Control of mould spoilage on apples using yeast-based biological agents

By

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DECLARATION

I, **Zukisani Gomomo**, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed **Date**

ABSTRACT

In orchards, storage and processing facilities, considerable quantities of fresh products are lost due to mould spoilage thus threatening the economic growth, food safety and security. Twenty five percent of the total fruits produced in industrialised countries is lost due to mould and more than 50% in developing countries. Fresh fruit and those used for beverage production must meet stringent quality and regulatory requirements in order to reach consumers in good quality. Currently, preservation can be achieved by application of synthetic chemicals to control the growth of mould. However, chemical fungicides pose serious health concerns to consumers such as skin and eyesight damage, as well as cardiovascular diseases. Synthetic fungicides can also have a negative impact on the environment, which results in the search for alternative methods of preservation. Biological control is a promising alternative to reduce or eliminate the use of synthetic chemicals, since it is more environmentally friendly and cost-effective. Yeasts can be used as alternative to synthetic chemicals because of their ability to compete for nutrients and produce inhibitory growth compounds The aim of this study was to screen yeasts for growth inhibition activity against fruit spoilage mould, *Botrytis cinerea*, *Penicillium expansum* and *Alternaria alstroemeriae* under *in vitro* and *in vivo* conditions.

Yeasts were screened against *Botrytis cinerea*, *Penicillium expansum* and *Alternaria alstroeneriae* using radial fungal plug inhibition assay, dual inhibition assay and the mouth-to-mouth plate inhibition assay. Three yeast strains were also evaluated for growth inhibition activity against the mould during post-harvest apple trials. Out of 104 yeasts screened, 67 showed growth inhibition activity against *Penicillium expansum,* 36 yeasts inhibited *Botrytis cinerea,* 47 yeasts inhibited *Alternaria alstroemeriae* and 22 yeasts showed inhibition against all three moulds. *Candida pyralidae, Meyerozyma guilliermondii* and *Zygoascus hellenicus* yeast strains showed highest inhibition activity against *P. expansum* (38%, 42% and 35%), *B. cinerea* (62%, 63% and 58%) and *A. alstroemeriae* (34%, 40% and 35%), respectively. Volatile

organic compounds produced by *Pichia kluyveri*, showed 81%, 91% and 76% growth inhibition activity against *P. expansum B. cinerea* and *A. alstroemeriae* respectively. While, *C. pyralidae* showed 57% and 68% against *B. cinerea* and *A. alstroemeriae* respectively, and two *M guilliermondii* yeast strains were effective in inhibiting the growth of all three mould with percentage inhibition ranging from 57% to 70%.

On apples, a commercial fungicide showed 100%, 51% and 24% growth inhibition activity against *A. alstroemeriae, B. cinerea* and *P. expansum,* respectively. While the selected *P. kluyveri* strain showed 100%, 57% and 26% inhibition against *A. alstroemeriae, B. cinerea* and *P. expansum,* respectively, and *M. guillermondii* showed 100%, 60% and 18% inhibition against *A. alstroemeriae, B. cinerea* and *P. expansum,* respectively. *C. pyralidae* showed least inhibition activity by showing 36%, 47% and 13% inhibition against *A. alstroemeriae, B. cinerea* and *P. expansum,* respectively. The *P. kluyveri* and *M. guilliermondii* strains showed good potential as biocontrol agents against spoilage moulds and needs further investigation. Yeast can be used to control the growth of mould, but the level of inhibition is species dependent. The bio-control yeasts that were used in this study were comparable to a commercial fungicide, therefore they can potentially be considered as alternatives to chemical fungicides. Future research should investigate the application of these yeasts as biofungicides during pre- and post-harvest trials of different fruit.

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DEDICATION

Gratefully dedicate this work to heavenly Father, my ancestors, my father Zoyisile Gomomo, my late mother Linda Gomomo, my two sisters Sinovuyo Gomomo and Liqhayiya Gomomo and the beloved Gomomo family at large under clan name (AmaGiqwa) with close friends for supporting me in difficult and good times, their prayers were always there motivating me and always seeing potential in me. They have been my providers throughout the duration of my study, I am truly grateful.

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GLOSSARY

Abbreviations/Symbols	Definition (units)
°C	Degree Celsius
ANOVA	Analysis of variance
YMA	Yeast Malt Peptone Agar
YMB	Yeast Malt Peptone broth
PDA	Potato Dextrose Agar
ANOVA	Analysis of variance
ARC	Agricultural Research Council
RPM	Revolutions per minute (rev/min)
GPB	Grape pomace extract broth
DNA	Deoxyribonucleic acid
ITS	Internal transcriber spacer
PCR	Polymerase Chain Reaction

OUTLINE OF THE THESIS

The research presented in this thesis was conducted at ARC Infruitec-Nietvoorbij (Fruit, Wine and Vine Institute of the Agricultural Research Council), Stellenbosch, South Africa, in collaboration with the Agricultural Research Group, Department of Agriculture, Cape Peninsula University of Technology, South Africa.

The thesis comprises six chapters:

Chapter 1: Gives a brief introduction to the thesis and objectives of the study, and provides an outline of the thesis.

Chapter 2: Contains the literature review.

Chapter 3: Covers the research design and the results that were generated. Submitted to Polish Journal of Food and Nutrition Sciences

Chapter 4: Focuses on the general discussion, conclusions and recommendations

Chapter 5: List bibliographical references consulted for the research

Chapter 6: Appendices

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

The control and maintenance of fruits is one of the most important aspects in agricultural industry, thus fruits play an important role in contributing to the sustainability of the global economy. Fruits meant for both local and export markets must meet stringent quality and regulatory requirements in order to reach consumers in good quality [Calvin *et al*., 2006; Jongwanich, 2009; Al-hindi *et al*., 2011]. Apples (*Malus domestica)* are the third most cultivated fruit crop in the world and the production of apples has increased to about 85 million tonnes in 2016 [FAOstat, 2019]. South Africa produces approximately 1.3 million tonnes of apples each year, with a total value of more than R8 billion and 92% of this income is generated by fresh fruit sales [Du Plessis, 2017].

Fruits are characterised by low pH, and high levels of sugars and nutrients, which make them particularly susceptible to fungal decay [Al-hindi *et al*., 2011]. Agricultural produce are subject to mould spoilage before, during and after harvest, as well as during transportation and processing [Romanazzi *et al*., 2016, 2017]. Fruit losses due to mould pose several challenges to the agrifood industry [Salman, 2005; Parveen *et al*., 2016]. Each year, 25% of the total fruits produced in industrialised countries are lost and more than 50% in developing countries. [Droby, 2005; Nunes, 2012]. These losses in return affect negatively on the economy of the producing countries. Extending the shelf life and improving the quality of fruit post-harvest and during transportation remains important to the agricultural industry. During the pre- and post-harvest stages, considerable amounts of fruits including apples are lost due to diseases caused by *Botrytis cinerea* (grey mould), *Penicillium expansum* (blue mould), *Alternaria* spp. (necrotic leaf blotch), *Venturia inaequalis* (apple scab), *Cladosporium* spp. (sooty spot),

Aspergillus spp. (black mould), *Mucor* spp. (Mucor rot), *Rhizopus stolonifer* (black bread mould) and *Colletotrichum gloeosporioides* (bitter rot) [Sharma *et al*., 2009].

Spoilage mould need to be controlled to maintain the quality and quantity of fruit produced around the world [Mercier & Lindow, 2001]. This can only be achieved by preservation or post-harvest control without altering the properties of the products, and also without posing health problems to consumers [Calvin *et al*., 2006]. Currently, fruit producers and exporters are using costly spraying programs incorporating synthetic chemicals, which are labour intensive and require the application of various classes of fungicides sprayed up to 20 times during the growing season. These practices can negatively affect the health of consumers, the environment, the taste and aroma of the food being preserved [Oliveira *et al*., 2014; Contarino *et al*., 2019]. In addition, prolonged exposure to those chemicals even at the lowest dose possible can lead to serious health problems in humans [Benito *et al*., 2009]. Some fungi can become resistant to fungicides as farmers use the chemicals regularly [Fernández-Ortuño *et al*., 2008].

Consumers are forcing the industries to reduce chemical residues and are promoting the search for alternatives to synthetic chemical fungicides [Quaglia *et al*., 2011; Robiglio *et al*., 2011]. The recent trend is shifting towards safer and environmentally friendly alternatives for the control of post-harvest decay [Sharma *et al*., 2009; Chitranshi *et al*., 2020]. Biocontrol agents are more environmentally friendly since they leave no residues behind after application and cost effective than synthetic chemicals, because the agents can reproduce and they are available in food, beverages and even on waste materials [Öberg, 2002; Suprapta, 2012].

Yeasts can be used as alternative to synthetic chemicals because of their ability to compete for nutrients, space, their ability to grow faster than fungal pathogens and the production of inhibitory growth compounds [Liu *et al*., 2013; Mewa-Ngongang *et al*., 2019a, b]. Advantage

of biological systems is their ability to secrete extracellular metabolites, such as Volatile organic compound (VOCs), Acetic acid, hydrogen sulphide and cell wall degrading enzymes such as laminarinases, glucanases, proteases, peroxidase and chitinases with antimicrobial properties against many fruit spoilage mould [Grevesse *et al*., 2003; Hua *et al*., 2014; Cordero-Bueso *et al*., 2017; Zhou *et al*., 2018; Mewa-Ngongang *et al*., 2019b]. The aim of this study was to screen yeasts for growth inhibition activity against fruit spoilage mould, *Botrytis cinerea, Penicillium expansum* and *Alternaria alstroemeriae in vitro* and *in vivo*.

1.2 Research questions

- Will the yeast-based biological agent be able to control mould growth of apples?
- Can the yeast-based formulation be used as pre and post-harvest biocontrol agent?
- What will be the nature of growth inhibition and the mode of inhibition associated with the biocontrol activity?
- What are the predominant species that will show antagonistic effect?

1.3 Aim and objectives

The aim of this study was to evaluate the efficacy of yeast-based biological agents to control spoilage of *Botrytis cinerea, Penicillium expansum* and *Alternaria alstroemeriae* on apples.

Objective 1: To screen and identify yeasts for growth inhibition activity against *Botrytis cinerea, Penicillium expansum* and *Alternaria alstroemeriae.*

Objective 2: To use a selection of biological control yeasts (n = 22) for further evaluation during *in vitro* plate assays against spoilage moulds.

Objective 3: Use selected biological control yeasts (n = 3) for *in vivo* bioassay based on activity against spoilage moulds.

1.4 Delineation of the research

This study did not investigate the application of the biocontrol agents in experimental or commercial orchards and no toxicology study of the biocontrol agents was conducted.

1.5 Significance of the research

This research will help in sustainable consumption and production of fruit, since the biocontrol agents are environmentally friendly, and are safe for human consumption. Since synthetic chemicals leave residues on fruits that cause harm to humans, biocontrol is the best alternative for good health and well-being on humans. This study also provide novel information about the growth inhibition activity of various yeast species against *Botrytis cinerea*, *Penicillium expansum* and *Alternaria alstroemeriae.*

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In this section, the importance of apples in human health, how this fruit play major role in human diet and inhibit some chronic diseases will be briefly discussed. This review will also cover the commercial importance and economic value of apples in South Africa. From planting until storage apples are exposed to fungal pathogens, which can cause huge losses to producers and can also affect the appearance of the fruit during marketing. Mould have been causing major problems to fruits for decades [Janisiewicz & Korsten, 2002]. The four major fungal pathogens affecting apples will be discussed briefly according to life cycle, symptoms, infection rate and sources. This section will also cover the prevention methods of these fungal pathogens, focusing on chemical control and biological control.

2.2 The importance of apples to human health

Apples (*Malus domestica*) are perishable crops, widely cultivated important economic fruit crop with nutritive and medicinal importance [Ben-Noun, 2016; Singh & Sharma, 2018]. Apples play a major role as a significant source of sugars, minerals, dietary fibre in human diet [Singh & Bedi, 1990; Vinson *et al*., 2001; Bondonno *et al*., 2017]. This fruit also contains antioxidants such as quercetin, catechin, phloridzin and chlorogenic acid that are capable of inhibiting cancer cell proliferation, lower cholesterol, prevent diabetes and heart disease [Singh & Bedi, 1990; Ben-Noun, 2016]. The concentration of nutrients required by the body depends on factors such as age, mass, sex, health and physical activity [Fourie, 1996]. Apples are a good source of soluble fibre, especially pectin a structurally and functionally complex polysaccharide [Mohnen, 2008], which helps to control insulin levels by slowing the release of

sugar into the bloodstream and it can also help to reduce cholesterol levels by lowering insulin secretion [Lee, 2012].

2.3 Commercial importance of apples

Apples are classified as deciduous fruits [Sacu, 2007]. The pomaceous fruit of the apple tree, species *Malus domestica* belong to the rose family (*Rosaceae*) [Sun *et al*., 2013]. Globally, apples are the third most produced fruit, with production increasing from 58 million tonnes in 2001 to 75.5 million tonnes in 2011 [FAOstat, 2013]. South Africa is a relatively small apple grower in terms of global hectares and produces approximately 1.3 million tonnes each year, with a total value of more than R8 billion [Du Plessis, 2017] . Ninety two percent of this income is generated by fresh fruit sales [Du Plessis, 2017].

There are over 6000 regionally important cultivars, but a few major cultivars dominate worldwide [Ramírez & Davenport, 2013]. Most commercial cultivars have an ancient origin and a long propagation history, such as McIntosh (1800s), Jonathan (1820s), Cox's Orange Pippin (1830s), Granny Smith (1860s), Delicious (1870s) and Golden Delicious (1890s) [Liang *et al*., 2015]. Most apples in South Africa are produced in Ceres, Groenland, Villiersdorp/Vyeboom in the Western Cape and Langkloof in the Eastern Cape [Sacu, 2007]. The South African apple industry is export-oriented with approximately half of the apples produced being absorbed by the export market [Department of Agriculture Forestry and Fisheries, 2018].

South Africa exports approximately 43% of produced apples every year, and the main buyers of South African apples are the UK and Malaysia [Garming *et al*., 2014]. The total area for apple cultivation in South Africa is 24 156 hectares [Department of Agriculture Forestry and Fisheries, 2018]. During the 2016 and 2017 seasons, apples contributed about 28.2% (5.5 billion Rand) of the total gross value for deciduous fruits in South Africa [Department of

Agriculture Forestry and Fisheries, 2018]. Due to increase in area planted and yields, available irrigation water and improved water management techniques by farmers, the production of apples is estimated to increase by 6% in the year 2019 and 2020 [Wellington, 2019]. Post-harvest losses in the world can range from 10 to 30% per year despite the use of modern storage facilities and chemicals applications [Singh *et al*., 2017].

2.4 Fungal pathogens of apples

Spoilage microorganisms require nutrients for their growth and proliferation. Fungal pathogens cause huge losses to harvested apples during transportation and storage [Sharma, *et al*., 2009]. Losses of fresh apples after harvest can be very high depending on the species, harvest methods, length of storage and marketing conditions [Ippolito & Nigro, 2000]. Post-harvest decay has been a major problem for decades and overcoming this problem has been difficult [Janisiewicz & Korsten, 2002]. Pre-harvest infection of apples may occur through direct penetration of the skin, infection through natural openings on the produce, and infection through the damaged parts [Singh & Sharma, 2018].

There are several moulds that are capable of initiating the infection process on the surface of floral parts and on developing fruits [Singh & Sharma, 2018]. Mould, such as *Botrytis cinerea* (grey mould), *Penicillium expansum* (blue mould), *Alternaria* spp. (necrotic leaf blotch), *Venturia inaequalis* (apple scab), *Cladosporium* spp. (Sooty spot), *Aspergillus* spp. (black mould), *Mucor* spp. (Mucor rot), *Rhizopus stolonifer* (black bread mould) and *Colletotrichum gloeosporioides* (bitter rot) are responsible for the majority of pre- and post-harvest losses [Sharma *et al*., 2009; Singh & Sharma, 2018]. The growth of mould in highly perishable fruits such as apples and pears is one of the major problems that may lead to significant economic losses [Sardella *et al*., 2016]. Mould can be found everywhere and can grow in most moist environments, such as ceilings, floors, walls, and drains or improperly sanitised surfaces

[Sardella *et al*., 2016]. Major fungal pathogens affecting apples will be discussed briefly in the following sections.

