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Integrated control of postharvest *Fusarium solani* of potatoes using UV-C irradiation and *Moringa oleifera* leaf extracts

By

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Submitted in partial fulfilment of the
requirements for the degree of

Master of Science in Agriculture (Plant Pathology)
In the

Discipline of Plant Pathology

School of Agricultural, Earth and Environmental Sciences

College of Agriculture, Engineering, and Science

University of KwaZulu-Natal

Pietermaritzburg

June 2024

DISSERTATION SUMMARY

Potatoes are a source of food, income and important nutrients which are beneficial to human health. However, production of potato tuber may be hindered by postharvest losses, which reduce the quality and quantity of potatoes reaching consumers. Major losses of potatoes are caused by poor harvesting, sorting, cleaning, handling, and packing. *Fusarium solani*, a causal agent of dry rot disease that mainly affects potatoes occurs during storage as the pathogen invades the potato tuber through tissue injuries inflicted during lifting or grading. *Fusarium* dry rot has been mainly controlled by using chemicals. However, the frequent use of chemicals has been recently reported to enhance fungicide resistance to potato pathogens. Chemical fungicides also negatively affect human health and the environment, as they introduce residues in treated potatoes and soil.

There is a need to investigate and develop sustainable agricultural strategies such as UV-C irradiation and plant extracts as alternative strategies which are human and environmentally friendly. Therefore, this research aimed to evaluate the effect of UV-C irradiation and *Moringa oleifera* leaf extract, independently and their combined effect in controlling *F. solani* on potatoes *in vitro* and *in vivo.* The antifungal effects of UV-C irradiation against *F. solani* were evaluated *in vitro* and *in vivo*. UV-C treatment for 10 and 15 minutes at a 10 cm distance successfully inhibited the mycelial growth of *F. solani* by ≥ 50% at 7-day post-inoculation. The *in vivo* results showed that 'Sifra' potatoes treated with UV-C for 10 minutes and 15 minutes had a disease incidence ≤ 33%. The increase in the duration of UV-C exposure to potatoes lowered the disease incidence on potatoes. The scanning electron micrographs showed the breakage and shrinkage of the mycelia *in vitro*, and the disruption of spores in UVC -treated potatoes.

Moringa leaf extracts were prepared and adjusted into different concentrations, MLE 1%, MLE 1.5%, MLE 2%, MLE 2.5%, and MLE 3%. These concentrations were evaluated for their efficacy against *F. solani in vitro* and *in vivo*. The findings demonstrated that MLE 1.5%, MLE 2.5%, and MLE 3% inhibited the mycelial growth of *F. solani* by ≥ 50% *in vitro*. The *in vivo* findings revealed that both MLE 2.5% and MLE 3% reduced incidence of dry rot in potatoes. The antifungal activity of moringa was increased at higher concentrations. The scanning electron micrographs showed mycelia distortion in samples treated with moringa and the disruption of *F. solani*

spores on the treated potatoes. It also indicated the formation of biofilms in moringa-treated potatoes.

Furthermore, this study evaluated the effect of integrated control of *F. solani* using UV-C and moringa leaf extracts. The *in vitro* results demonstrated that samples treated with MLE 2.5% and exposed to UV-C for 15 minutes inhibited the mycelial growth of *F. solani* by 100%. 'Sifra' potatoes exposed to UV-C for 15 minutes and treated with MLE 3% had the lowest disease incidence (8.33%). The scanning electron micrographs showed abnormal, shrinkage, disruption, aggregation and reduced hyphae length of mycelia in samples treated with both UV-C and MLE. Moreover, it indicated the formation of biofilms in UV and moringa-treated surfaces of potato wounds. These integrated treatments enhanced efficacy compared to the individual application of either treatment. UV-C can be integrated with moringa and be used as alternatives to synthetic fungicides to control dry rot of potatoes.

PREFACE

The research contained in this dissertation was completed by the candidate while based in the Department of Plant Pathology, School of Agricultural, Earth and Environmental Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa. The research was financially supported by Potatoes South Africa (PSA).

DECLARATION

I, GCINOKUHLE BUTHELEZI, declare that

I. The research reported in this thesis, except where otherwise indicated, is my original work.

II. This thesis has not been submitted for any degree or examination at any other university.

III. This thesis does not contain other people's data, pictures, graphs or other information unless specifically acknowledged as being sourced from other people.

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ACKNOWLEDGEMENTS

First and foremost, I would like to thank God for granting me the opportunity to enroll in this study and for giving me the strength and wisdom to complete it successfully.

I would like to extend my appreciation to my supervisors, Dr. N.C Mbili and Prof. A Mditshwa, for their support, patience, and encouragement. The guidance they gave carried me throughout the study. I am grateful for their input and insight throughout the process of writing this thesis. Their unwavering encouragement has helped me through some of the most challenging times.

I would like to thank our lab technicians, Miss B.S. Mazibuko and Mr K. Mkhonza, for their laboratory assistance. I would also like to thank the Horticulture lab technician, Mr Thokozani Nkosi for his assistance in the Horticulture lab.

I am grateful to the Biochemistry technician Mr Qiniso Mthethwa and Mr Sandile Mthembu for assisting me in the Biochemistry lab.

I am grateful to Potatoes South Africa for their financial support in the form of a bursary for the duration of the study.

I would like to thank Ms Carol Mhlaba, Ms Nonkanyiso Malevu, Ms Londeka Mbatha, Ms Cebo Mbutho and Ms Gugu Buthelezi for their support and words of couragement. I would like to thank Plant Pathology postgraduate students for their support and motivation.

Most importantly, I would like to thank my family for their support since the beginning of this study. Their encouragement, love, and prayers kept me going. *Izandla zedlula ikhanda bo Shenge!*

DEDICATION

I dedicate this study to my grandfather Mr M Buthelezi, my grandmother Mrs H.E Buthelezi, my mother, Miss N Buthelezi, my siblings, Nokubonga, Thubelihle, Asibonge and my baby boy Amukelwa.

TABLE OF CONTENTS

Chapter 1

General introduction

1.1 Background

Potato (*Solanum tuberosum L*) is one of the commonly consumed vegetables, it is a source of nutrients and income, especially for small-scale farmers. Potato is a main source of antioxidants in the human diet. It provides basic nutrients such as carbohydrates, and dietary fiber, as well as several vitamins and minerals including potassium, magnesium, iron, and zinc (Spooner and Hetterscheid, 2006; King and Slavin 2013; Lovat et al. 2016). Potatoes are generally eaten boiled, roasted, or fried and frequently served as a side dish or snack**.**

The potato plants develop by forming tubers and increase their yield until the foliage falls off (Wustman and Struik, 2007). About 5000 potato varieties have been reported to be grown worldwide (Zaheer and Akhtar 2016). In addition to being widely planted, potato plants are vigorous, high-yielding, and adaptable to various environmental conditions (Lingling et al., 2018). Total production of potatoes in South Africa was slightly reduced from 2, 595.28 million metric tons in 2021 to 2, 528.95 in 2022 (STATISTA, 2023). In South Africa, potato is mainly produced in four provinces; Limpopo, Free State, Western Free State and North west contributing to about 65% to 70% of the annual production (Haverkort et al., 2013). Limpopo is the leading province in the number of hectares planted which accounts for 22% of hectares of the total national production followed by Eastern Free State, Western Free State, and lastly Sandveld with 11 562 hectares, 7 372 hectares, and 5 498 hectares, respectively (Potatoes SA, 2022).

Potatoes are susceptible to a variety of plant pathogens, including fungi (Gao et al. 2000; Liu et al. 2019), bacteria (Charkowski 2018; Clarke et al. 2019), and viruses (Yellareddygar et al., 2018; Zhan et al. 2019) that cause disease at different stages of potato production. *Fusarium solani* (Mart.Sacc,1881) is one of the important fungal pathogens that is known for causing decay and postharvest losses in potato tubers, particularly during storage. *F. solani* is a species complex of at least 26 closely related filamentous fungi in the division *Ascomycota,* family *Nectriacea*e. It causes fungal dry rot which can cause damage to potatoes in storage as well as in seed pieces after

planting (Fiers et al., 2012). The dry rot of potato develops more rapidly at a high relative humidity. This disease can also reduce crop establishment by inhibiting the developing potato sprouts and causing crop losses of up to 25%, while more than 60% of tubers can be infected during storage (Wharton et al., 2007a).

1.2 Problem statement

Potato is known as a semi-perishable commodity, the storage of both seed and ware potatoes is a major challenge for most farmers. Storage losses can reach 50% and sometimes higher in worse cases (Wale et al., 2008). Major losses of potatoes occur due to poor harvesting techniques as well as poor storage facilities and during transportation. The pathogen then invades the potato tuber through tissue injuries inflicted during lifting or grading (O'Brien and Leach, 1983). Therefore, the quality and marketable yield of potatoes is remarkably reduced.

F. solani, a causal agent of dry rot is one of the most devastating postharvest pathogens of potatoes. Among *Fusarium* spp, *F. solani* causes about 50% of infections followed by *F. oxysporum* (Snyder and Hansen, 1940) which causes approximately 20% (Wharton et al., 2007). Dispersal of *F. solani* could be through infested soil attached to the tuber surface during harvesting (Theron and Holz, 1990).

The control of *Fusarium* dry rot has been mainly achieved by postharvest applications of chemicals such as cymoxanil (cyan acetamide oximes), carbamates (prothiocarb and propamocarb) and Thiabendazole™ (TBZ), as the tubers enter storage (Vatankhah et al., 2019). However, it has been reported that most of the *Fusarium* spp have developed resistance towards these fungicides (Vatankhah et al., 2019). These fungicides also leave residues in treated potatoes which could be detrimental to the environment and human health (Goswami et al., 2018). The problems associated with the use of fungicides have motivated researchers to search for novel and non-chemical methods for the potato industry.

1.3 Justification

Potato is ranked as the third most important food crop after rice and wheat and is consumed by over a billion people throughout the world (Haverkort et al., 2012;

Devaux et al., 2014). Potato production is increasing intensively in many developing countries accounting for more than half of the global harvest (Scott and Suarez, 2012). Recent trends indicate that potato production is increasing in highly populated developing nations (Bradeen et al., 2011). Potato yield may be limited in storage due to sprouting, and evaporation of water from tubers (Wustman and Struik, 2007). However, postharvest diseases are the major limiting factor affecting potato production and storage (Fiers et al., 2012). *F. solani* is a phytopathogenic fungus and is an important causal agent of various diseases such as root and fruit rot of *Cucurbita* spp., root and stem rot of pea, foot rot of bean and dry rot of potato (Wharton and Kirk, 2007).

