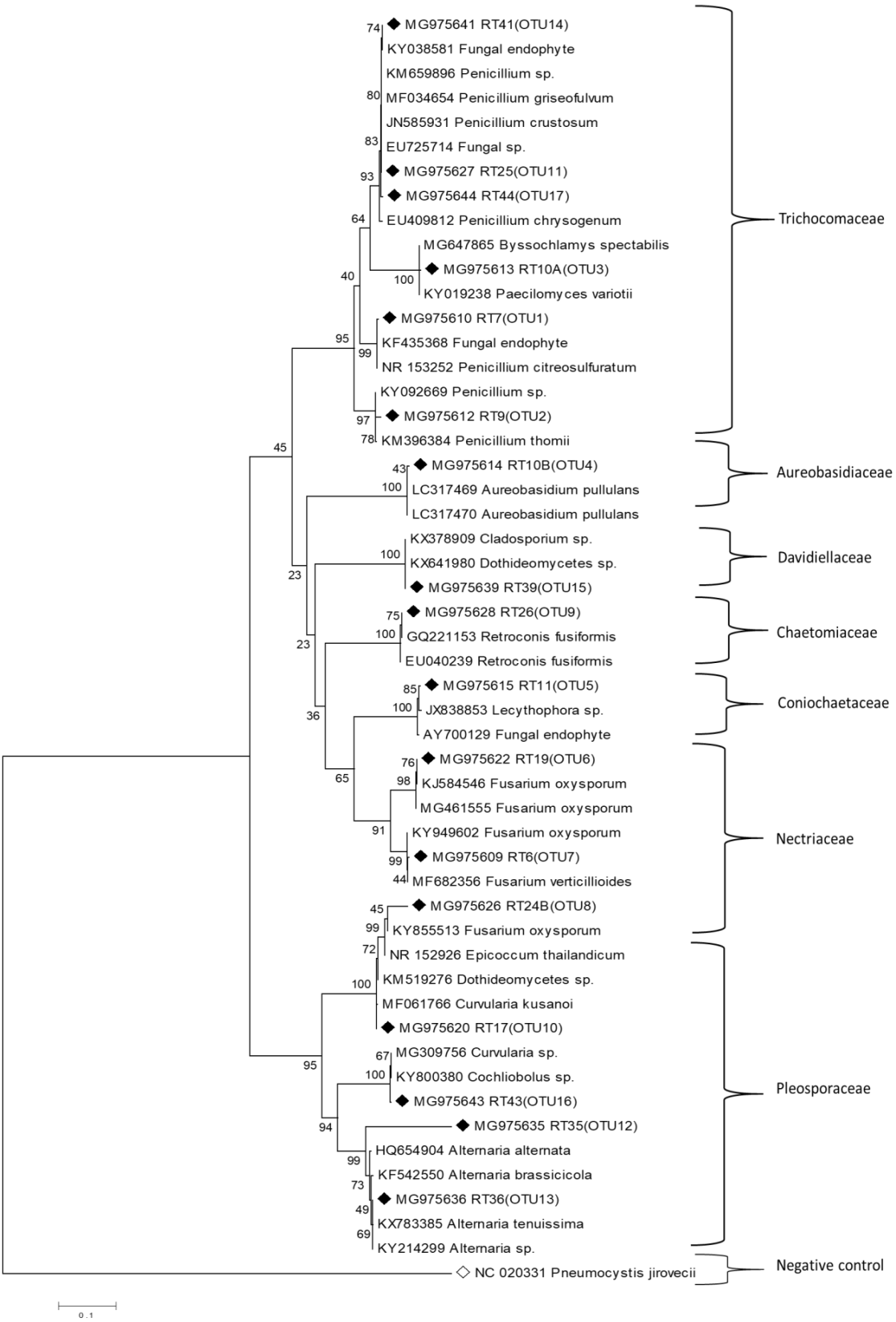


Figure S1: Phylogenic tree of different fungal isolates from tomatoes using the Neighbor-joining method. The statistical significance was estimated by 1000 bootstrap replications to estimate the stability and support of the branches. Bar, 0.1 nt substitution rate (Knuc).

Table S1: The viable cell counts before and after freeze drying

Fungal antagonists	Viable cell counts (cells/ml) Freeze drying	
	Before	After
B. spectabilis	3.20×10^5	3.04×10^5
C. kusanoi	4.08×10^5	3.20×10^5
E. thailandicum	3.0×10^5	2.52×10^5
R. fusiforms	3.95×10^5	3.24×10^5

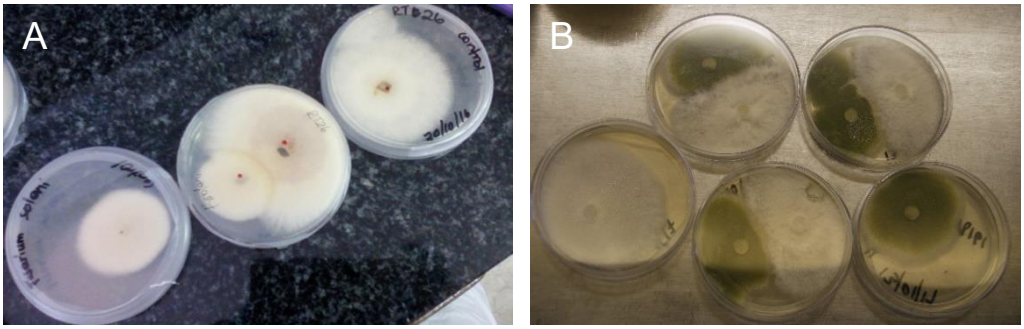


Figure S2: Dual culture assay of (A) *Retroconis fusiformis* against *Fusarium solani* ATCC 36031 and (B) *Penicillium crustosum* against *Geotrichum candidum* ATCC 34614