2.4.1 *Penicillium expansum* (blue mould)

Blue mould caused by *P. expansum* is one of the most economically significant post-harvest disease known to cause soft rot or wet rot and in pears, apples, peach and other fruits [Zhang *et al*., 2007b; Sanzani *et al*., 2009; Vico *et al*., 2014]. It causes the loss as well as shortens the shelf-life of harvested fruits [Yu *et al*., 2007]. Infection of fruits by *P. expansum* has potential public health significance, since the pathogen produces the mycotoxin patulin, a highly reactive unsaturated lacton that can be found in several fruit-derived food (including apples and pears) [Beretta *et al*., 2000; Spadaro *et al*., 2007; Sanzani *et al*., 2009; Vico *et al*., 2014]. There is no clear evidence that patulin is carcinogenic, however, it has been shown to cause immunotoxic and neurotoxic effects in animals [Deveraj, 1982; Paucod *et al*., 1990; Wouters & Speijers, 1996]. Beretta *et al*. [2000] explained that patulin may cause acute and chronic toxicity, including carcinogenic, mutagenic, and teratogenic effects. Acute symptoms of patulin consumption can include agitation, convulsions, edema, ulceration, intestinal inflammation and vomiting [Speijers, 2004].

Blue mould originates primarily from infection of wounds, such as punctures, bruises, and limb rubs on the fruit [Sardella *et al*., 2016]. *P. expansum* spore masses may appear on the decayed area starting at the infection site and decayed fruit has an earthy musty odour [Shim *et al*., 2002; Sardella *et al*., 2016]. The first symptoms are soft watery brown spots, which undergo rapid enlargement at temperatures between 20°C and 25°C [Snowdon, 1990].

Blue mould can occur in production areas where the most advanced post-harvest storage technologies are available [Sanzani *et al*., 2009]. *P. expansum* can also occur during shipping and marketing of the fruit, dramatically reducing shelf life (affects the quality properties of the

infected fruit making it unmarketable) and causing serious losses [Sanzani *et al*., 2009; Sanzani *et al*., 2013; Wang *et al*., 2014]. Under adequate storage conditions, losses due to this rot should be less than 1%, but if storage problems occur, or conditions are less than optimal, losses can be 50% or more [Mari *et al*., 2002; Monroe, 2009].

2.4.2 *Botrytis cinerea* (grey mould)

B. cinerea is the causal agent of grey mould, an airborne plant pathogen with a necrotrophic lifestyle (kills the living cells of its host and then feeds on the dead matter) attacking over 200 crop hosts worldwide [Have *et al*., 1998; Williamson *et al*., 2007]. Grey mould is considered as the second most important pathogen of fruits such as apples and pears [Pierson, 1970; Aldwinckle & Jones, 1990; Dean *et al*., 2012]. *B. cinerea* produces a range of cell wall degrading enzymes, toxins and other low molecular weight compounds such as oxalic acid [Williamson *et al*., 2007].

Grey mould originates mainly from wounds, such as punctures and bruises, which are created during harvest or post-harvest handling [Rosenberger, 1990b; Xiao & Kim, 2008; Sardella *et al*., 2016]. Generally, grey mould does not have a distinct odour, but in advanced stages, it may have a pleasant fermented odour that differentiates it from other storage rots [Pierson *et al*., 1970]. The infection commonly begins on attached senescent flowers and then as a soft rot, it spreads to affect the adjacent developing fruit (blossom-end rot) it is characterised by a dry and shallow red discoloration [Sholberg *et al*., 2003; Williamson *et al*., 2007]. When the entire fruit is decayed, it may appear like a baked apple [Xiao & Kim, 2008]. Under high relative humidity, greyish spore masses and fluffy white to grey mycelia may appear on the decayed area [Xiao & Kim, 2008].

Losses as high as 20% to 60%, due to *B. cinerea* are common, especially after an extended period of storage and is mostly found on fruits that were not treated with fungicides prior to

storage [Sardella *et al*., 2016]. This is because grey mould has the ability to spread from decayed fruit to healthy fruit through direct contact of fruit during storage [Xiao & Kim, 2008; Sardella *et al*., 2016]. The disease develops more quickly in cold storage temperatures unlike other postharvest decays [Aldwinckle & Jones, 1990]. The mould is able to cause spoilage during cold storage where the temperatures ranges to –0.6°C to 2°C by forming a nest rot growing from infected to healthy fruit [Ogawa &English, 1991; Have *et al*., 1998; Xiao & Kim, 2008].

2.4.3 *Venturia inaequalis* (apple scab)

Apple scab, caused by the mould *V. inaequalis*, is the most important disease of apples and has been found in all apple-growing regions around the world, reducing yield and quality of fruit [MacHardy *et al*., 2001; Spitaler *et al*., 2004; Gladieux *et al*., 2008; Hossain *et al*., 2009]. The mould is virulent on all green herbaceous organs of the apple tree [Lauret, 2011]. *V. inaequalis* can infect and colonise the sepals, leaves, fruits, petioles, blossoms and even twigs of the tree, but the symptoms are commonly observed on the leaves and fruits [Lauret, 2011; Doolotkeldieva & Bobusheva, 2017]. *V. inaequalis* reproduces sexually and asexually and it rapidly builds up resistance against fungicides [Reddel, 2015]. *V. inaequalis* is responsible for disease of apple trees worldwide and requires a high number of fungicide applications to control [Schmoock *et al*., 2008; Fiaccadori *et al*., 2011].

Infection is initiated by ascospores (a spore contained in an ascus or that was produced inside an ascus) that are released by rainfall from pseudothecia, the release coincides with host budburst and leaf unfurling, increasing the probability of host infection [Carisse & Rolland, 2004; Reddel, 2015; Scheer *et al*., 2015]. On leaves and fruits, the disease appears as olive green to dark brown to black, soft circular spots that consist of sporulating mycelia, growing under the epidermis [Lauret, 2011; Sardella *et al*., 2016]. Apple scab causes badly scabbed or misshapen fruits, the tree becomes susceptible to chilling and freezing injuries, and causes

premature leaf and fruit fall [Lauret, 2011; Thakur *et al*., 2013; Sardella *et al*., 2016]. Research has shown that the mould can overwinter as conidia on bud scales and it mostly overwinters in the apple leaf litter [Carisse & Rolland, 2004; Doolotkeldieva & Bobusheva, 2017].

Apples are susceptible, primarily early in the growing season, but the scab may occur throughout the growing season [Scheer *et al.*, 2015; Sardella *et al.*, 2016]. In order to limit the damage by *V. inaequalis*, focus has to be on the reduction of primary inoculum during overwintering in the leaf litter [Scheer *et al.*, 2015]. The losses from apple scab can be 70% or more of the total fruit value [Agrios, 2005; Schmoock *et al*., 2008].

2.4.4 *Alternaria* species

Alternaria is a very complex genus that involves hundreds of species, but specific data are difficult to obtain because of the proliferation of nomenclature of doubtful taxonomic validity [Pastor & Guarro, 2008]. *Alternaria* causes a range of diseases with economic impact on a large variety of important agronomic host plants including apples [Thomma, 2003; Kayalvizhi & Antony, 2011; Patriarca & Fernández Pinto, 2018; Mir *et al*., 2020]. *Alternaria* species are able to colonise ripening fruits in the field and that will lead to spoilage of fruits after harvest and during storage [Thomma, 2003; Patriarca & Fernández Pinto, 2018]. *Alternaria* spp. are well known for the production of toxic secondary metabolites that are powerful mycotoxins that have been linked to the development of cancer in mammals [Pastor & Guarro, 2008; Thomma, 2003]. In addition, *Alternaria* is gaining importance as an emerging human pathogen, especially in immune compromised patients [Thomma, 2003; Patriarca & Fernández Pinto, 2018].

Alternaria species are foliar pathogens that cause slow damage of host tissues through the reduction of photosynthetic potential [Thomma, 2003; Kayalvizhi & Antony, 2011]. The most susceptible plants to *Alternaria* are those that are physiologically old, weak, wounded by wind, sand, hail or insects and weakened tissue due to stresses [Thomma, 2003; Tsedaley, 2014].

Small, slightly sunken, light to medium brown spots appear on the lenticels of the fruit (apples , pears, citrus) often post-rainfall and usually no earlier than 6-8 weeks prior to harvest [Horlock, 2006; Kayalvizhi & Antony, 2011; Tsedaley, 2014). During late spring or early summer a small, round, blackish spots appear on leaves, later they gradually enlarge in diameter to have purplish border [Mir *et al*., 2020].

Most *Alternaria* species are saprophytes that are commonly found in soil or on decaying plant tissue [Thomma, 2003; Pastor & Guarro, 2008; Patriarca & Fernández Pinto, 2018]. These mould grow well at low temperatures, they are responsible for spoilage of fruits and vegetables in refrigerated storage [Patriarca & Fernández Pinto, 2018]. Although exact figures have not been estimated yet but it is estimated that the disease have 70% infection rate on Delicious apples [Kayalvizhi & Antony, 2011].

2.5 Prevention methods for mould

2.5.1 Chemical control

Despite the wide-spread use of modern storage facilities and techniques, chemical fungicides are the major means by which post-harvest diseases of certain fruits are controlled [Spadaro *et al*., 2002; Calvo *et al*., 2007; Sharma *et al*., 2009; Lima *et al*., 2011]. For post-harvest mould control, triazoles, hydroanilide fenhexamid, dicarboximides and succinate dehydrogenase can be used [Meissner & Stammler, 2010]. Benzimidazole fungicides, including benomyl, thiabendazole (TBZ) and thiophanate-methyl, were introduced in the late 1960s [Zhao *et al*., 2010]. Benzimidazoles were initially registered for use in the orchard to control powdery mildew and other mould diseases as well as in the packinghouse to control post-harvest diseases including grey mold and blue mold [Zhao *et al*., 2010].

However, resistance to benzimidazole fungicides was observed among populations of *B. cinerea* and *P. expansum* on pear in the mid-1970s [Bertrand & Saulie-Carter, 1978]. Chemical

control is being increasingly limited because these chemicals have some negative effects on the taste and aroma of the fruits as well as the onset of fungicide-resistant strains of fungal pathogens [Errampalli *et al*., 2006; Lima *et al*., 2011]. Prolonged exposure to some of chemicals even at the lowest dose possible can lead to serious health problems [Benito *et al*., 2009; Yang *et al*., 2011a].

2.5.1.1 *Penicillium expansum* (blue mould)

The use of chemical fungicides is an important strategy for controlling *P. expansum* in harvested commodities [Spadaro *et al*., 2013]. First-generation post-harvest fungicides, such as sodium o-phenylphenate (SOPP), dichloran, sec-butylamine, etc., are effective in preventing decay by wound invading pathogens (e.g., *Penicillium*, *Rhizopus*, etc.) [Singh & Sharma, 2018]. Errampalli & Crnko, [2004] found that individual as well as mixtures of isolates of *P. expansum* are sensitive to fungicides fludioxonil and cyprodinil. Lima *et al*. [2011] showed that cyprodinil (CYPR) and boscalid (BOSC) at full and low dosage are highly effective against isolates of *P. expansum* under *in vitro* and *in vivo* trials. On the other hand on experiments carried out on wounded apples, Lima *et al*. [2011] found that thiabendazole (TBZ), which is presently allowed for post-harvest treatment of pome fruit in different countries, either at full or at low dosage, is ineffective against *P. expansum*.

2.5.1.2 *Botrytis cinerea* (grey mould)

Compounds with excellent *B. cinerea* activity came to the market, the pyrimidines cyprodinil, pyrimethanil and droxyanilide fenhexamid [Rosslenbroich & Stuebler, 2000]. Metomeclan, dichlofluanid, myclozolin, *N*-phenylcarbamate plus carbendazim and iprodione were the most efficient chemicals to control grey mould of rose flowers during 20 incubation. [Elad, 1988].

Three out of 123 *B. cinerea* isolates sampled from floral parts of apple from commercial apple orchards were highly resistant to thiabendazole, while all remaining isolates were sensitive to

thiabendazole [Zhao *et al*., 2010]. Acibenzolar-S-methyl resulted in a greater protection of the fruit against grey mould by significantly reducing the pathogen growth on apples [Spadaro *et al*., 2004].

2.5.1.3 *Venturia inaequalis* (apple scab)

Fungicides are generally considered to be the only economically feasible control measure against apple scab [Carisse & Rolland, 2004]. Control of apple scab in orchards relies on repeated applications of fungicides during spring and summer to prevent primary infection of fruit and leaves [MacHardy 1996; Scheer *et al*., 2015]. Summer applications of fungicides also reduce the late infection of apple fruit, which may result in scab lesions on stored fruit [Scheer *et al*., 2015]. When fungicides are applied during late summer to prevent pre-harvest infection, there is the risk that trace residues of fungicides may remain on the fruit at harvest [Beresford *et al*., 2008]. Apple scab control was mainly based on spray intervals of no more than 6–7 days, but the introduction of strobilurin fungicides led to the extension of the application interval up to about 10 days [Fiaccadori *et al*., 2011; Köhl *et al*., 2015] For organic cultivation, only a few substances are able to control scab effectively [Spitaler *et al*., 2004]. *V. inaequalis* populations developed resistance to an increasing number of fungicides such as dodine, benomyl, the DMI fungicides (sterol demethylation inhibitors) and newly developed strobilurin-based fungicides in major apple growing areas [Carisse & Pelletier, 1994; Fiaccadori *et al*., 2011; Beresford *et al*., 2013].

2.5.1.4 *Alternaria* species

The most common and effective method for the control of *Alternaria*, is through the application of foliar fungicides [Tsedaley, 2014]. It is suggested that fungicides must be applied regularly in the early stages of the disease to prevent infection [Tsedaley, 2014]. Captan, mencozeb and benomyl failed to control *Alternaria*, hence, iprodione chlorothalonil, maneb, or macozeb chemicals were introduced and showed best control of the disease *Alternaria*, [Filajdac &

Sutton, 1992; Tsedaley, 2014]. From flowering onwards, 3–4 sprays fungicide should be applied [Tsedaley, 2014].

2.5.2 Biological control

There are several definitions for biological control. De Bach [1964] defined biological control as the action of parasites, predators or pathogens in maintaining another organism's population density at a lower average than would occur in their absence. Eilenberg *et al*. [2001] defined biological control as the intentional introduction of a biological control agent (natural enemy or predator) for permanent establishment and long-term pest control. Michaud *et al*. [2008] defined biological control as the reduction of pest populations brought about through the actions of other living organisms, often referred to as natural enemies or beneficial species. The definition of Eilenberg *et al*. [2001] will be used as working definition in the context of this study.

The development of pathogen resistance to chemical fungicides, together with growing concern in human demand for healthier preservative methods and environmental pollution associated with the use of pesticides, have stimulated the search for alternative control methods [Spadaro & Gullino, 2004; Calvo *et al*., 2007; Quaglia *et al*., 2011]. Biocontrol is becoming one of the most promising alternatives to chemical fungicides. The societal pressure for healthy food, free of chemical residues, compelled research to rapidly develop environmental friendly methods [Gerbore *et al*., 2014]. Control of mould using biocontrol agents has increased, fuelled by the legislative removal of certain chemicals in some countries [Andersen & Winding, 2004]. Biological control is a component of integrated pest management (IPM) and is often most effective when coupled with other pest control tactics in an IPM program. There are substantial research and publications on biological control of plant pathogens, but the application of biocontrol options to control pathogens such as apple scab are still limited because successful

application of biological controls requires more knowledge-intensive management [Carisse & Dewdney, 2002; Gardener & Fravel, 2002;

Köhl *et al*., 2015].

2.5.2.1 Yeasts as biocontrol agents

Yeasts have received little attention as biocontrol agents of soil-borne fungal plant pathogens in comparison with bacteria [Robiglio *et al*., 2011; Di Francesco *et al*., 2021]. Yeasts can be considered as an alternative to synthetic chemicals because of their ability to compete for nutrients, space, and to grow faster than fungal pathogens [Liu *et al*., 2013; Banjara *et al*., 2016; Mewa-Ngongang *et al*., 2018; Mewa-Ngongang *et al*., 2019a, b]. Robiglio *et al*. [2011] suggested that yeasts also have potential to control diseases caused by soil-borne fungal plant pathogens.

Three biocontrol agents (BCAs) *Metschnikowia pulcherrima* strain (MACH1), *M. pulcherrima* strain (GS9), and *M. fructicola* strain (AL27) effectively reduced the blue mould rot diameter and weight on apple [Spadaro *et al*., 2013]. These authors further explained that AL27 was the most effective antagonist and reduced the rot diameter similar to the highly used chemicals imazalil + pyrimethani. Experiments carried out on wounded apples with *P. expansum*, *Rhodosporidium kratochvilovae* LS11 and *Cryptococcus laurentii* LS28 significantly reduced the level of infection although they were applied at a relatively low concentration of cells (5×10^6 cells/mL) [Lima *et al*., 2011]. Zhang *et al*. [2007b], found that when infected wounds on peach fruit were treated with *Cryptococcus laurentii*, lesion development by *B. cinerea*, *P. expansum* or *R. stolonifer* were effectively reduced and prevented. These authors showed that *C. laurentii* has potential as a biocontrol agent for the control of postharvest grey mould rot, blue mould rot and Rhizopus rot caused by *B. cinerea*, *P. expansum* and *R. stolonifer*, respectively.