Fusarium dry rot is one of the most significant postharvest diseases affecting potatoes, and losses could range from 6 to 25% sometimes reaching 60% if poor handling techniques are used during harvesting and storage (Stevenson et al., 2001). Dry rot is caused by different *Fusarium* species, however, *F. solani* and *F.sambucinum* are the most common causes of dry rot (Stevenson et al., 2001). On a growing medium, *F. solani* grows slowly and produces a bluish-purple colour (figure 1.1). *Fusarium* dry rot spore-infected tubers or contaminated soil are the initial sources of *Fusarium* inoculum (Pandya and Patil, 2016).

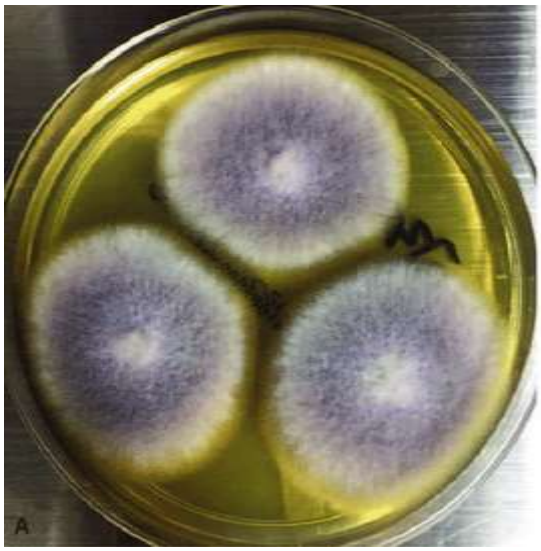

Figure 1.1: Mycelial growth of *F.solani* in Potato Dextrose Agar medium (Hafizi et al., 2013).

Fusarium dry rot is currently controlled by the application of either chemical or biological compounds. Fungicide treatment is the main method currently used to control postharvest pathogens. However, recent studies have reported that most of the *Fusarium* species develop resistance to fungicides used in potatoes (Vatankhah et al., 2019). Moreover, fungicide treatments may also have detrimental effects on human health and the environment (Goswami et al., 2018). Ultraviolet-C (UV-C) irradiation and plant extracts are recommended alternative control strategies that can be used to substitute synthetic fungicides (Allende and Artes, 2003).

UV-C irradiation is a cheap, safe, and appropriate method for postharvest storage which has been widely used in controlling microorganisms in a variety of fresh products during storage (Allende and Artes, 2003). It is used as a postharvest treatment to enhance fruit quality and extend the shelf-life of fresh fruits and vegetables. UV-C treatment has been reported to exhibit antifungal effects against a range of postharvest plant pathogens. The intensity of UV-C irradiation required to kill the pathogens may differ with types of pathogens. Fungi have a more complex cell structure compared to bacteria and viruses and therefore, require more intensity for it cells to be degraded (Yin et al., 2013). For instance, a recent *in vitro* study by Terao et al. (2015) revealed that UV-C treatment at 20 kJ m^{-1} reduced the mycelia growth of *Colletotrichum gloeosporioides* (Penz and Sacc, 1884) and *Botryosphaeria dothidea*(Moug. Petr, 1971). UV-C treatment (2.064 kJ/m^2 for 5 minutes) has also been reported to decrease *Escherichia coli* and *Listeri*a innocua growth in 'Tommy Atkins' sliced mango stored at 4°C for fifteen days (Romero et al., 2017).

Plant extracts are likely to be safer for the environment and humans, and fungi are less likely to develop resistance towards these natural pesticides. *Moringa oleifera* has antifungal, antiviral, antibacterial, and antioxidant properties effective against various phytopathogens (Emad El-Din et al., 2016; Belay and Sisay, 2014). Thus, moringa extracts have the potential to control fungal diseases, while improving the shelf life of crops due to their high antioxidant concentration. *M. oleifera* extracts (leaves, bark, and seeds) have been reported to inhibit the mycelial growth of *F. solani* f.sp. *radicicola* and *F. oxysporum* f.sp. *lycopersici* (Dwivedi and Enespa, 2012). It is envisioned that this study will contribute to developing safe and reliable methods of controlling worldwide postharvest pathogens of potatoes including dry rot caused by *F. solani*.

The success of this study will also help in improving quality and extending the shelf life of potato, therefore, increasing its marketable yield.

1.4 Research aim and objectives

The main aim of this research is to investigate the effect of integrating UV-C irradiation and *M. oleifera* extracts to control *F. solani* of potatoes *in vitro* and in postharvest storage.

To achieve the aim of this study, the study had the following specific objectives:

1. Evaluate the *in vitro* and *in vivo* effect of UV-C irradiation on *F. solani.*
2. Evaluate the *in vitro* and *in vivo* effect of moringa leaf extracts on *F. solani*.
3. Determine the mode of action of UV-C irradiation and moringa leaf extracts against *F. solani* of potato.
4. Evaluate the *in vitro* and *in vivo* effect of the integration of UV-C irradiation and moringa leaf extracts on postharvest dry rot of potato.

1.5 Research hypothesis

This study hypothesized that the integration of UV-C irradiation and moringa leaf extracts will be more effective against *F. solani* compared to an individual application of these treatments.

1.6 Dissertation structure

The dissertation consists of 6 chapters, and the details of each chapter are explained below:

Chapter 1 is the general introduction of the dissertation; Chapter 2 is the literature review of the postharvest diseases affecting potatoes and their management strategies. Chapter 3 investigated the *in vitro* and *in vivo* effects of UV-C irradiation treatment against *F. solani*; Chapter 4 investigated the *in vitro* and *in vivo* fungicidal effects of moringa leaf extracts against *F. solani*; Chapter 5 investigated the *in vitro* and in *vivo* effect of integrated control of *F. solani* using UV-C irradiation and moringa leaf extracts against *F. solani*; Lastly; Chapter 6 outlines the main findings of the study and their implications.

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Chapter 2

Literature review: Postharvest development and management of dry rot and other diseases in potatoes.

Abstract

Potato (*Solanum tuberosum*) is globally one of the economically important crops due to its contribution to food security. Postharvest pathogens mainly affect potato quality in storage, therefore reducing its market value. *Fusarium solani* is considered one of the most devastating postharvest pathogens of potatoes. Although there are various methods to reduce postharvest losses caused by *F. solani*, novel and environmentally friendly treatments are desired. UV-C irradiation and moringa leaf extracts have been reported to have antifungal effects against *F. solani*. Ultraviolet-C (UV-C) irradiation is a cheap, safe, and appropriate method for postharvest storage, which has been used to enhance fruit quality and extend the shelf life of various fresh horticultural produce. *Moringa oleifera* has antifungal, antiviral, antibacterial, and antioxidant properties effective against various phytopathogens. Thus, moringa extracts have the potential to control fungal diseases, while improving the shelf life of crops due to their high antioxidant concentration. This chapter reviews the occurrence of *F. solani* and the damage this pathogen causes to potatoes. It also reviews the current control and treatment methods that have been successfully found to be effective against *F. solani*. Importantly, it also highlights the research gaps that require further investigation.

Keywords: Tuber, Disease, Chemical fungicide, Postharvest quality, Shelf-life

2.1 Introduction

Potato (*Solanum tuberosum L*) belongs to the family *Solanaceae* and is the third non-cereal world food crop after rice and wheat (Pandey, 2008). This crop is grown in temperate, subtropical, and tropical regions. Potato crop is sensitive to heat because it originated in the cool Andes mountains (George et al. 2017). Tuber yield is reduced at temperatures above 18 °C, especially when combined with high ambient temperatures (above 30°C day / 23 °C night) (Monneveux et al. 2014). In South Africa, potatoes are commonly grown under relatively warm conditions, with frequent occurrences of climatic stresses such as heat, hail, and frost (van der Waals et al.

2016). Climate change leads to temperature increase in South Africa, with the already warm interior regions predicting temperature increases of twice those of the global average (Engelbrecht et al. 2015). Therefore, it is anticipated that climate change will lead to increased heat stress in potatoes in South Africa, which may be worsened by increase drought stress if supplemental irrigation is not provided (George et al. 2017).

Potato is cultivated for its nutritional, medicinal, and industrial values. There are more than 4000 varieties of native potatoes, mostly found in the Andes, they vary in size and shape (Bradshaw and Bonierbale, 2010). This crop is relatively cheap to grow, rich in nutrients, and are used in many delicious treats (Navarre et al., 2009). Potato serves as a food and income security source for many commercial and subsistence farmers. In terms of nutrition, tubers contain about 25% dry matter, including 10 to 23% starch, 1.4-3.0% high-quality proteins, and vitamins C, B1, B2, B6, PP, and K, which makes it an extremely important crop (Karpukhin, and Keita, 2020). Potato is also a source of antioxidants that can contribute in preventing both degenerative and age-related diseases.

Lutein and zeaxanthin compounds are predominant in yellow-fleshed potatoes (Burgos et al., 2009) and anthocyanins are present in purple and red-fleshed potato landraces (Burgos et al., 2013) commonly grown and eaten in the Andean highlands of Peru, Bolivia, Ecuador, and Colombia. Potatoes also contain glycoalkaloids, which in high concentrations can be toxic to humans but in low concentrations can have beneficial effects such as inhibition of the growth of cancer cells (Friedman 2015). Yellow-fleshed potatoes have a carotenoid concentration higher than white-fleshed potatoes while purple potatoes have a higher anthocyanin concentration than red- or white-fleshed potatoes (Hejtmánková et al., 2013).