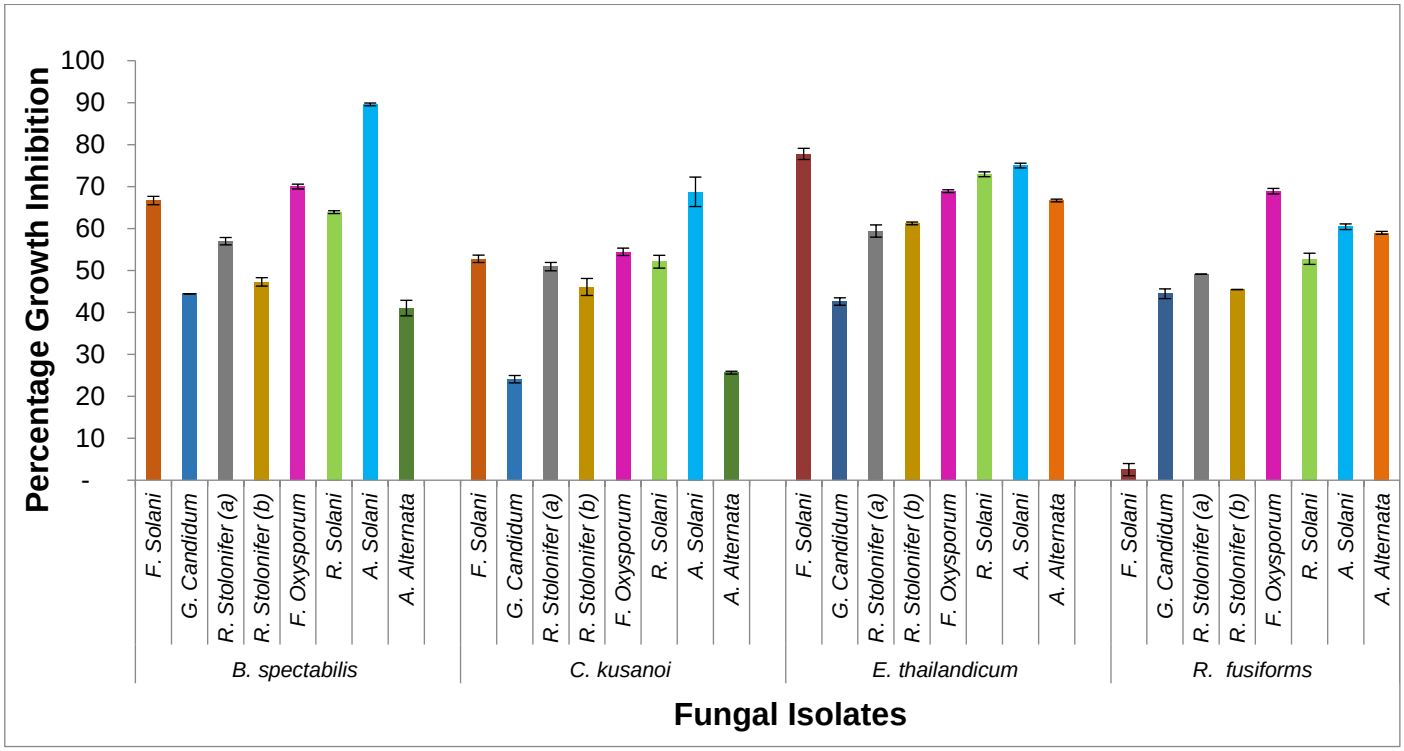


Figure S3: The 4 fungal antagonists against 8 fungal pathogens

Table S2: The weight loss of tomatoes during storage

A

Treatments	Weight loss (kg)			
	0	5	10	15
Control (-)	15.65	14.89	13.95	13.15
Control (+)	15.65	14.96	14.27	13.30
B. spectabilis	15.65	14.92	14.43	13.45
C. kusanoi	15.65	15.03	14.50	13.95
E. thailandicum	15.65	15.05	14.46	13.80
R. fusiforms	15.65	15.04	14.45	13.65

B

Treatments	Weight loss (kg)			
	0	5	10	15
Control (-)	15.65	14.75	13.49	12.15
Control (+)	15.65	15.27	13.82	13.10
B. spectabilis	15.65	14.90	13.99	12.65
C. kusanoi	15.65	15.35	14.50	13.25
E. thailandicum	15.65	14.91	13.16	12.90
R. fusiforms	15.65	14.94	13.95	12.75

A = 8 °C refrigeration and B = uncontrolled room. Negative control (-) are untreated tomatoes, whereas positive control (+) represents tomatoes treated with Sporekill® fungicide.

Table S3: The number of spoiled tomato during storage

Storage conditions	**Days**	**Number of spoiled tomatoes**					
		B. spectabilis	***C. kusanoi***	***E. thailandicum***	***R. fusiforms***	**Control (-)**	**Control (+)**
Controlled (8°C) temperature	5	2	1	1	2	4	0
	10	5	2	3	6	8	2
	15	9	6	6	12	14	6
Uncontrolled (Room) temperature	5	7	5	3	9	12	1
	10	10	7	7	14	16	3
	15	15	8	11	16	18	7

n= 30 tomato fruit

Reduction of post-harvest losses in tomato using fungal bio-preservative for smallholder farmers

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To whom it may concern

This is to certify that I, Alexa Kirsten Barnby, an English editor accredited by the South African Translators' Institute, have edited the dissertation submitted for the degree Masters of Science in Microbiology at North-West University, titled "Reduction of post-harvest losses in tomato using fungal bio-preservative: For smallholder farmers" by LR Moeng.

The onus is, however, on the author to make the changes and address the comments made.