Mewa-Ngongang *et al*. [2018] showed that *Candida pyralidae* strain Y1117, *Pichia kluyveri* strains Y1125 and Y1164 inhibited the growth of *Botrytis cinerea* at different inoculum doses. *Cryptococcus laurentii* effectively reduced the development of decay by *B. cinerea* on wounded apples at inoculum level of 10^4-10^5 cells per wound [Roberts, 1990]. Application of *Candida saitoana* to apple wounds significantly restricted the proliferation of *B. cinerea* and reduced the incidence of decay caused by *B. cinerea* [El Ghaouth *et al*., 2003]. *C. saitoana* applied on fresh apples either immediately or 24 h before inoculation with *B. cinerea* had no significant effect on lesion development caused by *B. cinerea*, but when *C. saitoana* was applied at 48 or 72 hours prior to inoculation with *B. cinerea*, *C. saitoana* effectively reduced lesion development [El Ghaouth *et al*., 2003]. The application of a cell suspension of the BIO126 strain of *Metschnikowia pulcherrima* at inoculum 10^8 cells/mL proved to be highly effective against grey mould on apples [Spadaro *et al*., 2004].

The application of yeast extract from *Saccharomyces cerevisiae* after leaf fall showed the greatest efficacy and significantly reduced ascospore discharge of *V. inaequalis* in 2013 and 2014 [Porsche *et al*., 2017]. These authors showed that the treatment of leaf litter with yeast extract can completely eliminate *V. inaequalis* inoculum in the course of the entire primary season.

During *in vivo* trials *Meyerozyma guilliermondii* reduced the fruit rot lesion size caused by *A. alternata*. on strawberries [Al-Rahbi *et al*., 2021]. The use of *M. guilliermondii* as the biocontrol of *A. alternata* was confirmed duirng *in vitro* and *in vivo* trials [Al-Maawali *et al*., 2021]. *Rhodosporidium paludigenum* showed antagonistic effect in controlling post-harvest decay of *A. alternata* and did affect fruit quality parameters [Wang *et al*., 2009]. *Candida sake* showed antagonistic effect by producing VOCs, which inhibited the growth of *A. alternata* [Arrarte *et al*., 2017]. This antagonistic effect showed by the yeast has the potential for use as a biofungicide for the control of *A. alternata* [Wang *et al*., 2009; Al-Rahbi *et al*., 2021].

2.5.2.2 Bacteria as biocontrol agents

As a group of important natural enemies of nematodes and fungi, bacteria have various modes of action: these include parasitizing; producing toxins, antibiotics, or enzymes; making the plant resistant to diseases and promoting plant health [Tian *et al*., 2007; Pane & Zaccardelli, 2015]. When a bacterial biological agent is used to control root pathogenic fungi, it is typically introduced to soil and rhizosphere by seed coating [Andersen & Winding, 2004). Among the control alternatives to chemicals, the application of beneficial bacteria living in association with plant roots is promising and several studies have documented improved plant traits by interaction with plant associated bacteria [Chowdhury *et al*., 2013]. Lactic acid bacteria (LAB) are widely used by food industries in the production of fermented products and also used to inhibit spoilage organisms for food safety [Paul Ross *et al*., 2002]. Cyclic dipeptides, phenyllactic acid, proteinaceous compounds, and 3-hydroxylated fatty acids are antifungal metabolites that have been isolated from LAB and used as biopreservatives [Schnürer & Magnusson, 2005]. Lactic acid bacteria and their antimicrobial metabolites have potential to be natural preservatives to control the growth of spoilage [Jeevaratnam *et al*., 2005].

Seventy-one *Pseudomonas syringae* strains have been evaluated as biological control agents (BCAs) and have been shown to inhibit/prevent post-harvest decay of apple fruit [Cirvilleri *et al*., 2005]. Experiments carried out by Quaglia *et al*. [2011] on wounded apples inoculated with *P. expansum* showed that the percentage of infection was significantly reduced in the wound sites treated with the *Pseudomonas syringae.*

Pseudomonas cepacia strongly inhibited fungal growth of grey mould during *in vitro* screening on nutrient yeast dextrose agar medium [Janisiewicz & Roitman, 1988]. These authors showed that at inoculum level of 10^3-10^5 conidia/mL, *P. cepacia* completely controlled *B. cinerea* on apples and pears. During *in vitro* assays, the antagonistic bacterium *Rahnella aquatilis* completely inhibited the germination of *B. cinerea* spores at a concentration of 10^6 cells/mL at

28°C [Calvo *et al*., 2007]. Assays using *R. aquatilis* for biological control of *B. cinerea* was performed at 4°C and 90% relative humidity. Under these cold storage conditions, the treatment with *R. aquatilis* significantly inhibited the development of *B. cinerea* on apples stored for 40 days [Calvo *et al*., 2007].

Bacterial suspension of *Pseudomonas fluorescens* Bk3 in minimal medium (2×10^9 cells/ml) with asparagine medium showed up to 73% inhibition of conidial growth of *V. inaequalis* after 7 days of pre-incubation [Hossain *et al*., 2009].

Enterobacter roggenkampii and *Pseudomonas aeruginosa* showed antagonistic effect against *Alternaria* fruit rot of tomato [Al-Maawali *et al*., 2021]. *Bacillus* species showed growth inhibition activity by decreasing severity of *Alternaria* disease on tomato (Pane & Zaccardelli, 2015). *Bacillus Licheniformis* and *B. subtilis* showed strong inhibitory effect against *A. alternata* [Sid *et al*., 2003]. Two strains of *Pseudomonas fluorescens* showed maximum inhibition (35 % and 38.6 % respectively) against *A. brassicae in vitro* and *in vivo* conditions (Gupta *et al*., 2020).

2.6 Conclusions

Both yeast and bacteria show promising results to control the growth of mould. The antimicrobial activity of biocontrol agent is mostly *in vitro* trials where the environment is controlled. The biocontrol agents show great potential i*n vitro* as alternative to chemical fungicides for the control of spoilage mould, but more research is needed where biocontrol agents are applied as preventive and curative treatments during post-harvest trials. More research is needed on the application of biocontrol agents as post-harvest treatments of fruit. There is limited research where the biocontrol agents are applied as pre-harvest treatments, therefore more work needs to be done where biological agents are evaluated as preventive and curative treatments as pre-harvest control of spoilage mould.

CHAPTER 3

Evaluation of yeasts as biological control agents against fruit spoilage mould

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Abstract

During pre- and post-harvest stages, considerable quantities of fresh products are lost due to mould spoilage. The aim of this study was to evaluate the antagonistic effect of selected yeasts against spoilage mould under *in vitro* and *in vivo* conditions. One hundred and four yeast isolates were screened for growth inhibition activity against *Botrytis cinerea*, *Penicillium expansum* and *Alternaria alstroemeriae* using plate assays. Three yeast strains were also tested for growth inhibition activity against the three mould species during post-harvest apple trials. Sixty-seven out of 104 yeasts showed growth inhibition activity against *P. expansum*, 36 yeasts

inhibited *B. cinerea*, 47 yeasts inhibited *A. alstroemeriae* and 22 yeasts showed inhibition against all three moulds. *Candida pyralidae*, *Meyerozyma guilliermondii* and *Zygoascus hellenicus* yeast strains showed highest inhibition activity against all three moulds. Volatile organic compounds produced by *Pichia kluyveri*, *C. pyralidae* and two *M. guilliermondii* yeast strains were effective in inhibiting the growth of all three moulds. The selected *P. kluyveri* strain showed 100%, 57% and 26% growth inhibition against *A. alstroemeriae*, *B. cinerea* and *P. expansum*, respectively, on apples and performed better than a commercial fungicide. While the selected *M. guillermondii* strain showed 100%, 60% and 18% inhibition on apples against *A. alstroemeriae*, *B. cinerea* and *P. expansum*, respectively. *Pichia kluyveri* and *Meyerozyma guilliermondii* yeast strains showed potential as biofungicides and warrant further investigation.

Keywords: Mould spoilage, fungicides, postharvest control, biocontrol agents (BCAs), apples

3.1 Introduction

Fruits are commercially and nutritionally important commodities, and play an important role in human health by supplying vitamins and minerals [Al-Hindi *et al*., 2011]. Globally, apples (*Malus domestica*) are the third most-produced fruit, with production increasing to 85 million tonnes in 2016 [FAOstat, 2019]. South Africa is a relatively small apple grower in terms of global hectares and produces approximately 1.3 million tonnes each year, with a total value of more than R8 billion [Du Plessis, 2017]. Ninety-two percent of this income is generated by fresh fruit sales [Du Plessis, 2017]. Agricultural produce is subject to mould spoilage before, during and after harvest, as well as during transportation and processing [Romanazzi *et al*., 2016; 2017]. Fruit losses due to spoilage mould pose several challenges to the agrifood industry [Salman, 2005; Parveen *et al*., 2016]. Each year, 25% of the total fruits produced in

industrialised countries is lost and more than 50% in developing countries [Droby, 2005; Nunes, 2012]. During the pre- and post-harvest stages, considerable amounts of fruits including apples are lost due to mould diseases caused by *Botrytis cinerea* (grey mould), *Penicillium expansum* (blue mould), *Alternaria* spp. (necrotic leaf blotch), *Venturia inaequalis* (apple scab), *Cladosporium* spp. (Sooty spot) and *Colletotrichum gloeosporioides* (bitter rot) [Sharma *et al*., 2009].

Spoilage mould needs to be controlled to maintain the quality and abundance of fruit produced [Mercier & Lindow, 2001]. Currently, fruit producers and exporters are using costly spraying programs incorporating synthetic chemicals, which are labour intensive and require the application of various classes of fungicides sprayed up to 20 times during the growing season. These practices can negatively affect the health of consumers, the environment, and the taste and aroma of the food being preserved [Oliveira *et al*., 2014; Contarino *et al*., 2019]. In addition, prolonged exposure to chemicals even at the lowest dose possible can lead to serious health problems in humans [Benito *et al*., 2009]. Some fungi can become resistant to fungicides as farmers use the chemicals regularly [Fernández-Ortuño *et al*., 2008].

The desire to minimise chemical residues and to offset rising prices of new synthetic chemicals is fostering the search for alternatives to synthetic chemical fungicides [Quaglia *et al*., 2011; Robiglio *et al*., 2011]. The recent trend is shifting towards safer and environmentally friendly alternatives for the control of post-harvest decay [Sharma *et al*., 2009]. Interest in biological control approaches is greater than ever because it showed great potential as alternatives to chemical fungicides [Liu *et al*., 2013; Mewa-Ngongang *et al*., 2019b]. Biocontrol agents are more environmentally friendly and cost effective than synthetic chemicals [Öberg, 2002].

Yeasts can be used as alternatives to synthetic chemicals because of their ability to compete for nutrients and space, their ability to grow faster than fungal pathogens and the production of

inhibitory growth compounds [Liu *et al*., 2013; Mewa-Ngongang *et al*., 2019a, b]. Yeasts such as *Meyerozyma guilliermondii*, *Candida pyralidae* and *Hanseniaspora* species have the ability to secrete extracellular metabolites, such as volatile organic compounds (VOCs), acetic acid, hydrogen sulphide and cell wall degrading enzymes, which have antimicrobial properties against many fruit spoilage moulds [Mewa-Ngongang *et al*., 2019b; Ruiz-Moyano *et al*., 2020; Al-Maawali *et al*., 2021; Han *et al*., 2021]. The aim of this study was to screen yeasts for growth inhibition activity against *Botrytis cinerea*, *Penicillium expansum* and *Alternaria alstroemeriae* under *in vitro* and *in vivo* conditions.

3.2 Materials and methods

3.2.1 Culturing conditions and inoculum preparation

One hundred and four yeast isolates were obtained for evaluation from the ARC Infruitec-Nietvoorbij (The Fruit, Vine and Wine Institute of the Agricultural Research Council, Stellenbosch, South Africa), the Instituto Superior de Agronomia (Lisbon, Portugal), the Centraal Bureau voor Schimmelcultures (Utrecht, Netherlands), the Gulbenkian Institute of Science (Oeiras, Portugal) and the Council for Scientific and Industrial Research (Pretoria, South Africa) (Table S1). The yeasts were cultured on yeast malt agar (YMA) (1% glucose, 0.3% malt extract, 0.5% peptone, 2% bacteriological agar) for 2 days at 28°C. A loopful of each pure yeast colony on the plates was transferred into test tubes containing 5 mL of sterilised yeast malt broth (YMB) (Sigma-Aldrich, South Africa) and incubated at 28°C for 2 days. Thereafter 1 mL of the culture was transferred to a sterile 2 mL microtube and centrifuged at 13500 rpm (20412 × g) for 5 minutes. The supernatant was discarded, and the pellet resuspended in 100 µL of sterile distilled water. Yeast cells were counted using a haemocytometer and a microscope (400X magnification) in order to prepare the yeast inoculum (1×10^8 cells/mL).

For the apple bioassay, grape pomace was obtained by pressing Chenin Blanc grapes from the ARC Infruitec-Nietvoorbij research farm (Stellenbosch) at 200 kPa. The resultant grape pomace extract was frozen in 25 L polypropylene buckets at −20°C. Prior to use, the grape pomace extract was thawed and diluted with sterile distilled water to the required sugar concentration. Yeast strains Y63 (*C. pyralidae*), Y88 (*M. guilliermondii*) and Y64 (*P. kluyveri*) from the ARC Infruitec-Nietvoorbij culture collection, were used. Yeasts were grown in 5 mL YMB for 2 days at 28°C and then transferred to Erlenmeyer flasks containing 50 mL of sterile grape pomace extract broth (GPB), incubated at 28°C and agitated at 150 rpm, using a rotary shaker (LM-53OR, RKC Instrument Inc., Ohta-ku Tokyo, Japan) for 2 days. The yeast cultures were then transferred to 500 mL GPB and grown at 28°C for 24 hours under agitation. The yeast inoculum of 1×10^8 cells/mL was used, as mentioned previously.

The fruit spoilage moulds, *B. cinerea*, *P. expansum* and *A. alstroemeriae*, were obtained from the Post-harvest Pathology laboratory at ARC Infruitec-Nietvoorbij and cultured for 7 to 14 days at 25°C on potato dextrose agar (PDA, Merck, South Africa). Spores were harvested by gently scraping them from the surface of the agar and rinsing with sterile distilled water to attain 50 mL of a spore suspension in a sterile 250 mL Schott bottle. The inoculum was prepared by adjusting the spore suspension to 1×10^5 cells/mL using a haemocytometer and a microscope (400X magnification).

3.2.2 Radial growth inhibition assay

The radial growth inhibition assay was applied as described by Núñez *et al*. [2015], with some modifications. In brief, a 5 mm mycelial disk, obtained from a 7-day old mould culture, was placed at the centre of a fresh YMA plate using a sterile cork borer. Subsequently, 15 µL of the yeast cells suspension (1×10^8 cells/mL) was spotted 25 mm away from the mould plug. Four different yeast isolates were spotted per plate and incubated at 25°C for 7 days. All yeast

treatments had three replicates. The control plates only contained the 5 mm diameter mycelial disk of the respective mould. Positive growth inhibition results were observed by the presence of C-shape growth around the yeast colonies and negative growth inhibition results were observed by the growth of the mould all around the yeast colony (Figure 1).

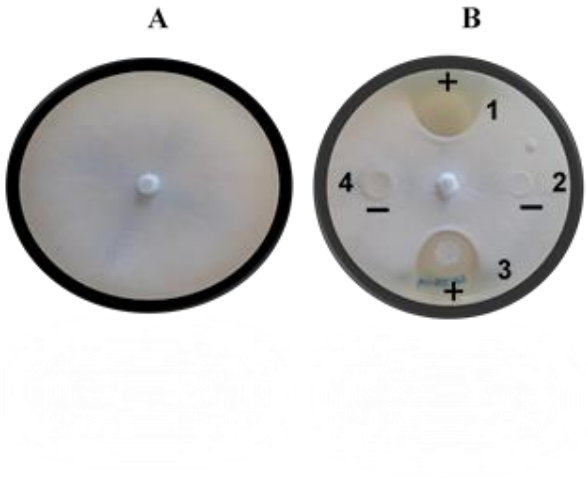

FIGURE 1. *Botrytis cinerea* growth (A) and antagonistic effect of selected yeast isolates *Candida pyralidae* (1), *Pichia kluyveri* (Y64) (2), *Meyerozyma guilliermondii* (Y88) (3) and *Debaryomyces hansenii* (Y8) (4) against *B. cinerea* (B) on yeast malt agar. The positive sign (+) represents growth inhibition activity and the negative sign (-) represents no inhibition. This is a representative example of three replicates

3.2.3 Diffusible metabolites assay

The dual assay described by Chen *et al*. [2018] was used to evaluate 23 yeasts that were selected based on the growth inhibition activity against the three mould species during the radial growth inhibition assay (Figure 2). Only those yeasts that showed growth inhibition activity against the three mould species during the radial growth inhibition assay were evaluated further. One yeast strain, Y64 (*P. kluyveri*), was also included as reference yeast. Similar to the radial growth inhibition assay, a 5 mm mould mycelial disk was placed at the edge of the YMA plate. Subsequently, 20 µL of the yeast suspension (1×10^8 cells/mL) was spotted 40 mm away from the mould plug (Figure 3) and incubated at 25°C for 9 days. The negative control plates contained only the 5 mm diameter mycelial disk of the respective mould. All treatments had

three replicates. The percentage inhibition was calculated using the mathematical expression: Fungal Radial Growth Inhibition (FRGI) = $(D_0 - D_t/D_0) \times 100$, with D_0 representing the horizontal growth average of the fungal colony on the negative control plates and D_t representing the horizontal growth average of fungal colony on the yeast treated plates (Figure 3), as described by Núñez *et al.* [2015].