Potatoes have higher calorie per unit area production potential than any grain and can be produced, stored, and consumed without major technological inputs (Devaux et al., 2021). About less than 50% of potatoes grown worldwide are consumed fresh. The rest are processed into potato food products and food ingredients; fed to cattle, pigs, and chickens; processed into starch for industrial use; and re-used as seed tubers for growing the next season's potato crop (FAO, 2008). Like other plant foods, the nutritional composition of potatoes is affected by different pre-harvest factors including, environment, cultural practices, maturity at harvest, biotic and abiotic

stresses, and post-harvest factors including processing, poor storage practices, transportation, and improper marketing (Pinhero et al., 2009). Damaged tubers lead to the development of infections by pathogens, which invade the tuber through tissue injuries inflicted during lifting or grading (Pinhero et al., 2009). Among all factors that reduce the quantity and quality of potatoes, diseases are major causes of postharvest losses and reduced shelf life (Pinhero et al., 2009). Hence, the marketable value of potatoes is also reduced. Significant losses of potatoes have motivated researchers to investigate safe and reliable methods to control the postharvest disease of potatoes. The current review aims to provide an overview of the epidemiology and pathogenesis of dry rot of potato caused by *Fusarium solani*, and advances in postharvest methods of rot management.

2.2. Origin and economic importance of potatoes

Potato is thought to have originated in the Andes region of South America and gradually spread throughout the world (Brown and Henfling, 2014). It was first cultivated by the native South Americans possibly 2000 years before the Spanish conquest. In 1537, the Spaniards had their first encounter with a potato in one of the villages of the Andes in Europe. It was introduced between 1580. A.D. to 1585 A.D. in Spain, Portugal, Italy, France, Belgium, and Germany. It was introduced in India by Portuguese sailors during the early 17th century and its cultivation was spread to North India by the British (Brown and Henfling, 2014).

Globally, potato is ranked as the third most consumed crop after rice and wheat and is the top non-grain food commodity (Caliskan et al., 2022). In 2022, global production of potato reached 375 million tonnes, with China and India producing the most at 95.5 and 56 million tonnes, respectively (FAOSTAT, 2023). Potato is a high-quality vegetable food crop and is utilized in a variety of ways, such as the preparation of chips, wafers, flakes, granules, flour, starch, soup, or gravy thickener (Burgos et al., 2020). It can be a supplement to meat and milk products for improving their taste, lowering energy intake, and reducing food costs, due to its high protein content with high biological value (Rajiv and Kawar, 2016).

2.3 Production of potato in South Africa and production constraints

According to STATISTA (2023), a total production of potatoes in South Africa was 2,528.95 million metric tons in 2022, a slight reduction from 2,595.28 in the previous year. During 2020 and 2021 there was an increase in potato production (Figure 2.1), and it was ranked third among the most produced crops in the country.

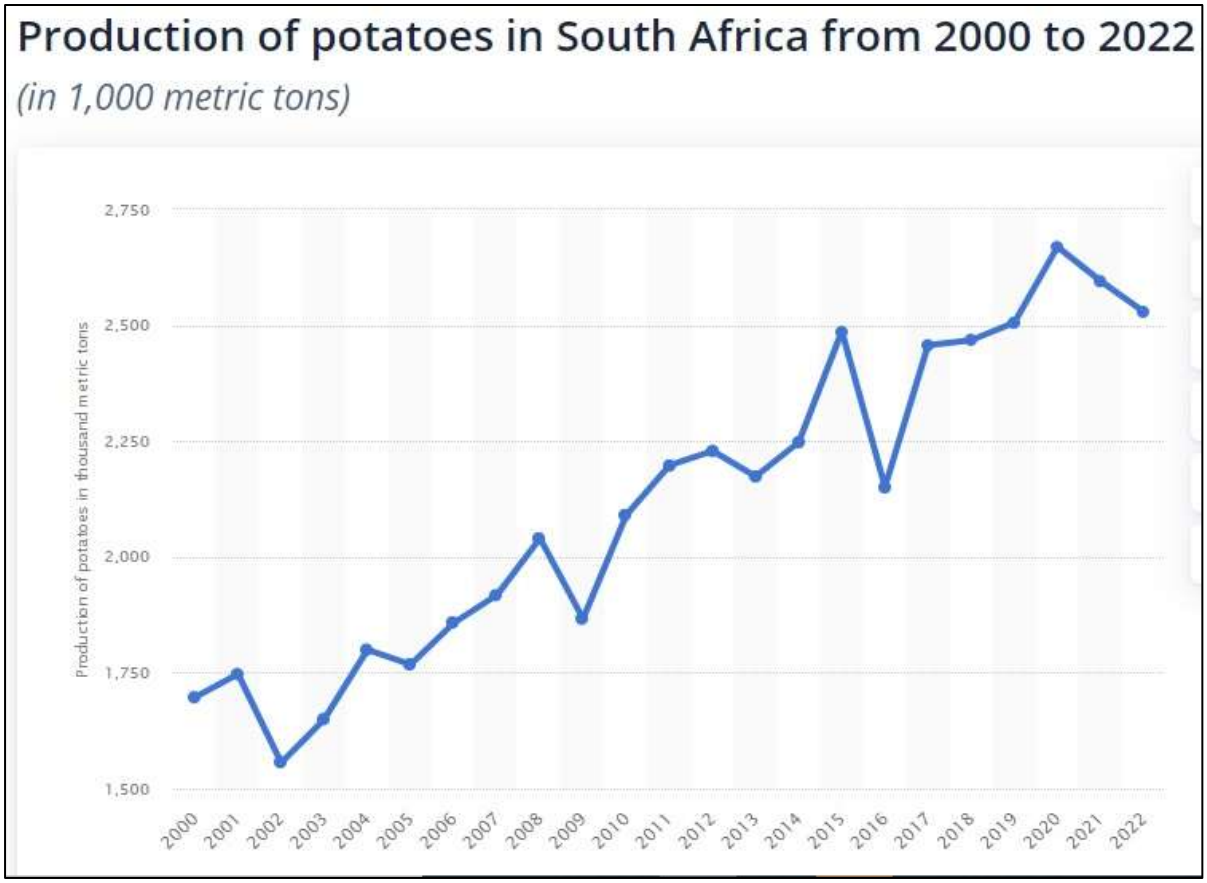

Figure 2.1: Fluctuations in potato production from 2000 to 2022 in South Africa (STATISTA, 2023).

In South Africa, potato production occurs in 16 regions, with the major producing potato provinces being Limpopo, Eastern Free State, Western Free State and Sandveld contributing to between 65% to 70% of the total annual production (Haverkort et al., 2013). In 2018, Limpopo province had the highest number of hectares planted, which accounts for 22% of hectares of the total national production (Potatoes SA). Eastern Free State was the second place with 11 562 hectares followed by Western Free State in third place with 7 372 hectares and lastly Sandveld with 5 498 hectares.

Potato crop production is affected by several diseases that result in reduced plant growth, low tuber quality and reduced marketable yield. Globally, approximately 60 soil-borne diseases affect potatoes and cause enormous damage mostly on tubers which is the economically most important part of the plant (Fiers et al., 2012). Diseases caused by fungal and fungal-like pathogens are the most detrimental to potato production. These postharvest diseases include late blight (*Phytophthora infestans* (de Bary, 1876) (Nyankanga et al., 2008), (pink rot (*Phytophthora erythroseptica* (Pethybr, 1913) (Salas et al., 2000), Pythium leak (*Pythium spp.)* (Pringsheim, 1858) and dry rot (*Fusarium solani* and *Fusarium spp*.) (Wharton and Kirk, 2007). These pathogens are both soilborne and seed-borne, hence the diseases can occur in both field and storage (Powelson and Rowe, 2008).

Fusarium solani is a species complex of at least 26 closely related filamentous fungi in the division Ascomycota, family *Nectriaceae. F. solani* is a phytopathogenic fungus and is a causal organism of several diseases including dry rot of potatoes, foot rot of bean, root and stem rot of pea and fruit rot of *Cucurbita* spp., root and stem rot of pea, foot rot of bean and dry rot of potato (Wharton and Kirk, 2007). This pathogen is widely distributed in soil and is considered an important plant pathogen in agriculture. Species of *F. solani* are the most significant potato pathogens that cause yield loss and can severely reduce their market value (Wharton and Kirk, 2007).

2.4. Dry rot

Dry rot caused by *Fusarium spp.* is one of the most important diseases affecting potatoes in storage (Bojanowski et al., 2013). Currently, there are 17 species and 5 variants of *Fusarium* recognized globally as causal agents of potato dry rot (Tiwari et al., 2020). However, *F*. *oxysporum* and *F. solani* are the predominant pathogens to cause *Fusarium* dry rot in potatoes in South Africa (Theron and Holz, 1990; Villarino et al., 2021). Dry rot disease can reduce crop establishment by affecting the developing potato sprouts and cause crop losses approximated to be up to 25%, while more than 60% of tubers can be infected during storage (Wharton et al., 2007).

2.4.1 Taxonomy and morphology

Fusarium solani is classified under the kingdom of Fungi, phylum Ascomycota and subphylum Pezizomycotina. It falls under the class Sordariomycetes, order Hypocreales, *Nectriaceae* family, and genus *Fusarium* (O'Donnell, 2000).

It grows as a white mycelium on potato dextrose agar (PDA) media and changes to pink as it gets older (Fig. 2.2A). Macroconidia have three to four septa on average, are slightly curved, are rather wide and thick-walled, and may have a slightly blunted apical end (Fig. 2.2B) (Peters et al., 2008).

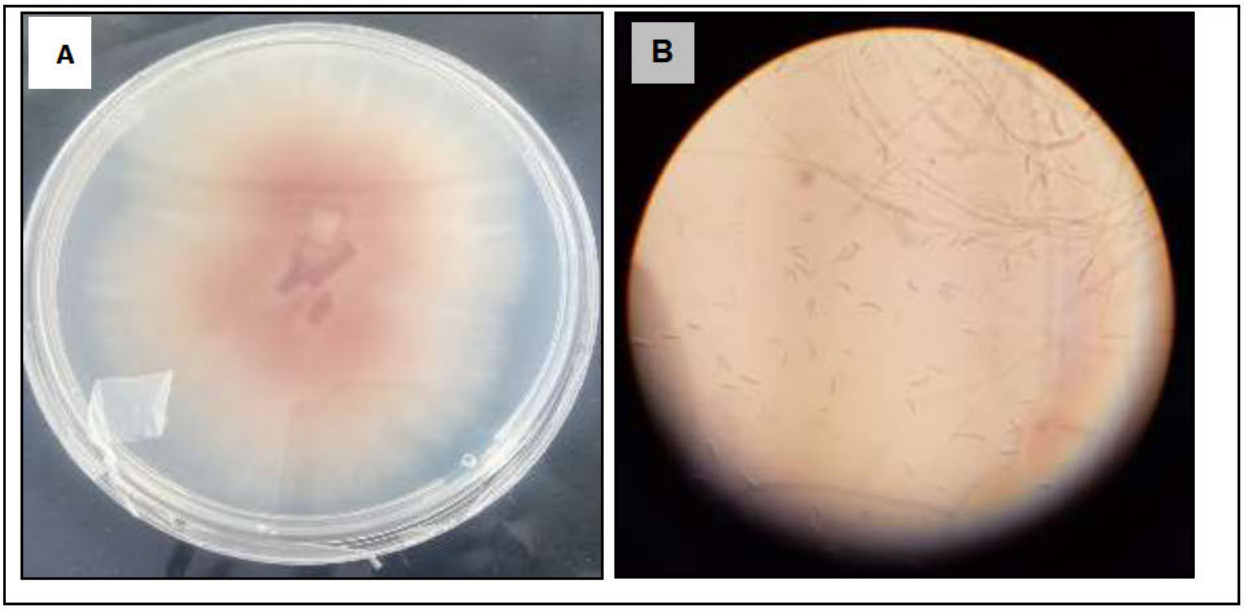

Figure 2.2. Mycelial growth of *F. solani* in potato dextrose agar after 7 days at 25 °C (A) and *F. solani* macronidia spores under a light microscope (B) (Buthelezi, 2024).

2.4.2 Host range

F. solani mainly affects the members of the *Solanaceae* family which includes tomato, eggplant, buffalo bur, and woody nightshade. It also affects members of the cucurbit family such as melon, cucumber, and pumpkin (Romberg and Davis, 2007).

2.4.3. Dispersal and distribution of *F. solani*

Fusarium spp. are found in soil with a worldwide distribution and are important plant pathogens. *F. solani* causes about 50% infections followed by *F. oxysporum* which

causes approximately 20% (Wharton et al., 2007). Dispersal of *F. solani* could be through infested soil attached to the tuber surface during harvesting. This pathogen then invades the potato tuber through tissue injuries inflicted during lifting or grading. The infection requires a fresh, unsuberized wound, with suberization of wounds preventing the infection (Powelson and Rowe, 2008; Estrada et al., 2010).

2.4.4 Economic importance

Fusarium spp. are responsible for significant economic losses in agricultural fields worldwide (Spolti et al., 2014) due to difficulties in the management of diseases caused by this species (Dalhoff, 2018). *F. solani* affects potatoes both pre and postharvest, but great loss occurs during storage and transportation to the marketplace (Benkeblia, 2012).

Dry rot of seed tubers can reduce crop establishment by affecting the growth of potato sprouts and causing crop losses of up to 25%, while more than 60% of tuber losses occur during storage (Wharton and Kirk 2007). This disease has been reported to cause postharvest losses costing US producers US$100–250 million annually (http://www.ars.usda.gov/is/AR/archive/jun02/fungus0602.pdf, 2008). Postharvest losses can be up to 28% in Dingxi located in Gansu Province, China, out of which, 88% are caused by dry rot (Suqin et al. 2004).

2.4.5 Epidemiology and disease cycle

Fusarium dry rot development is initiated by inoculum from infected seed tubers or infested soils (Estrada et al., 2010). Infection of potato tubers by *Fusarium* is through wounds that occur during harvesting or seed handling and cutting (Glass et al., 2001; Secor and Salas, 2001; Powelson and Rowe, 2008). The pathogen survives from one season to the other in infected tubers, decaying plant tissue, or in the soil as chlamydospores (Powelson and Rowe, 2008). Fusarium *dry rot* develops more rapidly in high relative humidity and at 15-20 °C. Potato rot development is accelerated at relative humidity of about 70% and hindered at lower relative humidity (Chen et al., 2020). *Fusarium* species are common in most soils where potatoes are grown and can survive as resistant spores free in the soil for a very long period. There are two stages in the potato crop cycle in which potato tubers can be infected by *Fusarium spp* in the spring and in the autumn (Wharton et al., 2007a; Fig. 2.3).

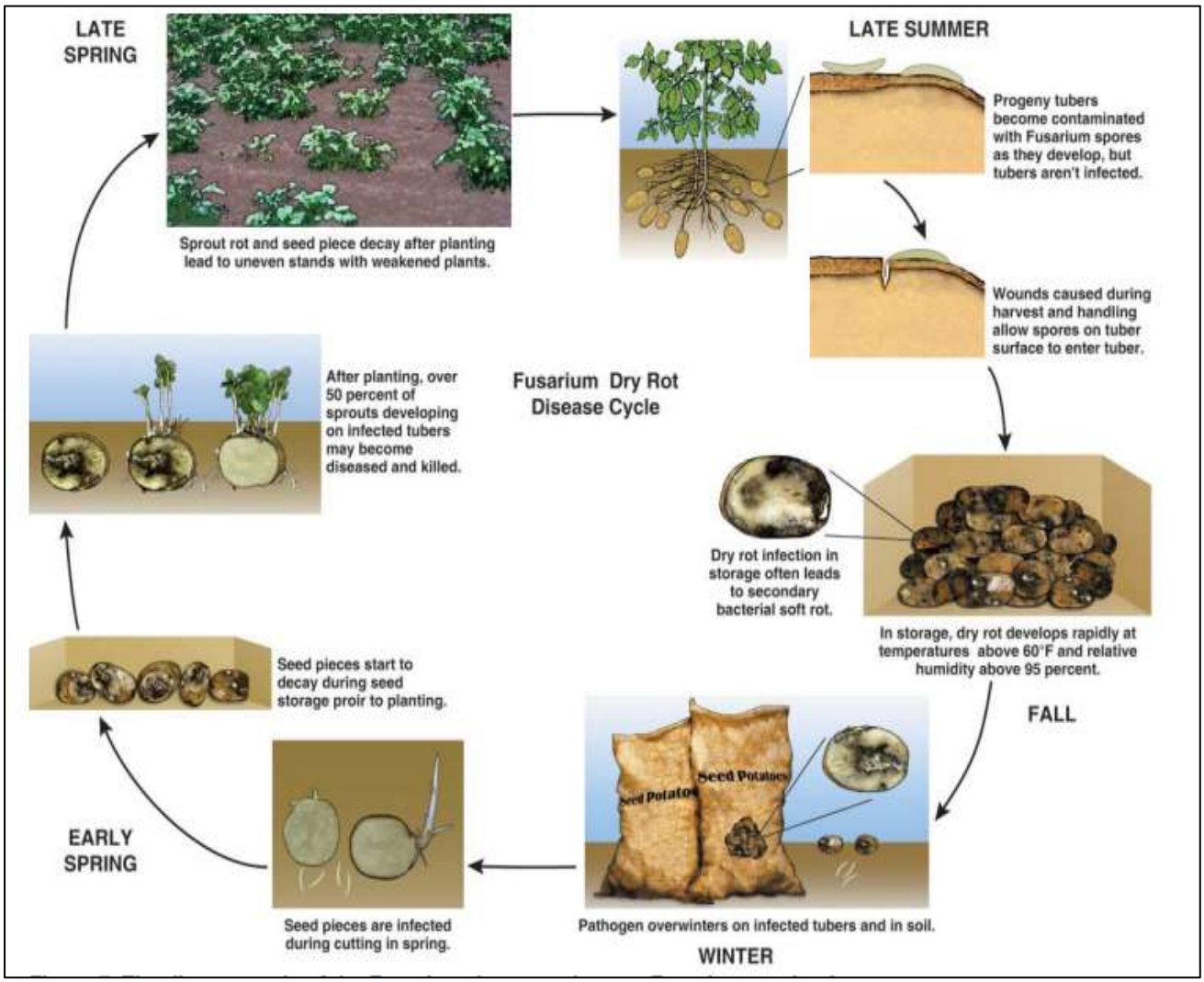

Figure 2.3: The disease cycle of *Fusarium* dry rot of potato pathogen, *Fusarium solani* (Wharton et al., 2007a).

2.4.6 Symptoms

Infections of dry rot in potatoes generally begin at wound sites inflicted during injuries. Once infection occurs, it slowly enlarges in all directions. The skin over the infected area sinks and wrinkles, sometimes in concentric rings, due to the fungus drying out the contents of the tuber (Figure 2.4A). Internally, infected areas change from light brown to black as the fungus kills the cells of the tuber (Figure 2.4B). Internal cavities produced by dry rot infections generally contain fungal mycelium of various colours (Figure 2.4C). The infected areas usually remain dry but at high moisture levels or

humidity, bacteria invade and cause smelly wet infections. If infected areas are not removed, the tuber can completely rot and become wrinkled (Tiwari et al., 2020).

In the field, symptoms include variable seed emergence and differences in plant size. *Fusarium* species can also cause wilting which includes stunted growth, chlorosis of leaves, and abscission of lower leaves (Cullen et al., 2005).