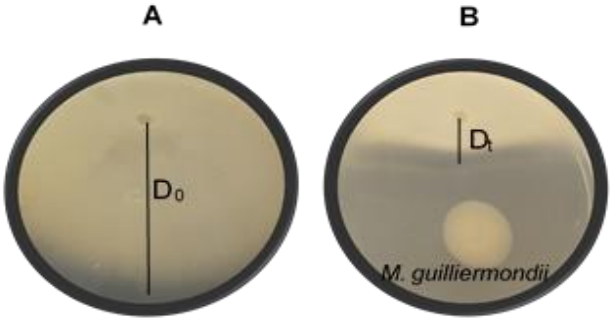

FIGURE 3. Visual representation of the growth of *Botrytis cinerea* (A) and the antagonistic effect of yeast isolate *Meyerozyma guilliermondii* (Y88) against *B. cinerea* (B) on yeast malt agar. D_0 representing horizontal growth of the fungal colony on the negative control plates and D_t representing the horizontal growth average of fungal colony on the yeast treated plates. Each plate is a representative example of three replicates.

3.2.4 Volatile organic compound (VOCs) detection

To assess the effect of VOCs produced by the 23 yeasts used in relation to their growth inhibition potential against fruit spoilage organisms, the mouth-to-mouth assay described by Medina-Córdova *et al*. [2016] was used. Two YMA plates facing each other were sealed with laboratory film, per experimental repeat. The bottom plate was spread with 100 µL of the yeast suspension (1×10^8 cell/mL), while the top plate contained a 5 mm mould mycelial disk placed at the centre. The negative control treatment only contained the 5 mm diameter mycelial disk in the centre of the plate, while no yeast was spread on the second plate. The plates were

incubated at 25°C for 7 days. All treatments had three replicates. The VOC inhibition activity (VOCIA) was calculated using the mathematical expression [Núñez *et al*., 2015]: VOCIA = $(D_0 - D_t/D_0) \times 100$, with D_0 representing the average diameter of the fungal colony on the negative control plates and D_t representing the diameter of the fungal colony on the yeast treated plates, as shown in Figure 4.

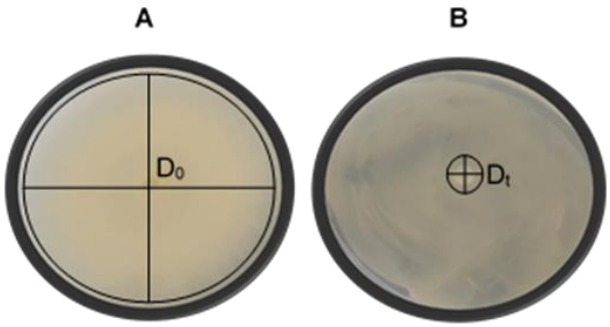

FIGURE 4. Visual representation of the growth of *Botrytis cinerea* (A) and the antagonistic effect of yeast isolate *Pichia kluyveri* (Y64) against *B. cinerea* (B) on yeast malt agar. D_0 representing the average diameter of the fungal colony on the negative control plates and D_t representing the diameter of the fungal colony on the yeast treated plates. Each plate is a representative example of three replicates.

3.2.5 Apple Bioassay

The post-harvest biocontrol efficacy assay was performed on the apple cultivar "Panorama Goldens" and sixteen treatments were applied (Table 1). Each treatment had five replicates. Each replicate consisted of a rectangular fruit-packaging box containing five apples. Ethanol (70% v/v) was sprayed on the apples to eradicate any microorganisms on the surface and allowed to dry completely before wound infliction. Apples were uniformly wounded (approximately 5 mm diameter and 3 mm deep), with a sterile cork borer. After 15 minutes, 15 µL of sterile purified water was inoculated into the wound of the control treatment, while the

other treatments received 15 µL of the respective mould spore suspension (1×10^5 cells/mL) and then allowed to dry for 30 minutes. Subsequently, 15 µL of a yeast inoculum (1×10^8 cells/mL) or commercial fungicide was introduced into the wound. The negative control treatments were only infected with *B. cinerea*, *P. expansum* or *A. alstroemeriae* and not treated with yeast or the commercial fungicide. The commercial fungicide N-(trichloromethylthio) cyclohex-4- ene-1,2-dicarboximide, common name Captan (800 g/kg) (South Africa) was used. Treated apples were maintained at ±20°C for 7-20 days. During the incubation period, the relative humidity was maintained at 80%. Growth inhibition results were characterised by the absence of mould development. Lesion diameters were measured, and percentage growth inhibition was calculated and analysed statistically to determine the effectiveness of the treatments.

TABLE 1. Treatments applied on apples during postharvest biocontrol trials*.

Treatment description
Sterile distilled water (Control)
Botrytis cinerea
Penicillium expansum
Alternaria alstroemeriae
B. cinerea and *Candida pyralidae* Y63
P. expansum and *C. pyralidae* Y63
A. alstroemeriae and *C. pyralidae* Y63
B. cinerea and *Meyerozyma guilliermondii* Y88
P. expansum and *M. guilliermondii* Y88
A. alstroemeriae and *M. guilliermondii* Y88
B. cinerea and *Pichia kluyveri* Y64
P. expansum and *P. kluyveri* Y64
A. alstroemeriae and *P. kluyveri* Y64
B. cinerea and Captan
P. expansum and Captan
A. alstroemeriae and Captan

*****Apples were incubated in rectangular fruit packaging boxes and five boxes (replicates) were used per treatment, with each box containing five apples.

3.2.6 Identification

Only yeast isolates that showed growth inhibition activity were identified to species level. Yeast DNA was extracted using the method described by Lõoke *et al*. [2011]. Yeast identification to the species level was performed by amplification of the 5.8S-internal transcriber spacer (ITS) ribosomal region, using primers ITS1 and ITS4 [Mitchell *et al*., 1994]. PCR reaction mixture (50 µL) contained 5 µL SuperTherm Taq buffer, 0.2 µL SuperTherm Taq polymerase, 1.5 µL of 25 mM $MgCl_2$, 1 µL of 2.5 mM dNTP, 3 µL of each primer (2.5 mM), 0.5 µL of BSA, 5 µL of template DNA (100 ng/µL) and 30.8 µL sterile dH_2O. The PCR conditions used were: initial denaturation at 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 1 minute, and a final extension at 72°C for 10 minutes. The PCR products were submitted to Inqaba Biotechnical Industries (Pty) Ltd (Pretoria, South Africa) for Sanger sequencing. The sequenced fragments were then compared to sequences on the NCBI database using the standard nucleotide homology search Basic Local Alignment Search Tool (BLAST, http://www.ncbi.nih.gov/BLAST).

3.2.7 Statistical analyses

Growth inhibition data was subjected to analysis of variance (ANOVA) using XLSTAT software (Version18.07.39157, Addinsoft, New York, USA) and GLM procedure of SAS software (version 9.4, SAS Institute Inc, Cary, NC, USA). Fisher's least significant difference (LSD) values were calculated at the 5% probability level ($p = 0.05$) to facilitate comparison between treatment means.

3.3 Results and discussion

3.3.1 Radial growth inhibition assay

Of the 104 yeasts, 83 showed growth inhibition activity against the selected mould species. Sixty-seven yeasts showed growth inhibition activity against *P. expansum*, 36 against *B.*

cinerea, 47 against *A. alstroemeriae* and 22 yeasts showed inhibition activity against all three moulds (Table 2). Most of the isolates that showed antagonistic effects against the spoilage moulds belong to the genus *Hanseniaspora*, with *H. uvarum* being the predominant species. From the 22 yeasts selected for further characterisation, most of the isolates belonged to the genus *Zygosaccharomyces*. Yeasts can inhibit the growth of mould in different ways, such as the ability to grow faster than the spoilage mould by rapidly colonising surfaces, competition for nutrients or by production of growth inhibition compounds [Liu *et al*., 2013; Banjara *et al*., 2016; Mewa-Ngongang *et al*., 2019b].

TABLE 2. Growth inhibition activity* of yeasts against selected mould on yeast malt agar.

Yeast codes	Species	*Penicillium expansum*	*Botrytis cinerea*	*Alternaria alstroemeriae*
Y1	*Rhodotorula dairenensis*	+	−	++
Y2	*Hanseniaspora uvarum*	+	−	−
Y3	*Hanseniaspora uvarum*	−	+++	−
Y5	*Saccharomyces uvarum*	+	−	+
Y6	*Aureobasidium melanogenum*	++	+++	++
Y7	*Aureobasidium melanogenum*	−	++	+++
Y8	*Debaryomyces hansenii*	+	−	−
Y10	*Saccharomyces uvarum*	+	−	+
Y11	*Debaryomyces hansenii*	+	++	++
Y12	*Rhodotorula dairenensis*	++	−	++
Y13	*Hanseniaspora opuntiae*	−	++	−
Y14	*Saccharomyces uvarum*	+	−	+++
Y15	*Hanseniaspora. uvarum*	−	−	+
Y16	*Hanseniaspora uvarum*	+	−	−
Y17	*Hanseniaspora occidentalis*	++	+++	+++
Y18	*Debaryomyces hansenii*	−	+	+
Y19	*Hanseniaspora uvarum*	+	−	+
Y20	*Hanseniaspora uvarum*	+++	−	−
Y21	*Debaryomyces hansenii*	+++	−	−
Y24	*Meyerozyma guilliermondii*	++	++	+++
Y25	*Hanseniaspora. uvarum*	+++	++	−
Y26	*Hanseniaspora. uvarum*	+	−	−
Y27	*Hanseniaspora uvarum*	−	−	+
Y30	*Candida oleophila*	+	−	+
Y31	*Candida oleophila*	+	+	−
Y32	*Candida oleophila*	+	−	−
Y34	*Candida oleophila*	+	−	−
Y35	*Rhodotorula dairenensis*	++	+++	++
Y36	*Candida oleophila*	++	−	−
Y37	*Candida oleophila*	++	−	−
Y38	*Hanseniaspora uvarum*	+	−	+
Y39	*Meyerozyma guilliermondii*	+	+++	+++
Y43	*Hanseniaspora guilliermondii*	+	−	−
Y45	*Zygosaccharomyces bailii*	+	−	−
Y47	*Hanseniaspora opuntiae*	++	−	−
Y50	*Candida stellimalicola*	+	−	+++
Y51	*Pichia kudriavzevii*	++	−	−
Y53	*Rhodotorula dairenensis*	−	−	+++
Y54	*Hanseniaspora guilliermondii*	+	−	−
Y55	*Pichia kudriavzerii*	+	−	−
Y56	*Pichia fermentans*	−	−	+
Y57	*Hanseniaspora valbyensis*	−	+	+
Y58	*Saccharomyces cariocanus*	+	−	−

Y61	*Dekkera anomala*	+	−	+
Y62	*Dekkera anomala*	−	+	−
Y63	*Candida pyralidae*	+++	+++	+++
Y65	*Meyerozyma guilliermondii*	+	+++	++
Y67	*Brettanomyces lambicus*	+	+	−
Y69	*Zygosaccharomyces bailii*	−	−	++
Y70	*Lanchancea thermotolerans*	−	−	+
Y71	*Torulaspora delbrueckii*	+	−	−
Y72	*Metschnikowia pulcherrima*	−	+	−
Y73	*Lanchancea thermotolerans*	+	−	−
Y74	*Torulaspora delbrueckii*	++	+++	++
Y75	*Saccharomyces cerevisiae*	+	+	++
Y76	*Zygosaccharomyces bailii*	+	−	−
Y78	*Meyerozyma guilliermondii*	+	−	−
Y79	*Pichia kluyveri*	+	−	−
Y80	*Zygoascus hellenicus*	+++	−	−
Y81	*Meyerozyma guilliermondii*	+	−	+
Y82	*Meyerozyma guilliermondii*	+	−	+
Y83	*Brettanomyces lambicus*	+	+++	+++
Y84	*Debaryomyces hansenii*	++	+++	++
Y85	*Pichia kluyveri*	+	−	+
Y87	*Meyerozyma guilliermondii*	+	−	−
Y88	*Meyerozyma guilliermondii*	+++	+++	+++
Y89	*Zygoascus helenicus*	+++	+++	+++
Y90	*Zygosaccharomyces bailii*	+	−	++
Y91	*Zygosaccharomyces rouxii*	+	+++	++
Y92	*Zygosaccharomyces rouxii*	++	+	++
Y93	*Zygosaccharomyces microellipsoides*	+	+++	++
Y94	*Zygosaccharomyces cidri*	+	−	−
Y95	*Zygosaccharomyces florentinus*	+	+++	++
Y96	*Zygosaccharomyces fermentati*	+	++	++
Y97	*Zygosaccharomyces bisporus*	+	+	++
Y98	*Zygosaccharomyces bisporus*	++	+	−
Y99	*Brettanomyces bruxellensis*	−	+	−
Y100	*Brettanomyces bruxellensis*	−	+	−
Y101	*Brettanomyces lambicus*	−	+	+
Y102	*Candida magnoliae*	+	+	++
Y103	*Saccharromyces cerevisiae*	+	++	++
Y104	*Saccharromyces bayanus/cerevisiae*	+	−	+
Y105	*Meyerozyma guilliermondii*	+	−	−

*(-) no activity, (+) mild activity, (++) medium activity, (+++) strong activity

3.3.2 Diffusible metabolites assay

The 23 selected yeasts showed varying levels of antagonistic effects against, *B. cinerea, P. expansum* and *A. alstroemeriae* (Figures 2a, b, c). In general, the selected yeasts showed higher inhibition activities against *B. cinerea* (39% mean inhibition) than the other two mould species. The selected yeasts showed the lowest inhibition activity against *P. expansum* (17% mean inhibition). Nally *et al.* [2012] and Mewa-Ngongang *et al*. [2019b] reported that different yeast species showed growth inhibition activity at different levels against fruit spoilage mould, which is in agreement with the findings of this study.

Y88 (*Meyerozyma guilliermondii*), Y63 (*Candida pyralidae*) and Y89 (*Zygoascus hellenicus*) had the highest growth inhibition activity against *B. cinerea*, with 63%, 62% and 58%, respectively (Figure 2a). Y88, Y63 and Y89 showed significantly higher inhibition activity than the other yeast treatments. Yeast Y64 (*Pichia kluyveri*), that was selected as the control yeast, showed low inhibition activity (3%) against *B. cinerea*. Based on the growth inhibition activity, the yeasts were more effective against *B. cinerea,* than against *P. expansum* and *A. alstroemeriae.*

Yeasts Y88, Y63 and Y89 also had the highest growth inhibition activity against *P. expansum*, with 42%, 38% and 35%, respectively and were significantly better than the other yeast treatments (Figure 2b). The control yeast, Y64 showed 2% inhibition against *P. expansum*. The same three yeasts (Y88, Y63 and Y89) also showed highest inhibition activity against *A. alstroemeriae*, with 41%, 37% and 35%, respectively (Figure 2c). The control yeast (Y64) showed 7% inhibition activity against *A. alstroemeriae*. *Meyerozyma guilliermondii* strain Y88 had the highest inhibition activity against all three mould species. This is the first report of growth inhibition activity of *M. guilliermondii* against *A. alstroemeriae.* Al-Rahbi *et al*. [2021] and Al-Maawali *et al*. [2021] reported that *M. guilliermondii* had an antagonistic effect against *Alternaria alternata* under *in vitro* conditions. Wang *et al*. [2018] reported that *M.*

guilliermondii had antagonistic effects against two strains of *B. cinerea*, while inhibition of *P. expansum* growth by *M. guilliermondii* was reported by Han *et al.* [2021].