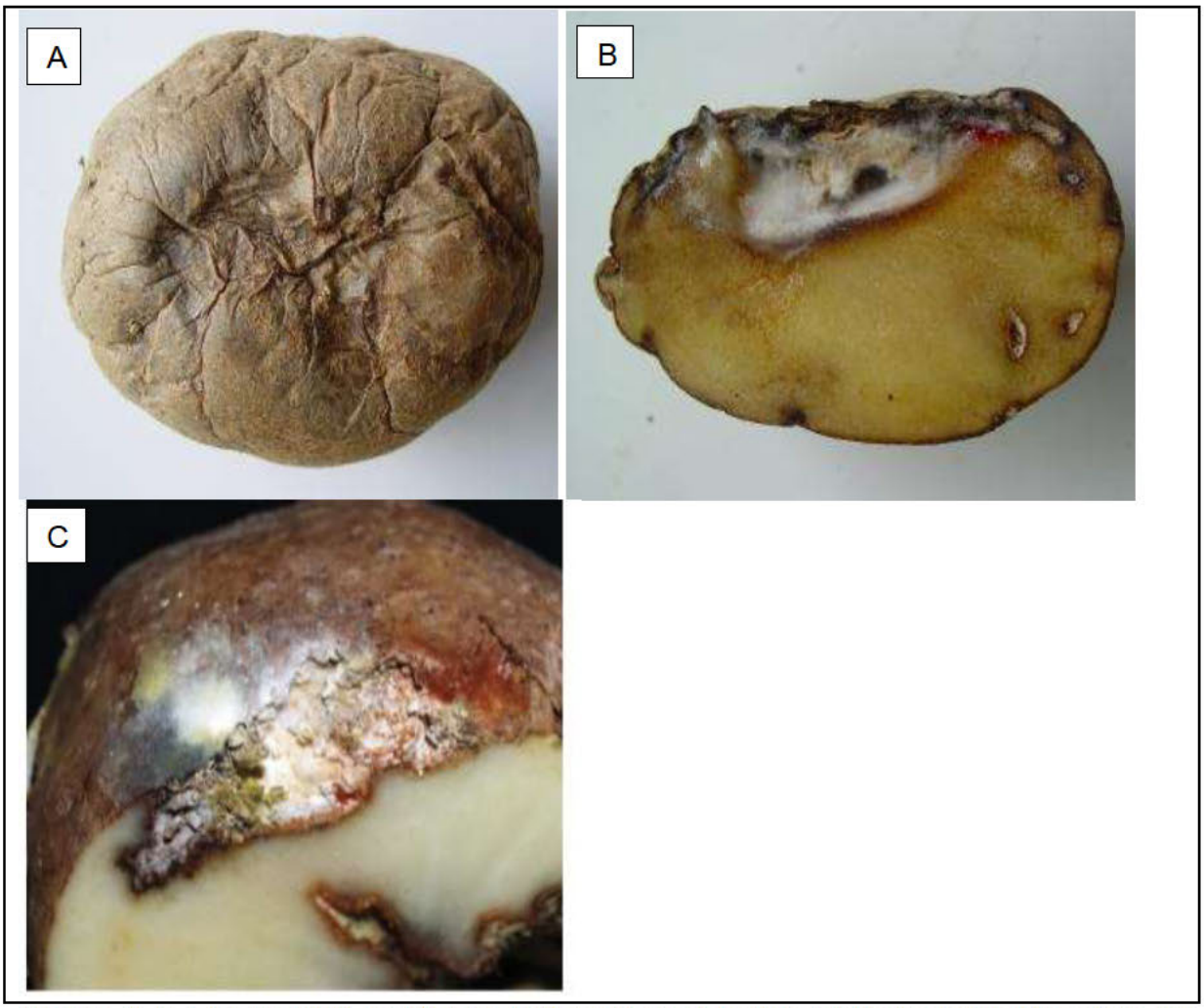

Figure 2.4: External (A) and internal view of a potato tuber infected with *Fusarium* dry rot, clumps of white to yellow mycelia line a dry necrotic cavity hollowed out from rotted tissue (B) and clumps of mycelium a white to pink to yellow sporulating masses form on the surface of dead skin (C) (Cullen et al., 2005).

2.5 Current control strategies

2.5.1 Cultural control

Cultural control is the practice of adjusting the crop environment to make it unfavourable for the development of pathogens, whilst maintaining the best conditions for high productivity (Viaene et al., 2006). *Fusarium solani* only infects tubers through wounds, therefore, a major effort should be placed on using practices that minimize tuber bruising and wounding (Xue et al., 2023). Currently, the primary methods used to control potential storage diseases include the elimination of infected tubers before storage, ventilation, and temperature and humidity manipulation (Xue et al., 2023). It is recommended to harvest potatoes when conditions allow the tubers to remain at temperatures between 10 and 18 °C.

Tubers should also be harvested 1-2 weeks after the aboveground potato plants have died to ensure maturation of tuber skin (Knowles and Plissey 2008). Potato tubers should be exposed to conditions favouring wound healing after they are harvested, this includes storage with high humidity (95–99%), a tuber pulp temperature between 13°C and 16°C, and adequate ventilation to prevent condensation on the tubers (Bojanowski et al. 2013). After the wound-healing period (7–10 days), temperature and relative humidity should be decreased to 2–5 °C (10 °C for processing tubers) and 90–95% RH. The planting of seeds that have a *Fusarium* problem in warm, well-drained soil to encourage rapid sprout growth and emergence and lessen the chance for infection can also reduce *Fusarium* dry rot (Fiers et al., 2012).

Seed storage facilities should be cleaned and disinfected before receiving seed to prevent the occurrence of *Fusarium* dry rot (Knowles and Plissey, 2008). Seed-cutting and handling equipment need to be disinfected using chlorine-based chemical disinfectants such as dilute household bleach (0.6% sodium hypochlorite) and freshly generated hypochlorous acid (800 ppm chlorine) before being used (Gilbert et al., 2023). The amount of initial inoculum of *Alternaria* species causing early blight of potato has been reduced through rotation with non-host crops such as small grains, corn or soybean (Sikora, 2004; Agrios, 2005; Schultz and French, 2009).

2.5.2 Chemical control

Chemical control refers to managing plant diseases using synthetic or naturally occurring fungicides. Several fungicides, including phosphorous acid, thiabendazole,

mancozeb, sodium hypochlorite, and calcium hypochlorite, have been registered for postharvest control of *Fusarium* dry rot (Wharton et al., 2007b). These fungicides also work against various potato postharvest diseases, including pink rot, late blight, silver scurf, early blight, and black scurf. Although phosphorous acid is registered for postharvest use, a few studies have assessed its potential during preharvest (Johnson et al., 2004). Studies using phosphorous acid as postharvest fungicides have shown potential efficacy against late blight of tubers and pinrot (Miller et al., 2006; Johnson, 2008).

Phosphorous acid applied on potato tubers after harvest and before storage significantly decreased disease development caused by *Phytophthora infestans* and *Phytophthora erythroseptica* (Miller et al., 2006) and continues to show great potential in controlling *P. infestans*, especially when the labelled rates are correctly used (Johnson, 2008). Phosphorous acid comprises phosphites that have direct antifungal activity against mycelial growth (Duff et al., 1994) and stimulate active plant defense responses (Trejo-Téllez and Gómez-Merino,2018).

Dry rot has been managed primarily by applying thiabendazole (TBZ), a benzimidazole fungicide, and mancozeb as tubers enter storage or before planting. However, Bojanowski et al. (2013) reported that *F. solani* species linked to potato dry rot had developed resistance to TBZ which led to the reduction of TBZ effectiveness. Thus, it has become more crucial to develop alternative strategies for controlling dry rot in potatoes.

2.5.3 Biological control

Biological control is the use of pests or other microorganisms to eliminate plant pathogens (Heimpel and Mills, 2017). Biological control does not only reduce the use of harmful fungicides, but it also helps in preventing environmental risks (Nega, 2014). Hence, researchers are particularly interested in evaluating biological control agents that can be used to control *F. solani.* Several biological control agents have been evaluated for their efficacy in controlling potato diseases. For instance, Ommati and Zaker (2012) evaluated *Trichoderma* isolates for biological control of potato wilt disease *in vitro* and greenhouse. The results indicated that *T. brevicompactum* and *T. asperellum* (Pers, 1801) showed the best performance in inhibiting the growth of *F. solani* in direct contact and coiled around the mycelia of *F. solani*. *T. longibrachiatum*

(Rifai,1969) exhibited the highest inhibitory effect against *F. solani* due to its production of volatile and non-volatile inhibitors (Ommati and Zaker, 2012). Furthermore, *T. longibrachiatum* significantly reduced the disease incidence in greenhouse trials.

The Bacillus strain, BACO3 was reported to show inhibitory effect on *Streptomyces spp in vitro*, and significantly reduce the severity of potato common scab. This strain displayed a strong antagonism against *Streptomyces* spp. It was further reported that the antagonism of BACO3 was associated with a proteinaceous fraction showing high similarity to an LCI protein which is an important factor for antimicrobial activities (Meng et al., 2012).

2.5.4 UV-C irradiation

Ultraviolet-C (UV-C) irradiation is a cheap, safe, and appropriate method for postharvest storage which has been widely used in controlling pathogens in various fruits and vegetables during storage (Allende and Artes, 2003). UV-C irradiation is used as a postharvest treatment to increase fruit quality and prolong the shelf life of fresh fruits and vegetables. A study by Ranganna et al (1997) evaluated the potential of UV-C irradiation in controlling *F. solani* and *E. carotovora* (Winslow et al.,1920) of potato in storage. The results revealed that UV-C irradiation completely suppressed the subsequent development of these diseases during 3 months in storage at 8ºC. Terao et al (2015), indicated that UV-C treatment at 20 kJm^{-1} reduced the development of *Colletotrichum gloeosporioides* and *Botryosphaeria dothidea* in 'Tommy Atkins' mango after 20 days of storage.

Romero et al. (2017) also reported that UV-C treatment (2.064 kJ/m^2 for 5 minutes) decreased *Escherichia coli* (Castellani Chalmers,1919) and *Listeri*a innocua (ex Seeliger and Schoofs, 1979) growth in 'Tommy Atkins' sliced mango stored at 4°C for fifteen days. UV-C irradiation activates defense-related enzymes including β-1,3-glucanase (GLU), peroxide (POD), ammonia-lyase (PAL), and chitinase (CHI) (Buraphaka et al., 2024). GLU and CHI, help with the degradation of pathogen cell walls, while PAL inhibits pathogen growth and development.

Tesfay and Magwaza (2017), indicated that moringa leaf extracts enhanced the quality and prolonged postharvest life of avocado (*Persea americana* Mill.) fruit. Although a

significant amount of research has been conducted on UV-C, there is a need for more investigation on its effect on potato tubers. Knowing the effect of UV-C on various pathogens, it is necessary to assess its impact on dry rot and other related potato diseases. Unlike most treatments which are quite expensive, UV-C could be a cheap alternative for the potato industry.

2.5.6 Plant extracts

Plant extract refers to a kind of substance with one or more biological functions derived from plants, which prevent plant pathogens and other functions (Gurjar et al., 2012). Plant extracts are likely to be safer for the environment and humans, and fungi are less likely to develop resistance towards these natural pesticides (Zaker, 2016). Natural plant extracts have been found effective against a wide range of plant pathogens. For example, ethanol and water extracts of Piper nigrum, *Ocimum sanctum* and *Citrus lemon* were effective against *Colletotricum lindemuthianum* (Saccardo and Magnus, 1878) *in vitro* and in the field (Amadioha, 2003).

Aloe vera ethanolic extracts were proved to exhibit antibacterial and antifungal activity against different bacterial and fungal strains (Danish et al., 2020). Njau et al (2014) evaluated the antibacterial activity of *Tetradenia riparia* crude extracts against *E. coli*, and *Enterococcuss faecalis* (Andrewes and Horder, 1906). The result suggested that ethanol, methanol, hexane and aqueous extracts of *T. riparia* were active *in vitro* against the tested pathogens. *Moringa oleifera* has antifungal, antiviral, antibacterial, and antioxidant properties effective against various phytopathogens (Belay and Sisay, 2014; Emad El-Din et al., 2016). Thus, moringa extracts have the potential to control fungal diseases, while improving the shelf life of crops due to their high antioxidant concentration. Several studies have reported moringa extracts to have inhibitory effects against *F. solani.*

For example, a study by Goss et al., (2017) evaluated the effect of moringa leaf and seed extracts on the growth of *F. solani* and *Rhizoctonia solani*. The results indicated that both seed and leaf extracts exhibited antifungal activity against both pathogens at all extract concentration levels. However, seed extract was the most effective in its inhibitory properties compared to the seed extract. It was assumed that the seed contained more antifungal bioactive compounds which made it more effective. *M.*

oleifera extracts have also been reported to inhibit the mycelial growth of *F. solani,* f.sp*. radicicola* and *F. oxysporum* f.sp. *lycopersici* (Dwivedi and Enespa, 2012).

Moringa leaf and seed extracts have also been reported to significantly affect the growth of *C. gloeosporioides* and *A. alternata* in avocado fruits (Tesfay et al., 2017b). Treated avocado fruits had significantly lower mass loss, ethylene, and respiration rates compared to the untreated control (Tesfay et al., 2017). Moringa plant extracts have also been reported to show a greater inhibition of *Aspergillus niger v. tieghema* associated with post-harvest rot of Onion bulbs (Joshi et al., 2022).

Although many reports are available on the efficacy of *M. oleifera* extracts as bio-fungicide against plants' pathogenic fungi, further investigation is still required to understand the *in vitro* and *in vivo* effect of *M. oleifera* extracts on *F. solani*.

The combined use of various treatments to control postharvest diseases has received attention in recent years. Treatment combination is mainly used to increase the effectiveness and decrease the negative effect of application by exposure to lower doses compared to a single application. UV-C radiation at 2 kJ/m^2 and hot water treatment (HWT) were tested against dry rot of 'Galia' melon caused by *F. pallidoroseum* (Cooke. Sacc, 188) (Terao et al., 2021). The *in vitro* tests revealed that the dose of 1.0 kJ/m^2 of UV-C and the heat treatment at 55 °C for 15 s provided a successful inhibition of the spore germination of *F. pallidoroseum.*

Moringa leaf extract-CMC coating has been combined with gaseous ozone in the postharvest treatment of mango fruit (Bambalele et al., 2021). The results obtained revealed that the combination of these treatments decreased the accumulation of sugars, delaying ripening in mango fruit, and therefore increasing the resistance of mango fruit to postharvest diseases (Bambalele et al.,2021). Combination of moringa leaf extracts with *Trichoderma* as an integrated control biological control agent has also been reported to successfully control *Sclerotium* damping-off and stem rot disease of cowpea in the field condition (Adandonon *et al*., 2006).

UV-C irradiation combined with sodium carbonate (SC) treatments on postharvest *Penicillium* decay in 'Mandarin' oranges stored at 20°C was investigated (Palou et al., 2007). SC treatment with 875 Gy, significantly reduced the disease severity, disease incidence, and sporulation of *P. digitatum* and *P. italicum* (Palou et al., 2007).

D'hallewin et al. (2005) evaluated the effect of the combination of UV-C irradiation and biocontrol treatments against decay caused by *P. digitatum* in orange fruit. The combination of the yeast *Candida oleophila* strain “13L” with UV-C irradiation exhibited a synergistic effect in reducing *P. digitatum* mould and only 11% of the artificially inoculated wounds were infected.

2.6 Conclusion

Potatoes are enormously influenced by numerous soilborne diseases including *F. solani*, a causal agent of *Fusarium* dry rot. This pathogen causes severe damage to potato tubers mainly during storage. *Fusarium solani* is currently controlled by implementing cultural practices such as the planting of certified seeds, cleaning of storage facilities, and disinfection of seed cutting and handling equipment. It is also controlled by the application of fungicides and biological control agents. However, some *Fusarium* species have developed resistance towards certain fungicides such as Thiabendazole™. The use of fungicides may also have detrimental effects on the environment and introduce fungicide residues on treated tubers. These risks associated with the use of fungicides necessitate the research of alternate control methods. Moringa leaf extracts and UVC irradiation treatments have been reported as safe and naturally friendly methods that can possibly be used to control dry rot in potatoes caused by postharvest *Fusarium solani*.

Although a single treatment of either UV-C irradiation or moringa leaf extracts may control postharvest disease, a combination of UV-C irradiation and moringa leaf extracts may be needed for improved control. However, no research is currently available to support this hypothesis. Thus, it is recommended that the effect of novel and environmental-friendly postharvest technologies such as UV-C, leaf extracts and biocontrol agents should be assessed against potato diseases, particularly dry rot. The mode of action of these treatments should also be investigated to fully understand their impact on the biochemistry and microbiology of the treated produce.

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Chapter 3

In vitro and *in vivo* effect of UV-C irradiation against *Fusarium solani* of potato

Abstract

Potatoes are semi-perishable and are highly susceptible to microbial attacks, particularly during postharvest handling and storage. *Fusarium solani* is one of the most significant postharvest pathogens affecting the quality and storability of potatoes. This experimental study evaluated the *in vitro* and *in vivo* effect of UV-C irradiation against *Fusarium solani* of potatoes. *In vitro* antifungal effects of UV-C irradiation were evaluated by exposing *F. solani*-inoculated potato dextrose agar (PDA) plates to UV-C light (254 nm) for 5 minutes, 10 minutes and 15 minutes at 10 cm, 15 cm and 20 cm. The plates were incubated at 25ºC for seven days. UV-C treatment for 10 and 15 minutes at a 10 cm distance successfully inhibited the *F. solani* mycelial growth by ≥ 50% at 7-day post-inoculation. The other treatments did not inhibit the pathogen *in vitro*. The control plates had the pathogen fully grown on the Petri plate *in vitro*. UV-C treatments (10 and 15 minutes at 10 cm) were selected for their best performance and were further screened against *F. solani in vivo*. Subsequently, 'Sifra' potatoes were treated with UV-C for 10 minutes and 15 minutes, and the results showed a ≤ 33% disease incidence compared to 96% for the untreated tubers. The scanning electron microscopy images showed the breakage and shrinkage of the mycelia *in vitro*, and the spores of *F. solani* were damaged on potatoes treated with UV-C irradiation. This study showed that UV-C irradiation has antifungal properties that inhibit the growth of *F. solani in vitro* and dry rot disease incidence in potatoes. Therefore, UV-C irradiation can be exploited as an alternative non-chemical and environmental-friendly control strategy for postharvest *F. solani* of potatoes.

Keywords: Tubers, Diseases, postharvest treatments, postharvest quality, shelf-life

3.1 Introduction

Potato is globally one of the most commonly consumed starchy vegetables. It is a source of carbohydrates, dietary fibre, proteins, vitamins and minerals such as iron and potassium. Although potatoes are quite popular, there are factors limiting potato production and quality. These factors include poor agronomic practices, poor handling during harvesting and storage, as well as inadequate marketing and transportation

systems (Stevenson et al., 2001). Diseases are also a major factor affecting quality and shelf-life of potato tubers. Bacterial, viral, and fungal pathogens have devastating effect on potato production pre- and postharvest (Ranjan et al., 2021).

Fusarium solani, the causal agent of dry rot, is one of the most destructive fungal pathogens of potato during storage and can severely reduce their market value (Wharton et al., 2007). *F. solani* has been primarily controlled by applying postharvest chemicals such as phosphorous acid, thiabendazole, mancozeb, sodium hypochlorite, calcium hypochlorite, and chlorine dioxide. These fungicides are effective against various postharvest diseases of potatoes including pink rot, late blight, silver scurf, early blight, and black scurf (Miller et al., 2006; Johnson, 2008). However, the continuous application of these fungicides results in *Fusarium* spp. developing resistant strains, which reduces their effectiveness.

Fungicides have also been reported to have detrimental effects on human health and environment due to their residues (Aktar et al 2009; de Chaves et al., 2022). Therefore, alternative non-chemical methods to control *F. solani* should be investigated. Ultraviolet-C irradiation (UV-C,180±280 nm) is a promising alternative method for controlling postharvest pathogens (Civello et al, 2006; Terao et al., 2015; Mditshwa et al., 2017). UV-C irradiation is also used as a postharvest treatment to increase fruit quality and prolong the shelf-life of fresh fruits and vegetables (Bambalele et al., 2021).

UV-C irradiation has been reported to provide a successful control against a range of potato diseases including dry rot and soft rot (Ranganna et al., 1997; Jakubowski, 2019). For instance, Stevens et al. (1999) demonstrated that UV-C treatment (λ = 254 nm of 3.6 kJ/m^2) enhanced the resistance of sweet potato roots to *F. solani*. UV-C treatment has also been reported to effectively reduce decay caused by most common postharvest pathogens, including *Penicillium digitatum* (Saccardo,1881) and *Penicillium italicum* (Wehmer, 1894), *Botrytis cinerea* (Von Haller, 1771) (Baka et al., 1999 and Marquenie et al., 2002), *Rhizopus stolonifera* (Ehrenberg,1818), *Alternaria citri* (Ellis and Pierce, 1902) and *Alternaria alternata* (Fr.Keissler, 1912). Furthermore, treating tomatoes with UV-C (1.3 – 40 kJ/m^2) at 10 cm has been reported to inhibit black and grey mould, delay ripening and extend shelf-life (Liu et al., 1993). Inducible resistance of UV-C irradiation against pathogens is related to the biosynthesis of substances (especially phenols) toxic to the pathogens, induced by an increase in

the activity of biosynthetic enzymes, including phenylalanine ammonia-lyase (Vanhaelewyn et al., 2020). Although considerable amount of research has been conducted on the UV-C on postharvest pathogens, there is limited literature on the effect of UV-C on *F. solani* of potato tubers. This is a crucial area of research as the food industry is shifting towards non-chemical postharvest technologies for extending the shelf-life of fresh produce. Thus, this study evaluated the efficacy of UV-C irradiation in inhibiting the mycelial growth of *F. solani in vitro* and its effect on the disease incidence of *F. solani* on 'Sifra' potatoes.