Candida pyralidae was the second best performing yeast against all three moulds. This is in agreement with the findings of Mewa-Ngongang *et al*. [2019b], who reported the antagonistic effects of *C. pyralidae* against the germination of *B. cinerea* spores under *in vitro* conditions. This is the first report of the growth inhibition properties of *C. pyralidae* against *P. expansum* and *A. alstroemeriae,* and of the growth inhibition properties of *Z. hellenicus* against *B. cinerea, P. expansum* and *A. alstroemeriae.*

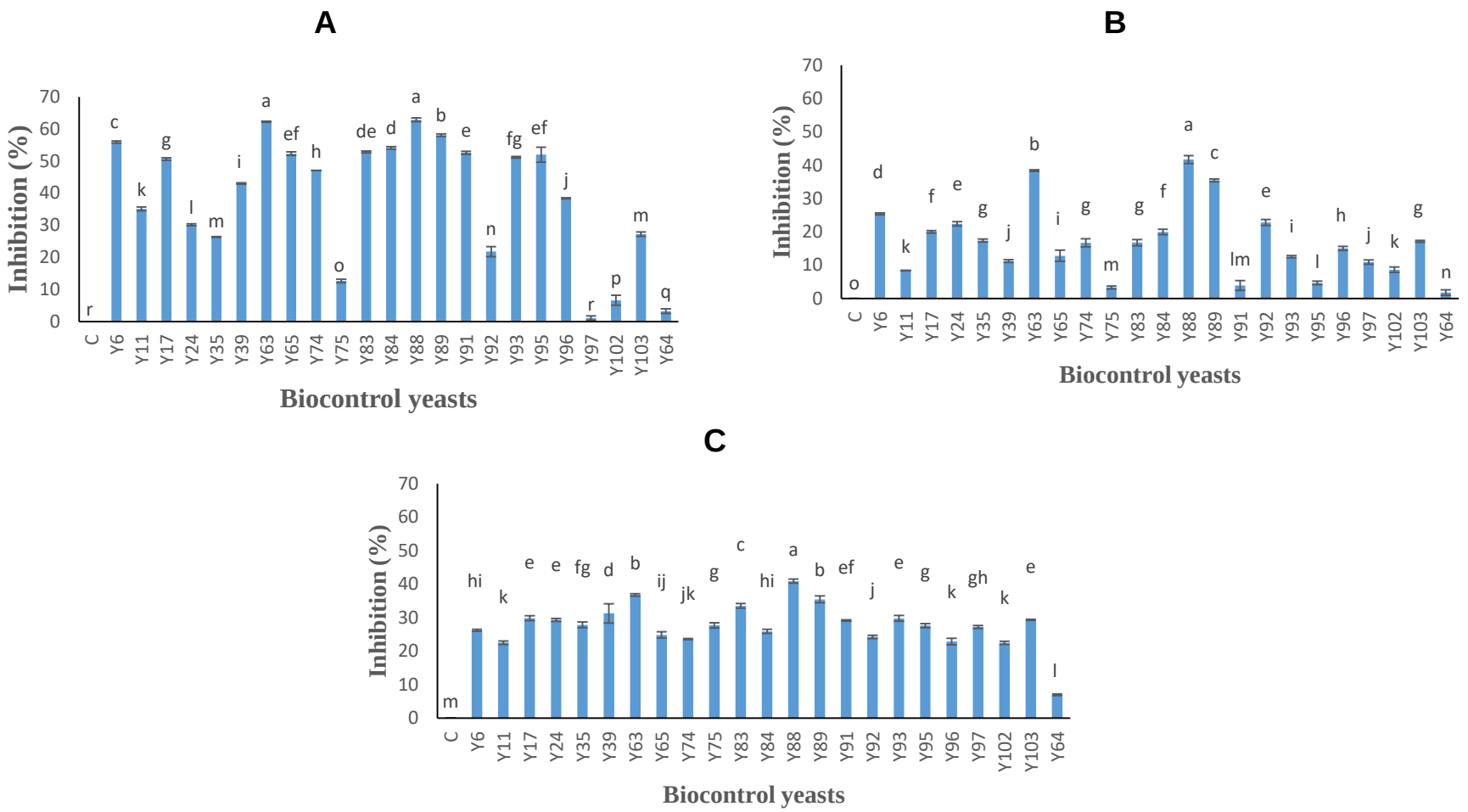

FIGURE 2. Growth inhibition activity expressed as a percentage (%) of 23 yeasts against *Botrytis cinerea* (A), *Penicillium expansum* (B) and *Alternaria alstroemeriae* (C) based on the diffusible metabolites assay results. Values are means of three replicates and the standard deviations are also shown. The different letters indicate significant differences ($p < 0.05$) between treatments.

3.3.3 Volatile organic compound (VOC) detection

The 23 yeasts produced VOCs that inhibited the growth of *B. cinerea* (Figure 5a), *P. expansum* (Figure 5b) and *A. alstroemeriae* (Figure 5c), but the level of growth inhibition varied among the yeasts. Yeast isolates Y64 (*P. kluyveri*), Y63 (*C. pyralidae*), and Y24 (*M. guilliermondii*) showed 91%, 57% and 56% growth inhibition activity against *B. cinere*a, respectively (Figure 5a). Against *P. expansum,* the highest growth inhibition was shown by *P. kluyveri* Y64, *M. guilliermondii* Y88 and Y65, with 81%, 70% and 69%, respectively (Figure 5b). The best performing yeasts against *A. alstroemeriae* were *P. kluyveri* Y64, *C. pyralidae* Y63 and *M. guillermondii* Y88, with 76%, 68% and 61% growth inhibition activity, respectively (Figure 5c).

Yeast isolate Y64 (*P. kluyveri*) showed the highest growth inhibition activity against all three moulds and was significantly better than the other yeast treatments (Figure 5) during the VOC trial. While the opposite was observed during the diffusible metabolite assay (Figure 2). It is clear that the mode of action of Y64 is linked to its ability to produce VOCs. The findings of this study are in agreement with Mewa-Ngongang *et al*. [2019b], who also reported on the ability of *P. kluyveri* and *C. pyralidae* to inhibit the growth of *B. cinerea* under *in vitro* conditions. Ruiz-Moyano *et al*. [2020] reported that *H. uvarum* also produced VOCs to control the growth of *B. cinerea* on fruits. Choińska *et al*. [2020] observed that *M. guilliermondii* produced VOCs to control the growth of *B. cinerea* and *P. expansum,* which is in agreement with the findings in this study. *Pichia kluyveri* showed the highest inhibition against *P. expansum*. Cordero-Bueso *et al*. [2017] also reported that VOCs produced by *P. kluyveri* have antagonistic activity against *P. expansum.* This the first report of VOCs from *P. kluyveri, C. pyralidae* and *M. guilliermondii* inhibiting the growth of *A. alstroemeriae.* However, Al-Maawali *et al*. [2021] showed that VOCs produced by *M. guilliermondii* inhibited the mycelial growth of *A. alternata.*

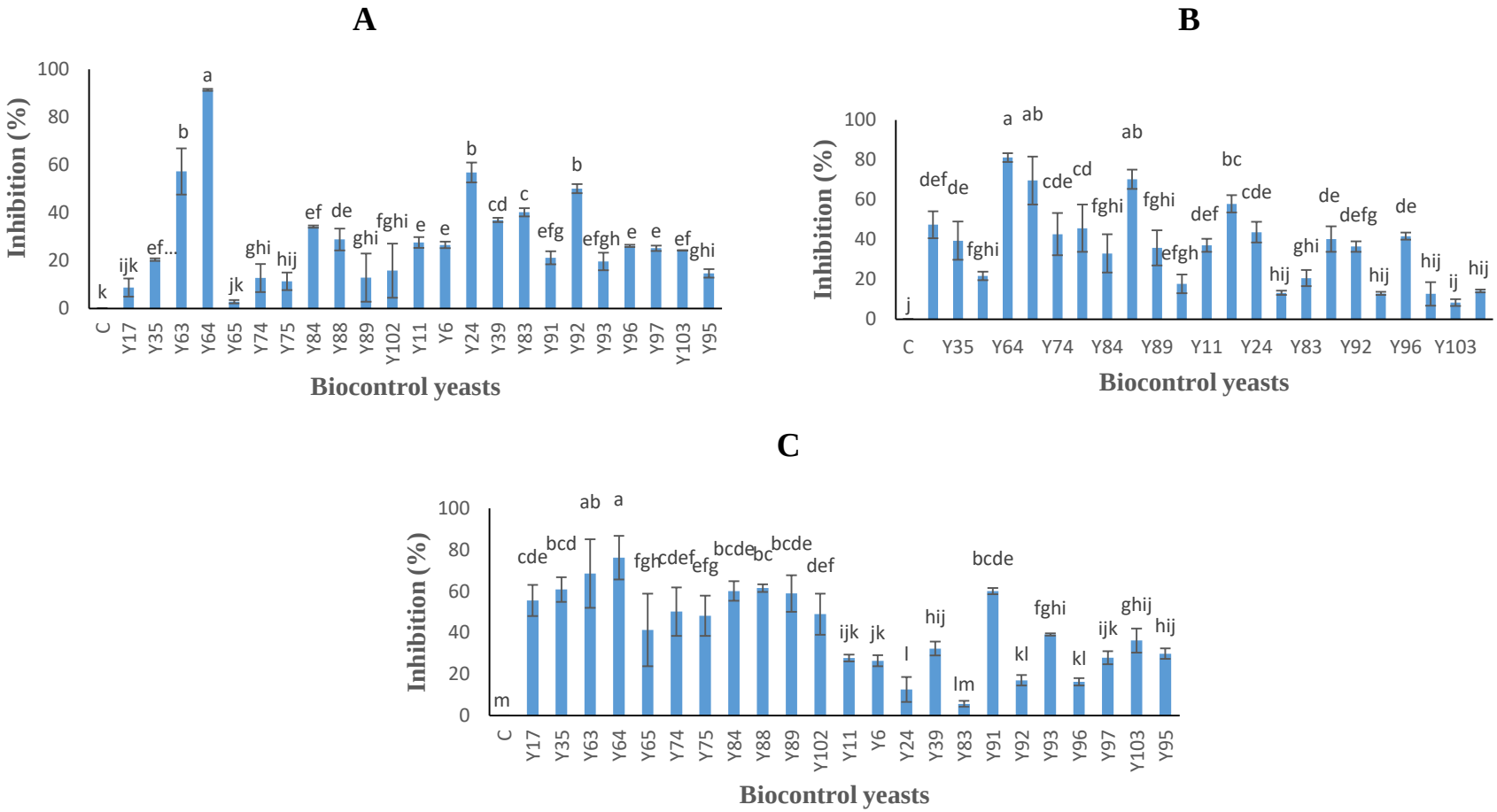

FIGURE 5. The growth inhibition activity expressed as a percentage (%) of 23 yeasts against *Botrytis cinerea* (A), *Penicillium expansum* (B) and *Alternaria alstroemeriae* (C) based on the volatile organic compound production. Values are means of three replicates and the standard deviations are also shown. The different letters indicate significant differences ($p < 0.05$).

3.3.4 Postharvest application of biocontrol yeasts on apples

The yeasts were effective in preventing mould spoilage of apples and reducing decay considerably (Figures 6a and 6b). However, the inhibition responses were yeast and mould species-dependent. *Meyerozyma guilliermondii* Y88 and *P. kluyveri* Y64 were effective in suppressing mould growth on apples, with 100% inhibition activity against *A. alstroemeriae.* The commercial fungicide (Captan) also provided 100% inhibition. *Candida pyralidae* Y63 showed 36% inhibition against *A. alstroemeriae,* which was significantly lower than the other treatments. This is the first report on growth inhibition activity of *P. kluyveri, C. pyralidae* and *M. guillermondii* against *A. alstroemeriae* on apples. However, Al-Rahbi *et al*. [2021] and Al-Maawali *et al*. [2021] showed that *M. guilliermondii* reduced the fruit rot lesions of *A. alternata* on strawberries and tomatoes by 68% and 50%, respectively.

Against *B. cinerea, M. guilliermondii* Y88 and *P. kluyveri* Y64 showed 61% and 57% inhibition, respectively, which was higher than the 52% obtained by the Captan (Figure 6a). *Candida pyralidae* Y63 showed 47% inhibition against *B. cinerea,* which was significantly lower than the other treatments. These findings are is in agreement with those of Mewa-Ngongang *et al*. [2019b] who also reported on the antagonistic effects of *C. pyralidae* against *B. cinerea* on apples. Wang *et al*. [2018] reported that *M. guilliermondii* showed an antagonistic effect against *B. cinerea* isolates on grape berries, while Mewa-Ngongang *et al*. [2021] showed that *P. kluyveri* when applied preventively, was effective in suppressing *B. cinerea* growth by 95% on apples.

All the yeasts showed the lowest growth inhibition activity against *P. expansum* (Figure 6a). *Pichia kluyveri* Y64 showed the highest growth inhibition activity (26%) against *P. expansum* and performed better than the commercial fungicide, which showed 24% inhibition. *Meyerozyma guilliermondii* Y88 and *C. pyralidae* Y63 showed 19% and 13% inhibition against *P. expansum,* respectively. This study confirmed the findings of Cordero-Bueso *et al*. [2017],

who reported that *P. kluyveri* has antagonistic activity against *P. expansum*. Han *et al*. [2021] showed that *M. guilliermondii* has antagonistic activity against *P. expansum* on pears. This is the first report on the growth inhibition properties of *C. pyralidae* against *P. expansum* on apples. These observations on apples could be of great importance to the agricultural industry because these biocontrol yeasts can potentially be used as alternatives to chemical fungicides.

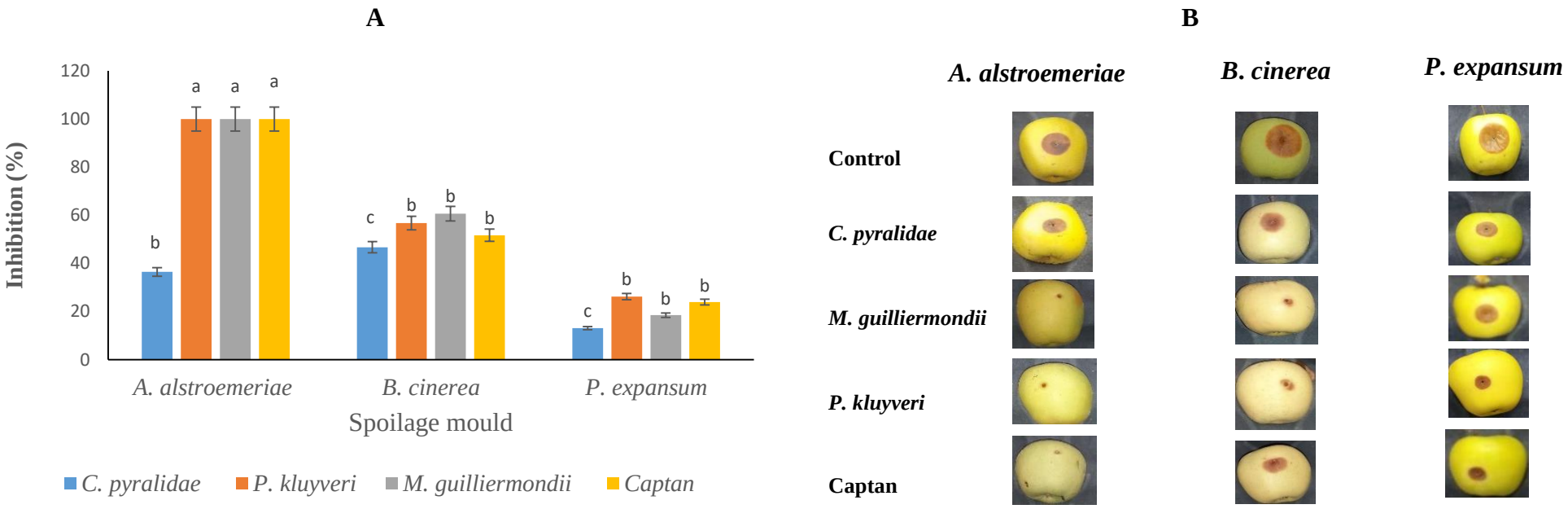

FIGURE 6. The growth inhibition activity expressed as a percentage (%) of Y63 *Candida pyralidae*, Y88 *Meyerozyma guilliermondii* and Y64 *Pichia kluyveri* yeasts against *Alternaria alstroemeriae*, *Botrytis cinerea* and *Penicillium expansum* during postharvest trials on apples (A). Values are means of five replicates and the standard deviations are also shown. The different letters indicate significant differences ($p < 0.05$) between treatments. (B) Photographs of apples showing lesion diameters. Each set is a representative example of 25 apples.