3.2 Materials and methods

3.2.1. Source of pathogen (*Fusarium solani*)

A pure culture of *F. solani* (Accession number: PPRI 10342) used in this study was purchased from the Agricultural Research Council- Plant Protection Research Institute (ARC-PPRI), Pretoria, Roodeplaat, South Africa. The fungal culture was grown on PDA and stored in 30 % glycerol at - 80 ºC for use throughout the study.

3.2.2 Pathogenicity test of *F. solani* on potato cultivar

Potatoes (cv. Sifra) were purchased from Pick n' Pay, disinfected with 70% ethanol, and rinsed with distilled water. Thereafter, they were placed on a paper towel to dry out at room temperature. Wounded potatoes were inoculated with an inoculum of *F. solani* with a conidial suspension of the concentration 1×10^3, 1×10^4, and 1×10^5 conidia/ml. The control potatoes were only inoculated with distilled water. The fungal suspension was inoculated into three potatoes with four replicates. The inoculated potatoes were incubated for 21 days at 25 °C with a relative humidity of 95%. The disease incidence was evaluated every seven days until the last day of the incubation period. The concentration of *F. solani* conidial suspension, which exhibited the highest percentage of disease incidence in potatoes, was further used throughout the *in vivo* trials.

3.2.4 *In vitro* screening of UV-C against *F. solani*

Ultraviolet (UV) lamps (TUT, 30W, Philipps, South Africa) emitting quasi-monochromatic UV radiation at 254 nm were used. The *in vitro* screening of UV-C

against *F. solani* was conducted by excising a 3-day-old mycelial plug of *F. solani* into 3mm x 3mm using a flame-sterilized scalpel. The mycelial plug was placed at the centre of freshly prepared PDA plates. The inoculated plates were exposed to UV-C treatment for 5 minutes, 10 minutes, and 15 minutes at 10 cm , 15 cm , and 20 cm distances. For control plates, PDA plates were inoculated with the pathogen and not exposed to UV-C treatment. Three replicates per treatment were used. The plates were sealed using a parafilm and incubated at 25°C. The mycelial growth of *F. solani* was observed and measured at 3-, 5-, and 7 days post-inoculation (dpi). The mycelial growth inhibition was calculated using the following formula:

$\%\ \text{Inhibition} = \frac{\text{MGC} - \text{MGT}}{\text{MGC}} x\ 100$, where MGC is the average diameter of mycelia growth in control sample and MGT is the average diameter of mycelia growth in treated sample.

The distance which showed great performance was further used in *in vitro* secondary and *in vivo* screening.

3.2.5 *In vivo* screening of UV-C against *F. solani*

A conidial suspension was prepared using a 14-day-old *F. solani* culture. Briefly, the surface of the sporulating culture was washed with sterile distilled water using a hockey stick. The haemocytometer was used to measure spore suspension concentration and adjusted to 1×10^4 conidia/ml. A sample of fresh 'Sifra' potatoes was surface sterilized with 70% ethanol for 1 min, rinsed in distilled water for 1 min, and allowed to dry at room temperature. Wounded potatoes were exposed to UV-C treatment for 10 and 15 minutes at 10 cm. After 24 hours, the *F. solani* conidial suspension was pipetted into the treated potatoes. For the control treatment, potatoes were only inoculated with the conidial suspension. There were three replicates, with four potatoes per replicate. Inoculated potatoes were placed in boxes properly sealed with plastics and incubated at 25°C storage with 95% relative humidity for 21 days. Disease incidence was measured at 7, 14, and 21 of post-inoculation. The following formula was used to calculate the disease incidence:

$$\%\ \text{disease incidence} = \frac{\text{The number of infected wounds in a treated potatoes sample}}{\text{Total number of wounds in a treated potato}} x\ 100$$

3.2.6 Scanning electron microscopy analysis of the interaction of *F. solani* and UV-C irradiation *in vitro* and *in vivo*

Treatments that successfully inhibited *F. solani in vitro* and *in vivo* were selected for scanning electron microscopy (SEM) analysis. The mycelial plugs (3 mm x 3mm) of *F. solani* were inoculated at the center of the freshly prepared PDA plate and exposed to UV-C irradiation. For *the in vivo* trial, potatoes were sterilized with 70% ethanol, wounded, and inoculated with 1×10^4 conidia/ml suspension. The inoculated plates and potatoes were incubated at 25 °C. After 7 and 14 days, the inhibition of mycelial growth and sporulation of *F. solani in vitro* and *in vivo*, respectively, was observed under SEM (Zeiss EVO LS15, Carl Zeiss NTS Ltd., Germany) at the Microscopy and Microanalysis Unit of the University of KwaZulu-Natal, Pietermaritzburg.

Samples were excised from inoculated PDA plates and potatoes, fixed for 3 hours in 3% buffered glutaraldehyde, and double-washed with 0.05M sodium cacodylate buffer for 5 minutes. The samples were then dehydrated in 2 mL aliquots of 10%, 30%, 50%, and 70% ethanol for 10 minutes each. The samples were rinsed three times with 100% ethanol for 10 minutes each to complete the dehydration process. Thereafter, the samples were put in the Quorum K850 critical drying point dryer (CPD) basket with 100% ethanol. The ethanol was replaced with liquid carbon dioxide (CO_2) during CPD.

The liquid CO_2 was heated and pressurized to the critical point where it transformed into a gas without damaging the samples due to surface tension, leaving the samples dry and undamaged. The dried samples were mounted onto SEM stubs using black tape. The sample stubs were transferred to the Quorum Q150R ES sputter coater and coated twice with gold and palladium so they could be conductive to the electron beam. After drying, the samples were viewed under the Zeiss EVO LS15 SEM.

3.2.3 Statistical analysis

All experiments were set up in a completely randomized design. The data obtained was subjected to analysis of variance (ANOVA) using Genstat® 23rd Edition. Duncan's Multiple Range Test (DMRT) was performed to test the significance of the mean differences obtained among the treatments at the 5% level of significance.

3.3 Results

3.3.1 Pathogenicity and morphology of *F. solani*

The pathogenicity test showed that *F. solani* was pathogenic to 'Sifra' potato tubers. There was a significant difference ($p < 0.05$) between the pathogen-inoculated potatoes and the control treatment (Figure 3.1). Potatoes inoculated with 1×10^4 spores/ml exhibited the highest disease incidence (90%) of dry rot (Figure 3.2A). The control potatoes exhibited the lowest dry rot disease incidence (2.08%) (Figure 3.2B).

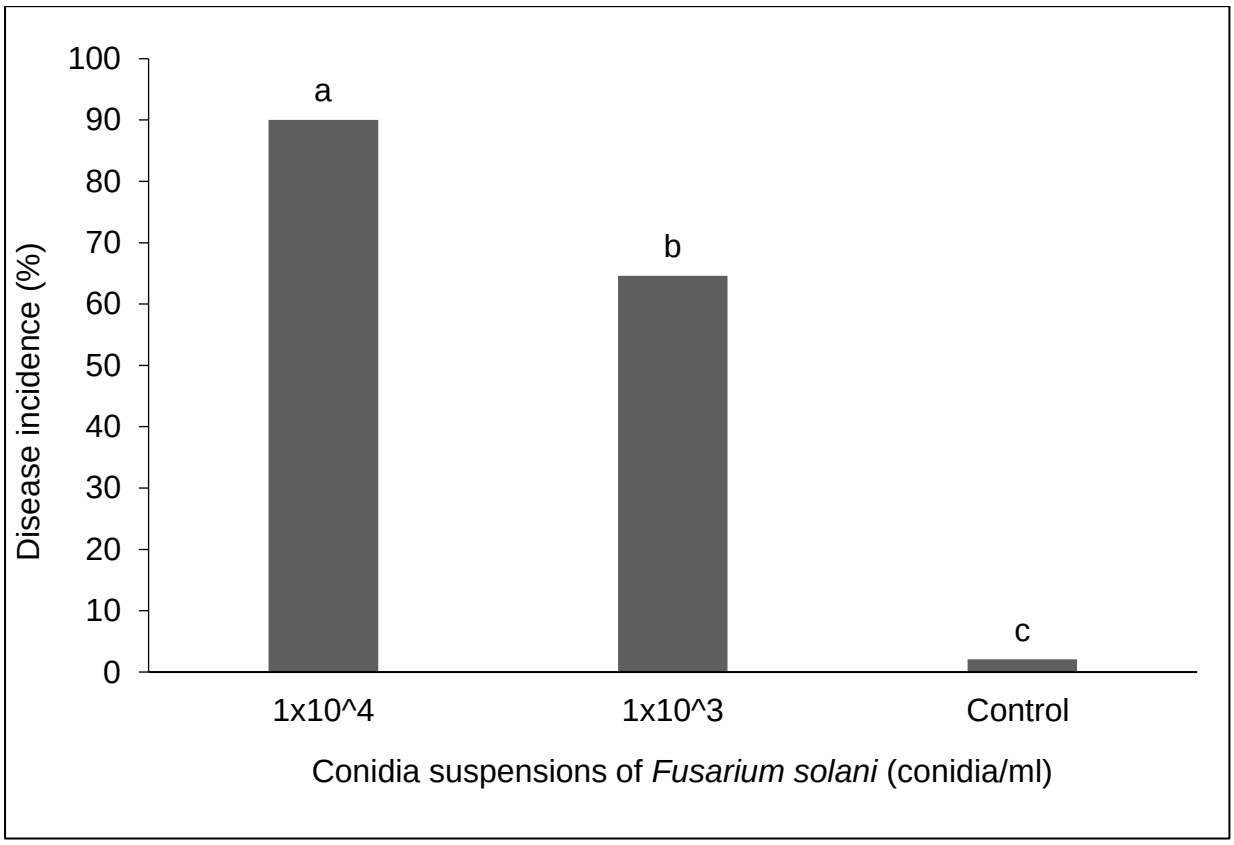

Figure 3.1: Pathogenicity test of *Fusarium solani* on 'Sifra' potatoes after 21 days of inoculation at 25°C, n = 3.

Figure 3.2: Incidence of *Fusarium* dry rot on 'Sifra' potatoes inoculated with 1×10^4 conidia/ml (A) and uninoculated potatoes (B) at 25°C, 95% relative humidity after 21 days.