3.4 Conclusions

The cell suspensions of yeast strains *C. pyralidae* Y63, *M. guillermondii* Y88 and *Z. hellenicus* Y89 showed the best antagonistic effects against *B. cinerea*, *P. expansum* and *A. alstroemeriae.* The production of VOCs by *P. kluyveri* was the mechanism of inhibition against *B. cinerea*, *P. expansum* and *A. alstroemeriae. Candida pyralidae* Y63, *M. guillermondii* Y88 and *P. kluyveri* Y64 showed effective inhibition against all three mould species on apples and were comparable to the commercial fungicide. These yeasts can potentially be considered as alternatives to chemical fungicides. However, more research is needed to determine how to apply these yeast-based biocontrol agents and the minimum dosage or inhibitory concentration that is needed. The VOCs that are responsible for inhibition should be identified and the production process needs to be optimised. Future research should also investigate the application of the yeast-based biological agents on fruit and trees for pre-harvest control of mould.

CHAPTER 4

General Discussion, Conclusions and Recommendations

4.1 General discussion

Yeasts occur naturally on fruit and are used in several fermentation processes and are generally regarded as safe. This study focused on the application of yeasts as natural biocontrol agents against *Botrytis cinerea*, *Penicillium expansum* and *Alternaria alstroemeriae.* The antimicrobial efficacy evaluations of different yeast species against fungal pathogens showed that some yeasts, grown in a culture suspension grown to (1×10^8 cells mL), inhibited the growth of the spoilage mould, *B. cinerea*, *P. expansum* and *A. alstroemeriae.* The mode of action of the various yeasts was not one of the objectives of this study, but it was evident that yeasts can inhibit the growth of mould by the production of antimicrobial compound such as extracellular metabolites and/or volatile organic compounds (VOCs), which is in agreement with other studies [Ruiz-Moyano *et al*., 2020; Al-Maawali *et al*., 2021; Han *et al*., 2021]. The production of VOCs by the various yeasts was effective in inhibiting the growth of the three selected spoilage moulds.

Only yeast isolates that showed growth inhibition activity were identified during this study. Overall, eighty-three yeasts showed growth inhibition activity against the selected moulds. Most of the isolates that showed broad antagonistic effects against the spoilage moulds belong to the genus *Hanseniaspora*, with *Hanseniaspora uvarum* being the predominant species. From the 22 yeasts selected for further characterisation, most of the isolates belonged to the genus *Zygosaccharomyces*, even though they did not show the highest growth inhibition activity. Identification of the yeasts to strain level was not part of the study, but based on the activity of the yeasts and their origins; we observed that there are differences among the species, but also strain differences within species. This aspect should be considered in future evaluations.

Three different yeast species *Candida pyralidae, Meyeromzyma guilliermondii* and *Pichia kluyveri* were selected as the best yeasts because of the antagonistic effect against *B. cinerea*, *P. expansum* and *A. alstroemeriae* on plate assays (*in vitro*) and apples (*in vivo*). Mewa-Ngongang *et al.* [2019b], confirmed that *C. pyralidae* and *P. kluyveri* showed inhibition activity *in vitro* against fruit spoilage mould. *M. guilliermondii*, known as a spoilage organism of alcoholic and non-alcoholic beverages [Comitini *et al.*, 2004], showed antagonistic effects against fruit spoilage mould, which are in agreement with Al-Rahbi *et al.* [2021] and Al-Maawali *et al.* [2021]. This study clearly indicate that although some of the yeast might be spoilage organisms, they might have antimicrobial activity against moulds and can be used as biocontrol agents. However, more investigation is needed in terms of mode of action and minimum inhibitory concentration for application.

The study is the first to report on yeasts showing growth inhibition activity against *A. alstroemeriae*. This is also the first report of the growth inhibition properties of *C. pyralidae* against *P. expansum* and *A. alstroemeriae* and of the inhibition properties of *Z. hellenicus* against *B. cinerea*, *P. expansum* and *A. alstroemeriae*. This is also the first report of VOCs produced by *P. kluyveri*, *C. pyralidae* and *M. guilliermondii* inhibiting the growth of *A. alstroemeriae*.

4.2 Conclusions

Mould management in agricultural industry is primary depended on chemical fungicides, which leave harmful residues on food products, therefore researchers has come to a decision to use best alternative which is the biological control. Yeast can effectively control the growth of mould, but the level of inhibition is species dependent. *C. pyralidae, M. guilliermondii* and *P. kluyveri* were found to produce antimicrobial compounds that are effective against *B. cinerea*, *P. expansum* and *A. alstroemeriae*. The yeasts inhibited the growth of the mould by production of extracellular metabolites and volatile organic compounds (VOCs). The yeasts that are

generally known as spoilage organisms, showed antimicrobial activity against moulds and can also be used as biocontrol agents. The effective inhibition activity of these yeasts on apples was comparable to a commercial fungicide. Yeasts can potentially be considered as alternatives to chemical fungicides

4.3 Recommendations

The biocontrol yeasts showed great potential as alternative to synthetic chemicals but, before applying these yeast-based agents on a commercial scale, the production process needs to be optimised. The mode of action and the antimicrobial compounds of the selected yeasts needs to be determined. Future research should determine what the minimum inhibitory concentration or dosage that is needed to inhibit the growth of moulds. Future research should also investigate the application of the yeast-based biological agents against different spoilage moulds and on different fruits such as grapes, peaches, citrus and strawberries and on trees for pre-harvest and postharvest control of mould. Although the yeast are generally regarded as safe and occur naturally on different sources, it will be important to do biotoxicity test on these yeasts to cornfirm that they or their products are safe for human consumption.

References

Agrios, G.N. (2005). Plant diseases caused by fungi. *Plant Pathology*, *4*, pp.385-614 fifth ed. Academic Press Ltd., San Diego, California, USA.

Aldwinckle, H.S., Jones, A.L. (1990). *Compendium of apple and pear diseases.* APS press, Saint Paul, MN. *Mycologia*, 82(6), p. 802.

Al-Hindi, R.R., Al-Najada, A.R., Mohamed, S.A. (2011). Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes. *African Journal of Microbiology Research*, *5*(4), pp.443-448.

Al-Maawali, S.S., Al-Sadi, A.M., Ali Khalifa Alsheriqi, S., Nasser Al-Sabahi, J., Velazhahan, R. (2021). The potential of antagonistic yeasts and bacteria from tomato phyllosphere and fructoplane in the control of *Alternaria* fruit rot of tomato. *All Life*, *14*(1), pp.34-48.

Al-Rahbi, B.A.A., Al-Sadi, A.M., Al-Mahmooli, I.H., Al-Maawali, S.S., Al-Mahruqi, N.M.T., Velazhahan, R. (2021). *Meyerozyma guilliermondii* SQUCC-33Y suppresses postharvest fruit rot of strawberry caused by *Alternaria alternata*. *Australasian Plant Pathology*, 50(3), 349-352.

Andersen, K. S., Winding, A. (2004). Non-target effects of bacterial biological control agents on soil Protozoa. *Biology and Fertility of Soils*, 40(4), pp. 230–236.

Arrarte, E., Garmendia, G., Rossini, C., Wisniewski, M., Vero, S. (2017). Volatile organic compounds produced by Antarctic strains of *Candida sake* play a role in the control of postharvest pathogens of apples. *Biological Control*, *109*, pp.14-20.

Banjara, N., Nickerson, K. W., Suhr, M. J., Hallen-Adams, H. E. (2016). Killer toxin from several food-derived *Debaryomyces hansenii* strains effective against pathogenic

Candida yeasts. *International Journal of Food Microbiology*, 222, pp. 23–29.

Benito, S., Palomero, F., Morata, A., Uthurry, C., Suárez-Lepe, J.A. (2009). Minimization of ethylphenol precursors in red wines via the formation of pyranoanthocyanins by selected yeasts. *International Journal of Food Microbiology*, *132*(2-3), pp.145-152.

Ben-Noun, L. (2016). Roots/Health Benefits of Apples. *Researchgate.* www. Researchgate.net/publication/288944473[Accessed November 2021]

Beresford, R. M., Wood, P.N., Shaw, P.W., Taylor, T.J. (2008). Application of fungicides during leaf fall to control apple scab (*Venturia inaequalis*) in the following season. *New Zealand Plant Protection*, 61, pp. 59–64.

Beresford, R. M., Wright, P.J., Wood, P.N., Park, N.M., Larsen, N.J., Fisher, B.M. (2013). Resistance of *venturia inaequalis* to demethylation inhibitor and dodine fungicides in four New Zealand apple-growing regions. *New Zealand Plant Protection*, 66, pp.274-283.

Beretta, B., Gaiaschi, A., Galli, C. L., Restani, P. (2000). Patulin in apple-based foods: Occurrence and safety evaluation. *Food Additives and Contaminants*, 17(5), pp. 399–406.

Bertrand, P.F., Saulie-Carter, J.L. (1978). The occurrence of benomyl-tolerant strains of *Penicillium expansum* and *Botrytis cinerea* in the Mid-Columbia region of Oregon and Washington'. *Plant Diseases*. Report. 62, 302–305.

Bondonno, N.P., Bondonno, C.P., Ward, N.C., Hodgson, J.M., Croft, K.D. (2017). The cardiovascular health benefits of apples: Whole fruit vs. isolated compounds'. *Trends in Food Science & Technology*, 69, pp.243-256.

Braun, P.G., McRae, K.B. (1992). Composition of a population of *Venturia inaequalis* resistant to myclobutanil. *Canadian Journal of Plant Pathology*, *14*(3), pp.215-220.

Calvin, L., Gale, F., Hu, D., Lohmar, B. (2006). Food Safety Improvement Underway in China, Economic Research Service. *Amber Waves, US Department of Agriculture Report*, pp.17-21.

Calvo, J., Calvente, V., de Orellano, M.E., Benuzzi, D., de Tosetti, M.I.S. (2007). Biological control of postharvest spoilage caused by *Penicillium expansum* and *Botrytis cinerea* in apple by using the bacterium *Rahnella aquatilis*. *International Journal of Food Microbiology*, 113(3), pp.251-257.

Carisse, O. and Pelletier, J.R. (1994). Sensitivity distribution of *Venturia inaequalis* to fenarimol in Québec apple orchards. *Phytoprotection*, *75*(1), pp.35-43.

Carisse, O., Dewdney, M. (2002). A review of non-fungicidal approaches for the control of apple scab. *Phytoprotection*, *83*(1), pp.1-29.

Carisse, O., Rolland, D. (2004). Effect of timing of application of the biological control agent *Microsphaeropsis ochracea* on the production and ejection pattern of Ascospores by *Venturia inaequalis*. *Phytopathology*, 94(12), pp. 1305–1314.

Chen, P.H., Chen, R.Y, Chou, J.Y. (2018). Screening and evaluation of yeast antagonists for biological control of *Botrytis cinerea* on strawberry fruits. *Mycobiology*, 46(1), 33-46.

Chitranshi, S., Dubey, N, Sajjad, M. (2020). Sustainable botanical products for safe post-harvest management of perishable produce: A review.*Journal of Horticulture and Postharvest Research*, *3* pp.125-140.

Choińska, R., Piasecka-Jóźwiak, K., Chabłowska, B., Dumka, J., Łukaszewicz, A. (2020). Biocontrol ability and volatile organic compounds production as a putative mode of

action of yeast strains isolated from organic grapes and rye grains. *Antonie van Leeuwenhoek*, 113(8), 1135-1146.

Chowdhury, S. P., Dietel, K., Rändler, M., Schmid, M., Junge, H., Borriss, R., Hartmann, A., Grosch, R. (2013). Effects of *Bacillus amyloliquefaciens* FZB42 on lettuce growth and health under pathogen pressure and its impact on the rhizosphere bacterial community. *PLoS ONE*, 8(7), pp. 1–10.

Cirvilleri, G., Bonaccorsi, A., Scuderi, G., Scortichini, M. (2005). Potential biological control activity and genetic diversity of *Pseudomonas syringae* pv. *syringae* strains. *Journal of Phytopathology 153*(11-12), pp. 654-666.

Comitini, F., Ingeniis De, J., Pepe, L., Mannazzu, I., Ciani, M. (2004). *Pichia anomala* and *Kluyveromyces wickerhamii* killer toxins as new tools against *Dekkera/Brettanomyces* spoilage yeasts. *FEMS Microbiology Letters*, 238(1), pp.235-240.

Contarino, R., Brighina, S., Fallico, B., Cirvilleri, G., Parafati, L., Restuccia, C. (2019). Volatile organic compounds (VOCs) produced by biocontrol yeasts. *Food Microbiology*, *82*, pp.70-74.

Cordero-Bueso, G., Mangieri, N., Maghradze, D., Foschino, R., Valdetara, F., Cantoral, J.M., Vigentini, I. (2017). Wild grape-associated yeasts as promising biocontrol agents against *Vitis vinifera* fungal pathogens. *Frontiers in Microbiology*, 8, p.20-25.

De Bach, P., (1964). Biological control of insect pests and weeds. *Biological control of insect pests and weeds.* Chapman and Hall Ltd., London, Chapter 22 and 23, pp. 49-70-pp. 631-650.

Dean, R., Van Kan, J.A., Pretorius, Z.A., Hammond-Kosack, K.E., Di Pietro, A., Spanu, P.D., Rudd, J.J., Dickman, M., Kahmann, R., Ellis, J., Foster, G.D. (2012). The Top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology*, *13*(4), pp.414-430.

Department of Agriculture Forestry and Fisheries (2018). A profile of the south african apple market value chain. *Directorate Marketing* pp. 1–57.

Devaraj, H., Radha, S.K., Shanmugasundaram, E.R. (1982). Neurotoxic effect of patulin. *Indian Journal of Experimental Biology*, *20*(3), p.230.

Di Francesco, A., Di Foggia, M., Corbetta, M., Baldo, D., Ratti, C., Baraldi, E. (2021). Biocontrol Activity and Plant Growth Promotion Exerted by Aureobasidium pullulans Strains. *Journal of Plant Growth Regulation*, *40*(3), pp.1233-1244.

Doolotkeldieva, T., Bobusheva, S. (2017). Scab disease caused by *Venturia inaequalis* on apple trees in Kyrgyzstan and biological agents to control this disease. *Advances in Microbiology*, *7*(06), p.450.

Droby, S. (2005). Improving quality and safety of fresh fruits and vegetables after harvest by the use of biocontrol agents and natural materials. In *International Symposium on Natural Preservatives in Food Systems 709* (pp. 45-52).

Du Plessis, K. (2017). Post-harvest Innovation Programme Innovate flipbook. pp. 82-83. https://www.postharvestinnovation.org.za/PHI-2017/mobile/index.html [Accessed 01 March 2021]

Eilenberg, J., Hajek, A., Lomer, C. (2001). Suggestions for unifying the terminology in biological control. *BioControl*, *46*(4), pp.387-400.

Elad, Y. (1988). Latent infection of Botrytis cinerea in rose flowers and combined chemical and physiological control of the disease. *Crop Protection*, *7*(6), pp.361-366.

El Ghaouth, A., Wilson, C. L., Wisniewski, M. (2003). Control of postharvest decay of apple fruit with *Candida saitoana* and induction of defense responses. *Phytopathology*, 93(3), pp. 344–348.

Errampalli, D., Brubacher, N.R., De Ell, J.R. (2006). Sensitivity of *Penicillium expansum* to diphenylamine and thiabendazole and postharvest control of blue mould with fludioxonil in 'McIntosh' apples. *Postharvest Biology and Technology* 39, 101–107.

Errampalli, D., Crnko, N. (2004). Control of blue mold caused by *Penicillium expansum* on apples "Empire" with fludioxonil and cyprodinil', *Canadian Journal of Plant Pathology*, 26(1), pp. 70–75.

FAOstat. (2019). Food and Agricultural Organisation of the United Nations – Statistical Division. Production – Crops. www.fao.org/faostat/ [Accessed 26 January 2022]

Fernández-Ortuño, D., Torés, J.A., De Vicente, A., Pérez-García, A. (2008). Mechanisms of resistance to QoI fungicides in phytopathogenic fungi. *International Microbiology*, *11*(1), p.1-9.

Fiaccadori, R., Cicognani, E., Alberoni, G., Collina, M., Brunelli, A. (2011). Sensitivity to strobilurin fungicides of Italian *Venturia inaequalis* populations with different origin and scab control. *Pest Management Science*, 67(5), pp. 535–540.

Filajdic, N., Sutton, T.B. (1992). Chemical control of *Alternaria* blotch of apples caused by *Alternaria mali*. *Plant disease*, *76*(2), pp.126-130.

Fourie, P.C. (1996). Fruit and human nutrition. In *Fruit processing* (pp. 20-39). Springer, Boston, MA.

Garming, H., Strohm, K., Dirksmeyer, W. (2014). Effects of structural change and international trade on profitability of apple production in Europe and South Africa. In *XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): XVII 1103* (pp. 143-150).

Gerbore, J., Benhamou, N., Vallance, J., Le Floch, G., Grizard, D., Regnault-Roger, C., Rey, P. (2014). Biological control of plant pathogens: advantages and limitations seen through the case study of *Pythium oligandrum*'. *Environmental Science and Pollution Research*, *21*(7), pp.4847-4860.

Gladieux, P., Zhang, X.G., Afoufa-Bastien, D., Sanhueza, R.M.V., Sbaghi, M., Le Cam, B. (2008). On the origin and spread of the scab disease of apple: out of central Asia. *PloS one*, *3*(1), p. e1455.

Grevesse, C., Lepoivre, P., Jijakli, M.H. (2003). Characterization of the exoglucanase-encoding gene PaEXG2 and study of its role in the biocontrol activity of *Pichia anomala* strain K. *Phytopathology*, *93*(9), pp.1145-1152.

Gupta, S., Didwania, N., Singh, D. (2020). Biological control of mustard blight caused by *Alternaria brassicae* using plant growth promoting bacteria. *Current Plant Biology*, *23*, p.100166.

Han, J., Zhao, L., Zhu, H., Dhanasekaran, S., Zhang, X., Zhang, H. (2021). Study on the effect of alginate oligosaccharide combined with *Meyerozyma guilliermondii* against *Penicillium expansum* in pears and the possible mechanisms involved. *Physiological and Molecular Plant Pathology*, *115*, p.101654.

Have, A.T., Mulder, W., Visser, J., van Kan, J.A. (1998). The endopolygalacturonase gene Bcpg1 is required for full virulence of *Botrytis cinerea*. *Molecular Plant-Microbe Interactions*, *11*(10), pp.1009-1016.

Horlock, C. (2006). Management of *Alternaria* leaf and fruit spot in apples. Department of Primary Industries and Fisheries, Queensland.

Hossain, M. B., Piotrowski, M., Lensing, J., Gau, A.E. (2009). Inhibition of conidial growth of *Venturia inaequalis* by the extracellular protein fraction from the antagonistic bacterium *Pseudomonas fluorescens* Bk3. *Biological Control*, 48(2), pp. 133–139.

Hua, S.S.T., Beck, J.J., Sarreal, S.B.L., Gee, W. (2014). The major volatile compound 2-phenylethanol from the biocontrol yeast, *Pichia anomala*, inhibits growth and expression of aflatoxin biosynthetic genes of *Aspergillus flavus*. *Mycotoxin research*, *30*(2), pp.71-78.

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

Janisiewicz, W. J. and Korsten, L. (2002).Biological control of postharvest diseases of fruits. *Annual Review of Phytopathology*, 40(1), pp. 411–441.

Janisiewicz, W.J. and Roitman, J. (1988). Biological control of blue mould and grey mould on apple and pear with *Pseudomonas cepacia*. *Phytopathology*, *78*(12), pp.1697-1700.

Jeevaratnam, K., Jamuna, M., Bawa, A. S. (2005). Biological preservation of foods Bacteriocins of lactic acid bacteria. *Indian Journal of Biotechnology*, 4(4), pp. 446–454.

Jongwanich, J., (2009). The impact of food safety standards on processed food exports from developing countries. *Food Policy*, *34*(5), pp.447-457.

Kayalvizhi, V., Antony, U. (2011). Microbial and Physico-chemical changes in tomato juice subjected to pulsed electric field treatment. *African Journal of Microbiology Research*,

8(37), pp.3382-3391.

Köhl, J., Scheer, C., Holb, I.J., Masny, S., Molhoek, W. (2015). Toward an integrated use of biological control by *Cladosporium cladosporioides* H39 in apple scab (*Venturia inaequalis*) management. *Plant Disease*, *99*(4), pp.535-543.

Lauret, J. (2011). Innovative strategies for the control of apple scab (*Venturia inaequalis* [Cke] Wint .) in organic apple production', (Doctoral dissertation, Université de Liège, Gembloux, Belgium), p. 188.

Lee, C.Y. (2012) Common nutrients and nutraceuticals quality of apple'. *New York Fruit Quarterly*, *20*(3), pp.1-8.

Liang, W., Dondini, L., De Franceschi, P., Paris, R., Sansavini, S., Tartarini, S. (2015). Genetic Diversity, Population Structure and Construction of a Core Collection of Apple Cultivars from Italian Germplasm', *Plant Molecular Biology 54500237674Reporter*, 33(3), pp. 458–473.

Lima, G., Castoria, R., De Curtis, F., Raiola, A., Ritieni, A., De Cicco, V. (2011). Integrated control of blue mould using new fungicides and biocontrol yeasts lowers levels of fungicide residues and patulin contamination in apples. *Postharvest Biology and Technology*. Elsevier B.V., 60(2), pp. 164–172.

Liu, J., Sui, Y., Wisniewski, M., Droby, S., Liu, Y. (2013). Utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit. *International Journal of Food Microbiology*, *167*(2), pp.153-160.

Lõoke, M., Kristjuhan, K., Kristjuhan, A. (2011). Extraction of genomic DNA from yeasts for PCR-based applications. *Biotechniques*, 51(6), 325-328.

MacHardy, W.E. (1996). Apple scab: biology, epidemiology, and management. *American*

Phytopathological Society (APS Press).

MacHardy, W.E., Gadoury, D.M., Gessler, C. (2001). Parasitic and biological fitness of *Venturia inaequalis*: relationship to disease management strategies. *Plant Disease, 85*(10), pp.1036-1051.

Mari, M., Leoni, O., Iori, R., Cembali, T. (2002). Antifungal vapour-phase activity of allyl-isothiocyanate against *Penicillium expansum* on pears. *Plant Pathology*, *51*(2), pp.231-236.

Medina-Córdova, N., López-Aguilar, R., Ascencio, F., Castellanos, T., Campa-Córdova, A.I., Angulo, C. (2016). Biocontrol activity of the marine yeast *Debaryomyces hansenii* against phytopathogenic fungi and its ability to inhibit mycotoxins production in maize grain (*Zea mays* L.). *Biological Control*, 97, 70-79.

Mercier, J., Lindow, S.E. (2001). Field performance of antagonistic bacteria identified in a novel laboratory assay for biological control of fire blight of pear. *Biological Control*, *22*(1), pp.66-71.

Mewa-Ngongang, M., du Plessis, H. W., Hlangwani, E., Ntwampe, S. K. O., Chidi, B. S., Hutchinson, U. F., Jolly, N. P. (2019a). Activity interactions of crude biopreservatives against spoilage yeast consortia. *Fermentation*, 5(3), 53.

Mewa-Ngongang, M., Du Plessis, H.W., Chidi, B.S., Hutchinson, U.F., Ntwampe, K.S.O., Okudoh, V.I. and Jolly, N.P. (2021). Physiological and antagonistic properties of *Pichia kluyveri* for curative and preventive treatments against post-harvest fruit fungi. *Polish Journal of Food and Nutrition Sciences*, 71(3), 245-253.

Mewa-Ngongang, M., Du Plessis, H.W., Ntwampe, S.K.O., Chidi, B.S., Hutchinson, U.F., Mekuto, L., Jolly, N.P. (2018). Fungistatic and Fungicidal Properties of *Candida*

pyralidae Y1117, *Pichia kluyveri* Y1125 and *Pichia kluyveri* Y1164 on the Biocontrol of *Botrytis cinerea*. In *Proceedings of the 10th International Conference on Advances in Science, Engineering, Technology and Healthcare (ASETH-18), Cape Town, South Africa* (pp. 19-20).

Mewa-Ngongang, M., Du Plessis, H.W., Ntwampe, S.K.O., Chidi, B.S., Hutchinson, U.F., Mekuto, L., Jolly, N.P. (2019b). The use of *Candida pyralidae* and *Pichia kluyveri* to control spoilage microorganisms of raw fruits used for beverage production. *Foods*, 8(10), 454.

Michaud, J. P., Sloderbeck, P. E., Nechols, J. R. (2008). Biological Control of Insect Pests on Field Crops in Kansas. *Kansas State University Agricultural Experiment Station and Cooperative Extension Service*, pp. 1–11.

Miessner, S., Stammler, G. (2010). *Monilinia laxa*, *M. fructigena* and *M. fructicola*: Risk estimation of resistance to QoI fungicides and identification of species with cytochrome b gene sequences. *Journal of Plant Diseases and Protection*, *117*(4), pp.162-167.

Mir, J. I., Raja, W. H. and Sheikh, M. A. (2020). *Alternaria* leaf and fruit spot in apple : Symptoms , cause and management. *European Journal of Biotechnology and Bioscience,* 8 (3),pp. 24-26

Mitchell, T.G., Freedman, E.Z., White, T.J. and Taylor, J.W. (1994). Unique oligonucleotide primers in PCR for identification of *Cryptococcus neoformans*. *Journal of Clinical Microbiology*, 32(1), 253-255.

Mohnen, D. (2008). Pectin structure and biosynthesis. *Current Opinion in Plant Biology*, *11*(3), pp.266-277.

Monroe, A. (2009). Integrated pest management for Australian apples and pears'. *Integrated pest management for Australian apples and pears.* NSW *Industry and Investmen*t *Management* pp. viii-212, Orange Region, Australia.

Nally, M.C., Pesce, V.M., Maturano, Y.P., Muñoz, C.J., Combina, M., Toro, M.E., De Figueroa, L.C., Vazquez, F. (2012). Biocontrol of *Botrytis cinerea* in table grapes by non-pathogenic indigenous *Saccharomyces cerevisiae* yeasts isolated from viticultural environments in Argentina. *Postharvest Biology and Technology*, 64(1), 40-48.

Nunes, C.A. (2012). Biological control of postharvest diseases of fruit. *European Journal of Plant Pathology*, 133(1), 181-196.

Núñez, F., Lara, M.S., Peromingo, B., Delgado, J., Sánchez-Montero, L., Andrade, M.J. (2015). Selection and evaluation of *Debaryomyces hansenii* isolates as potential bioprotective agents against toxigenic penicillia in dry-fermented sausages. *Food Microbiology*, 46, 114-120.

Oberg, S. (2002). Interactions between two ladybird species attacking a host alternating aphid. *Masters thesis, Department of Ecology and Plant Production Science, Swedish University of Agricultural Sciences*.

Ogawa, J.M., English, H. (1991). *Diseases of temperate zone tree fruit and nut crops*. (Vol. 3345). UCANR Publications.

Oliveira, C.M., Auad, A.M., Mendes, S.M., Frizzas, M.R. (2014). Crop losses and the economic impact of insect pests on Brazilian agriculture. *Crop Protection*, 56, 50-54.

Pane, C., Zaccardelli, M. (2015). Evaluation of *Bacillus* strains isolated from solanaceous phylloplane for biocontrol of *Alternaria* early blight of tomato. *Biological Control*, 84, pp.11-18.

Parveen, S., Wani, A.H., Bhat, M.Y., Koka, J.A., Wani, F.A. (2016). Management of postharvest fungal rot of peach (*Prunus persica*) caused by *Rhizopus stolonifer* in Kashmir Valley, India. *Plant Pathology and Quarantine*, 6(1), 19-29.

Pastor, F.J., Guarro, J., (2008). *Alternaria* infections: laboratory diagnosis and relevant clinical features. *Clinical Microbiology and Infection*, *14*(8), pp.734-746.

Patriarca, A., Fernández Pinto, V. (2018). *Alternaria*. Alternaria Species and Their Associated Mycotoxins. *Reference Module in Food Science*.

Paucod, J.C., Krivobok, S., Vidal, D. (1990). Immunotoxicity testing of mycotoxins T-2 and patulin on Balb/c mice'. *Acta Microbiologica Hungarica*, *37*(4), pp.331-339.

Paul Ross, R., Morgan, S., Hill, C. (2002). Preservation and fermentation: Past, present and future. *International Journal of Food Microbiology*, 79(1–2), pp. 3–16.

Pierson, C.F., Ceponis, M.J., McColloch, L.P. (1970). *Market diseases of apples, pears, and quinces* (No. 375-377), p. 1–96. In: Agriculture Handbook, vol. 376. Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C.

Porsche, F. M., Pfeiffer, B., Kollar, A. (2017). A new phytosanitary method to reduce the ascospore potential of *venturia inaequalis. Plant Disease*, 101(3), pp. 414–420.

Quaglia, M., Ederli, L., Pasqualini, S., Zazzerini, A. (2011). Biological control agents and chemical inducers of resistance for postharvest control of *Penicillium expansum* Link. on apple fruit. *Postharvest Biology and Technology*, 59(3), 307-315.

Ramírez, F., Davenport, T. L. (2013) Apple pollination: A review. *Scientia Horticulturae*. Elsevier B.V., 162, pp. 188–203.

Reddel, A.L. (2015). Fungicide Resistance Genetics of Apple Scab fungus *Venturia*

inaequalis'. *Honors Theses and Capstones*. 254. University of New Hampshire - Main Campus.

Roberts, R.G. (1990). Postharvest biological control of grey mould of apple by *Cryptococcus laurentii'*. *Phytopathology*, *80*(6), pp.526-530.

Robiglio, A., Sosa, M. C., Lutz, M. C., Lopes, C. A., Sangorrín, M. P. (2011). Yeast biocontrol of fungal spoilage of pears stored at low temperature. *International Journal of Food Microbiology*, 147(3), 211-216.

Romanazzi, G., Feliziani, E., Baños, S.B., Sivakumar, D. (2017). Shelf life extension of fresh fruit and vegetables by chitosan treatment. *Critical Reviews in Food Science and Nutrition*, *57*(3), pp.579-601.

Romanazzi, G., Smilanick, J.L., Feliziani, E., Droby, S. (2016). Integrated management of postharvest grey mould on fruit crops. *Postharvest Biology and Technology*, *113*, pp.69-76.

Rosenberger, D. A. (1990b). Grey mould in *Compendium of Apple and Pear Diseases* (eds A.L. Jones and H.H Aldwinkle). APS Press, St Paul, Minnesota, pp 55-56.

Rosslenbroich, H.J., Stuebler, D. (2000). *Botrytis cinerea*—history of chemical control and novel fungicides for its management. *Crop protection*, *19*(8-10), pp.557-561.

Ruiz-Moyano, S., Hernández, A., Galvan, A.I., Córdoba, M.G., Casquete, R., Serradilla, M.J., Martín, A. (2020). Selection and application of antifungal VOCs-producing yeasts as biocontrol agents of grey mould in fruits. *Food Microbiology*, *92*, p.103556.

Sacu, P. (2007) 'Deciduous fruit', *Processing*, 700 (August), pp. 36–46.

Salman, M.A.M. (2005). Biological control of Rhizopus soft rot on apple, pear and peach by *Trichoderma harzianum* (Doctoral dissertation). AnNajah National University, Nablus, Palestine.

Sanzani, S. M., De Girolamo, A., Schena, L., Solfrizzo, M., Ippolito, A., Visconti, A. (2009) Control of *Penicillium expansum* and patulin accumulation on apples by quercetin and umbelliferone. *European Food Research and Technology*, 228(3), pp. 381–389.

Sanzani, S. M., Montemurro, C., Rienzo, V. Di, Solfrizzo, M., Ippolito, A. (2013) International Journal of Food Microbiology Genetic structure and natural variation associated with host of origin in *Penicillium expansum* strains causing blue mould. *International Journal of Food Microbiology*, 165(2), pp. 111–120.

Sardella, D., Muscat, A., Brincat, J. P., Gatt, R., Decelis, S., Valdramidis, V. (2016). A Comprehensive Review of the Pear Fungal Diseases. *International Journal of Fruit Science*. Taylor & Francis, 16(4), pp. 351–377.

Scheer, C., Holb, I.J., Köhl, J., Masny, S., Molhoek, W. (2015). Toward an Integrated Use of Biological Control by *Cladosporium cladosporioides* H39 in Apple Scab (*Venturia inaequalis*) Management. April, pp. 535–543.

Schmoock, S., Kürkcüoglu, S., Gau, A. E. (2008). Biological control of apple scab and fire blight by the application of the non-pathogenic bacterium *Pseudomonas fluorescens* Bk3 to the leaf surface. *Ecofruit - 13th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing*, (1985), pp. 306–309.

Schnürer, J., Magnusson, J. (2005). Antifungal lactic acid bacteria as biopreservatives', *Trends in Food Science and Technology*, 16(1–3), pp. 70–78.

Sharma, R.R., Singh, D., Singh, R. (2009). Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. *Biological Control*, *50*(3), pp.205-221.

Shim, J.O., Choi, K.D., Hahn, K.D., Lee, J.H., Hyun, I.H., Lee, T.S., Ko, K.I., Lee, H.P., Le, M.W. (2002). Blue mould of pear caused by *Penicillium aurantiogriseum* in Korea. *Mycobiology*, *30*(2), pp.105-106.

Sholberg, P. L., Bedford, K. E., Stokes, S. (2003). Effect of preharvest application of cyprodinil on postharvest decay of apples caused by *Botrytis cinerea. Plant Disease*, 87(9), pp. 1067–1071.

Sid, A., Ezziyyani, M., Egea-Gilabert, C., Candela, M.E. (2003). Selecting bacterial strains for use in the biocontrol of diseases caused by *Phytophthora capsici* and *Alternaria alternata* in sweet pepper plants. *Biologia Plantarum*, 47(4), pp.569-574.

Singh, B.K., Yadav, K.S., Verma, A. (2017). Impact of postharvest diseases and their management in fruit crops: An overview. *Journal Biology Innovation*, *6*, pp.749-60.

Singh, D., Sharma, R. R. (2018). Postharvest Diseases of Fruits and Vegetables and Their Management, *Postharvest Disinfection of Fruits and Vegetables*. pp. 1-52 Elsevier Inc.

Singh, J.P., Bedi, R.K. (1990). FTO/SnSe heterojunction for photovoltaic conversion. *Japanese Journal of Applied Physics*, *29*(5A), p.L792.

Smith, F.D., Parker, D.M., Köller, W. (1991). Sensitivity distribution of *Venturia inaequalis* to the sterol demethylation inhibitor flusilazole: baseline sensitivity and implications for resistance monitoring. *Phytopathology*, *81*(4), pp.392-396.

Snowdon, A.L. (1990). Pome fruits. *A colour atlas of post-harvest diseases and disorders of fruits and vegetables', 1*, pp.170-218. Volume 1. Wolfe Scientific, London, UK.

Spadaro, D., Ciavorella, A., Frati, S., Garibaldi, A., Gullino, M. L. (2007). Incidence and level of patulin contamination in pure and mixed apple juices marketed in Italy. *Food Control*, 18(9), pp. 1098–1102.

Spadaro, D., Garibaldi, A. and Gullino, M. L. (2004) 'Control of *Penicillium expansum* and *Botrytis cinerea* on apple combining a biocontrol agent with hot water dipping and acibenzolar-S-methyl, baking soda, or ethanol application', *Postharvest Biology and Technology*, 33(2), pp. 141–151.

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*(2), pp.185-194.

Spadaro, D., Lorè, A., Garibaldi, A., Gullino, M. L. (2013). A new strain of *Metschnikowia fructicola* for postharvest control of *Penicillium expansum* and patulin accumulation on four cultivars of apple. *Postharvest Biology and Technology*, 75, pp. 1– 8.

Spadaro, D., Vola, R., Piano, S., Gullino, M.L. (2002). Mechanisms of action, efficacy and possibility of integration with chemicals of four isolates of the yeast *Metschnikowia pulcherrima* active against postharvest pathogens on apples. *Postharvest Biology. Technology*, *24*, pp.123-134.

Speijers, G.J.A. (2004). Patulin. *Mycotoxins in food: detection and control*, pp.339-352.

Spitaler, R., Marschall, K., Zidorn, C., Kelderer, M., Zelger, R., Stuppner, H. (2004). Apple scab control with grapefruit seed extract: no alternative to chemical fungicides. *Ecofruit-11th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing: Proceedings to the Conference from 3rd February to 5th February 2004 at Weinsberg/Germany*, pp. 208–211.

Sun, L., Guo, Y., Fu, C., Li, J., Li, Z. (2013). Simultaneous separation and purification of total polyphenols, chlorogenic acid and phlorizin from thinned young apples. *Food Chemistry*,136(2),pp.1022–1029.

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

Thakur, K., Chawla, V., Bhatti, S., Swarnkar, M.K., Kaur, J., Shankar, R., Jha, G. (2013). De novo transcriptome sequencing and analysis for *Venturia inaequalis*, the devastating apple scab pathogen. *PloS one*, *8*(1).

Thomma, B.P. (2003). *Alternaria* spp.: from general saprophyte to specific parasite. *Molecular Plant Pathology*, *4*(4), pp.225-236.

Tian, B., Yang, J., Zhang, K. Q. (2007). Bacteria used in the biological control of plant-parasitic nematodes: Populations, mechanisms of action, and future prospects. *FEMS Microbiology Ecology*, 61(2), pp. 197–213.

Tsedaley, B. (2014). Review on Early Blight (*Alternaria* spp.) of Potato Disease and its Management Options, *Journal Biology Agriculture and Healthcare* 4(27), pp. 191–199.

Vico, I., Duduk, N., Vasić, M., Nikolić, M., (2014). Identification of *Penicillium expansum* causing postharvest blue mould decay of apple fruit. *Pesticidi ifitomedicina*, *29*(4), pp.257-266.

Vinson, J.A., Su, X., Zubik, L., Bose, P. (2001). Phenol antioxidant quantity and quality in foods: fruits'. *Journal of Agricultural and Food Chemistry*, *49*(11), pp.5315-5321.

Wang, L., Wu, H., Qin, G., Meng, X. (2014). Chitosan disrupts *Penicillium expansum* and controls postharvest blue mold of jujube fruit. *Food Control*, 41(1), pp. 56–62.

Wang, X., Glawe, D.A., Kramer, E., Weller, D., Okubara, P.A. (2018) Biological control of *Botrytis cinerea*: interactions with native vineyard yeasts from Washington State. *Phytopathology*, 108(6), 691-701.

Wang, Y., Yu, T., Li, Y., Cai, D., Liu, X., Lu, H., Zheng, X.D. (2009). Postharvest biocontrol of *Alternaria alternata* in Chinese winter jujube by *Rhodosporidium paludigenum*. *Journal of Applied Microbiology*, *107*(5), pp.1492-1498.

Wellington, S. (2019). Deciduous Fruit Production Forecast to Fully Rebound from Drought. *Fresh Deciduous Fruit Deciduous.* Republic of South Africa, Pretoria, p-5

Williamson, B., Tudzynski, B., Tudzynski, P., van Kan, J.A. (2007). *Botrytis cinerea*: the cause of grey mould disease. *Molecular Plant Pathology*, *8*(5), pp.561-580.

Wouters, M. F. A., Speijers, G. J. A. (1996). Patulin. In *Food additives series 35*. Toxicological evaluation of certain food additives and contaminants (pp. 337–402). Geneva, Switzerland: World Health Organisation.

Xiao, C. L., Kim, Y. K. (2008). Postharvest Fruit Rots in Apples Caused by *washingtonensis* , and *Sphaeropsis pyriputrescens*, *Plant Health Progress Plant Health Progress*.

Yang, Z., Cao, S., Cai, Y., Zheng, Y. (2011a). Combination of salicylic acid and ultrasound to control postharvest blue mold caused by *Penicillium expansum* in peach fruit. *Innovative Food Science and Emerging Technologies*, 12(3), pp. 310– 314.

Yu, T., Ye, H. and Dong, X. (2007) 'Synergistic effect of chitosan and *Cryptococcus laurentii* on inhibition of *Penicillium expansum* infections', *International Journal of Food Microbiology*, 114, pp. 261–266.

Zhang, H., Zheng, X., Yu, T. (2007b). Biological control of postharvest diseases of peach with *Cryptococcus laurentii*', *Food Control*, 18(4), pp. 287–291.

Zhao, H., Kim, Y.K., Huang, L., Xiao, C.L. (2010). Resistance to thiabendazole and baseline sensitivity to fludioxonil and pyrimethanil in *Botrytis cinerea* populations from apple and pear in Washington State. *Postharvest Biology and Technology, 56*(1), pp.12-18.

Zhou, Y., Li, W., Zeng, J., Shao, Y. (2018). Mechanisms of action of the yeast *Debaryomyces nepalensis* for control of the pathogen *Colletotrichum gloeosporioides* in mango fruit. *Biological Control*, 123, 111-119.

Appendix

TABLE S1 Yeasts used in this study.

Yeast codes	Species name	Culture collection	Source
Y1	*Rhodotorula dairenensis*	ARC[a]-Genebank	Jaboticaba fruit
Y2	*Hanseniaspora uvarum*	ARC-Genebank	Jaboticaba fruit
Y3	*Hanseniaspora uvarum*	ARC-Genebank	Jaboticaba fruit
Y4	Unknown	ARC-Genebank	Jaboticaba fruit
Y5	*Saccharomyces uvarum*	ARC-Genebank	Jaboticaba fruit
Y6	*Aureobasidium melanogenum*	ARC-Genebank	Jaboticaba fruit
Y7	*Aureobasidium melanogenum*	ARC-Genebank	Jaboticaba fruit
Y8	*Debaryomyces hansenii*	ARC-Genebank	Jaboticaba fruit
Y9	Unknown	ARC-Genebank	Jaboticaba fruit
Y10	*Saccharomyces uvarum*	ARC-Genebank	Jaboticaba fruit
Y11	*Debaryomyces hansenii*	ARC-Genebank	Jaboticaba fruit
Y12	*Rhodotorula dairenensis*	ARC-Genebank	Jaboticaba fruit
Y13	*Hanseniaspora opuntiae*	ARC-Genebank	Jaboticaba fruit
Y14	*Saccharomyces uvarum*	ARC-Genebank	Jaboticaba fruit
Y15	*Hanseniaspora. uvarum*	ARC-Genebank	Jaboticaba fruit
Y16	*Hanseniaspora uvarum*	ARC-Genebank	Jaboticaba fruit
Y17	*Hanseniaspora occidentalis*	ARC-Genebank	Jaboticaba fruit
Y18	*Debaryomyces hansenii*	ARC-Genebank	Jaboticaba fruit
Y19	*Hanseniaspora uvarum*	ARC-Genebank	Jaboticaba fruit
Y20	*Hanseniaspora uvarum*	ARC-Genebank	Jaboticaba fruit
Y21	*Debaryomyces hansenii*	ARC-Genebank	Jaboticaba fruit
Y22	Unknown	ARC-Genebank	Jaboticaba fruit
Y23	Unknown	ARC-Genebank	Jaboticaba fruit
Y24	*Meyerozyma guilliermondii*	ARC-Genebank	Jaboticaba fruit
Y25	*Hanseniaspora. uvarum*	ARC-Genebank	Jaboticaba fruit
Y26	*Hanseniaspora. uvarum*	ARC-Genebank	Jaboticaba fruit
Y27	*Hanseniaspora uvarum*	ARC-Genebank	Jaboticaba fruit
Y28	Unknown	ARC-Genebank	Jaboticaba fruit
Y29	Unknown	ARC-Genebank	Jaboticaba fruit
Y30	*Candida oleophila*	ARC-Genebank	Jaboticaba fruit
Y31	*Candida oleophila*	ARC-Genebank	Jaboticaba fruit
Y32	*Candida oleophila*	ARC-Genebank	Jaboticaba fruit
Y33	Unknown	ARC-Genebank	Jaboticaba fruit
Y34	*Candida oleophila*	ARC-Genebank	Jaboticaba fruit
Y35	*Rhodotorula dairenensis*	ARC-Genebank	Jaboticaba fruit
Y36	*Candida oleophila*	ARC-Genebank	Jaboticaba fruit
Y37	*Candida oleophila*	ARC-Genebank	Jaboticaba fruit
Y38	*Hanseniaspora uvarum*	ARC-Genebank	Jaboticaba fruit
Y39	*Meyerozyma guilliermondii*	ARC-Genebank	Jaboticaba fruit
Y40	Unknown	ARC-Genebank	Mobola plum
Y42	Unknown	ARC-Genebank	Jaboticaba fruit

Y43	*Hanseniaspora guilliermondii*	ARC-Genebank	Marula pulp
Y44	Unknown	ARC-Genebank	Marula pulp
Y45	*Zygosaccharomyces bailii*	ARC-Genebank	Marula pulp
Y46	Unknown	ARC-Genebank	Marula pulp
Y47	*Hanseniaspora opuntiae*	ARC-Genebank	Marula pulp
Y48	Unknown	ARC-Genebank	Marula pulp
Y49	Unknown	ARC-Genebank	Marula pulp
Y50	*Candida stellimalicola*	ARC-Genebank	Marula pulp
Y51	*Pichia kudriavzevii*	ARC-Genebank	Marula pulp
Y52	Unknown	ARC-Genebank	Marula pulp
Y53	*Rhodotorula dairenensis*	ARC-Genebank	Marula pulp
Y54	*Hanseniaspora guilliermondii*	ARC-Genebank	Marula pulp
Y55	*Pichia kudriavzevii*	ARC-Genebank	Marula pulp
Y56	*Pichia fermentans*	ARC-Genebank	Marula pulp
Y57	*Hanseniaspora valbyensis*	ARC-Genebank	Palm wine
Y58	*Saccharomyces cariocanus*	ARC-Genebank	Palm wine
Y59	Unknown	ARC-Genebank	Palm wine
Y60	*Pichia kluyveri*	ARC-Genebank	Shiraz Fermentation
Y61	*Dekkera anomala*	ARC-Genebank	Wine barrel
Y62	*Dekkera anomala*	ISA[b] 1653	Unknown
Y63	*Candida pyralidae*	ARC-Genebank	Shiraz Fermentation
Y64	*Pichia kluyveri*	ARC-Genebank	Shiraz Fermentation
Y65	*Meyerozyma guilliermondii*	ARC-Genebank	Grape must (Chardonnay)
Y66	*Dekkera bruxellensis*	ARC-Genebank	Wine barrel
Y67	*Brettanomyces lambicus*	CBS[c]	Unknown
Y68	Unknown	ARC-Genebank	Palm wine
Y69	*Zygosaccharomyces bailii*	ARC-Genebank	Chardonnay
Y70	*Lanchancea thermotolerans*	ARC-Genebank	Chardonnay must
Y71	*Torulaspora delbrueckii*	ARC-Genebank	Grape must,
Y72	*Metschnikowia pulcherrima*	ARC-Genebank	Chardonnay
Y73	*Lanchancea thermotolerans*	CHR Hansen	Unknown
Y74	*Torulaspora delbrueckii*	ARC-Genebank	Spontaneous Shiraz fermentation
Y75	*Saccharomyces cerevisiae*	ARC-Genebank	Grapes
Y76	*Zygosaccharomyces bailii*	IGC[d] 4242	Unknown
Y77	*Brettanomyces. lambicus*	CBS 3093	Unknown
Y78	*Meyerozyma guilliermondii*	ARC-Genebank	Apple
Y79	*Pichia kluyveri*	ARC-Genebank	Apple
Y80	*Zygoascus hellenicus*	ARC-Genebank	Apple
Y81	*Meyerozyma guilliermondii*	ARC-Genebank	Apple
Y82	*Meyerozyma guilliermondii*	ARC-Genebank	Apple
Y83	*Brettanomyces lambicus*	ARC-Genebank	Unknown
Y84	*Debaryomyces hansenii*	ARC-Genebank	Mobola plum
Y85	*Pichia kluyveri*	ARC-Genebank	Mobola plum
Y87	*Meyerozyma guilliermondii*	ARC-Genebank	Apple
Y88	*Meyerozyma guilliermondii*	ARC-Genebank	Apple
Y89	*Zygoascus helenicus*	ARC-Genebank	Apple

Y90	*Zygosaccharomyces bailii*	CBS 4689	Grape must
Y91	*Zygosaccharomyces rouxii*	CBS 731	Grape must
Y92	*Zygosaccharomyces rouxii*	CBS 681	Soya product
Y93	*Zygosaccharomyces microellipsoides*	CBS 2734	Unknown
Y94	*Zygosaccharomyces cidri*	CBS 2951	Cider
Y95	*Zygosaccharomyces florentinus*	CSIR H576	Unknown
Y96	*Zygosaccharomyces fermentati*	CBS 4506	Fruit fly
Y97	*Zygosaccharomyces bisporus*	CSIR[e] Y849	Soil
Y98	*Zygosaccharomyces bisporus*	CBS 702	Unknown
Y99	*Brettanomyces bruxellensis*	ARC-Genebank	Wine barrel
Y100	*Brettanomyces bruxellensis*	ARC-Genebank	Wine barrel
Y101	*Brettanomyces lambicus*	CBS 2910	Unknown
Y102	*Candida magnoliae*	ARC-Genebank	Shiraz
Y103	Unknown	ARC-Genebank	Spontaneous Shiraz fermentations
Y104	*Saccharromyces cerevisiae*	ARC-Genebank	Apples
Y105	*Meyerozyma guilliermondii*	ARC-Genebank	Apples

[a]ARC-Agricultural Research Council

[b]ISA-Instituto Superior de Agronomia

[c]CBS-Centraal Bureau voor Schimmelcultures

[d]IGC-Gulbenkian Institute of Science

[e]CSIR-Council for Scientific and Industrial Research