3.3.2 *In vitro* effect of UV-C against *F. solani*

The 10 cm distance showed best performance compared to 15 cm. (appendix 1) and 20 cm (appendix 2) distances. The highest percentage (57.96%) inhibition of mycelial growth of *F. solani* was observed at 15 minutes exposure of UV-C treatment. On the other hand, 10 and 15 minutes of exposure to UV-C treatment inhibited *F. solani* mycelial growth by more than 50% at 7 days post-inoculation (Table 3.1). UV-C treatment for 5 minutes was the least effective in inhibition mycelia of the pathogen. The control plates had the pathogen fully grown on the Petri dish (Figure 3.4C).

Table 3.1 *In vitro* effect of UV-C irradiation on *Fusarium solani* at 10 cm distance. According to Duncan's multiple range test, means with the same letters are not significantly different ($p < 0.05$).

Treatments	Mycelia growth 7 dpi	Percentage (%)inhibition 7 dpi
10 minutes	39.67 a	51.43
15 minutes	34.33 a	57.96
5 minutes	63.00 b	22.86
Control	81.67 c	
P- value	<0.001	
LSD	5.803	
CV	5.6	
SED	2.517	

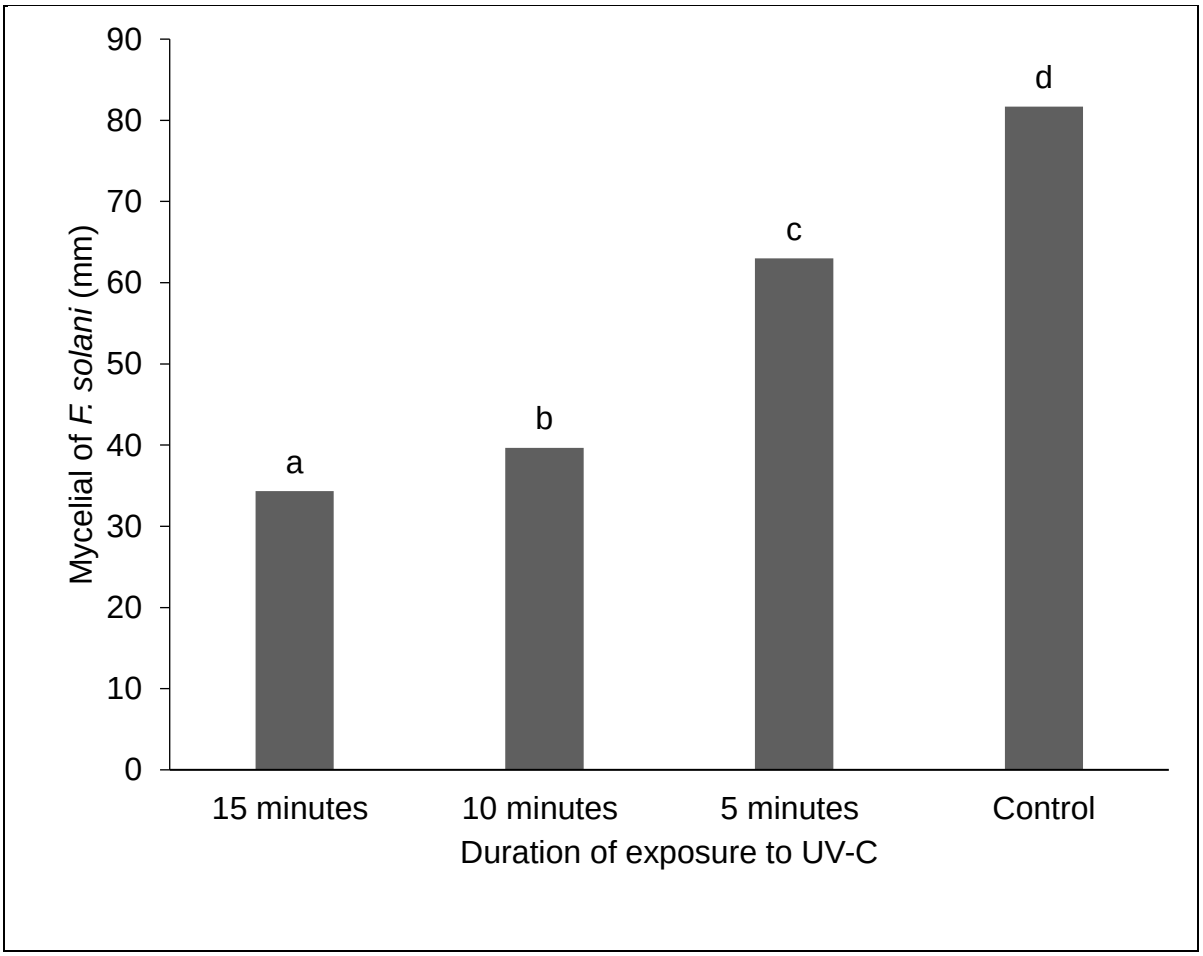

Figure 3.3: Effect of UV-C irradiation on mycelial growth of *F. solani* on potato dextrose agar after 7 days post inoculation at 25°C, n = 3.

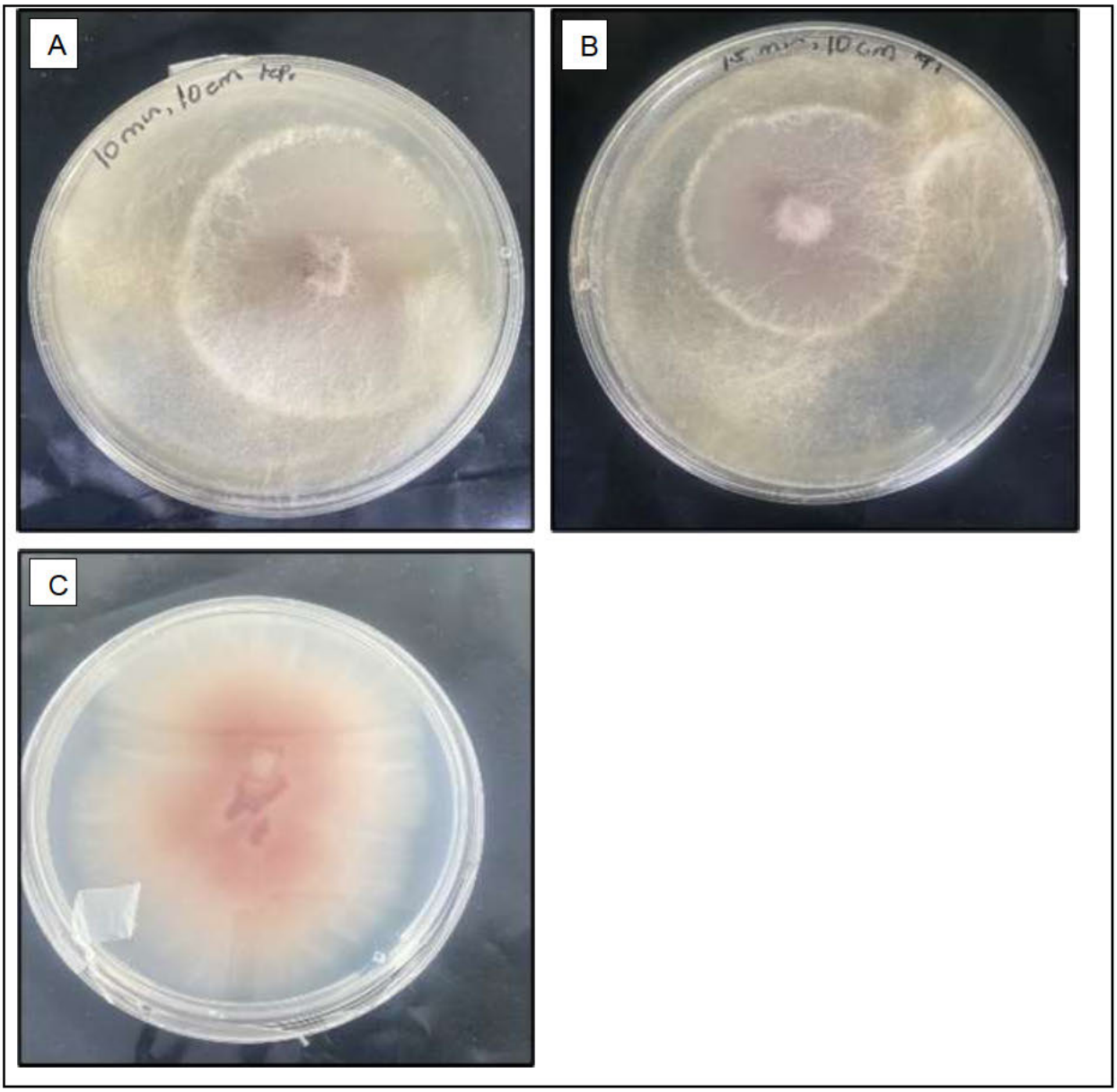

Figure 3.4: Best performing UV-C treatment of 10 minutes (A), 15 minutes (B) against *Fusarium solani* and control (C) on potato dextrose agar media after 7 days at 25°C *in vitro* secondary screening.

3.3.4 *In vivo* effect of UV-C treatment against *F. solani* on potatoes

Potatoes were observed and evaluated for disease incidence at day 21 post-inoculation at 25°C (Figure 3.5). Potatoes exposed to UV-C treatment for 15 and 10 minutes had low disease incidence of 22% and 33%, respectively, compared to pathogen control treatment which had the 96% disease incidence. Notably, secondary infection by soft rot was observed in pathogen control treatment (Figure 3.5 A) at day 21 post-inoculation.

Figure 3.5: Disease incidence of *Fusarium solani* on untreated (A), 10 minutes (B) and 15 minutes (C) UV-C treated 'Sifra' potatoes at 21 days of post-inoculation at 25°C, 95% RH.

3.3.5 Scanning electron microscopy analysis of the interaction between *F. solani* and UV-C treatment

The interaction among UV-C, mycelia and conidia of *F. solani* was observed under SEM after 7 days at 25°C. Changes were observed in the mycelial structures of the pathogen treated with UV-C (Figure 3.6 A and B) compared to the control (3.6C). Notably, UV-C treatment caused significant shrinkage, distortion and breaking of the mycelia of *F. solani* (Figure 3.6 A and B).

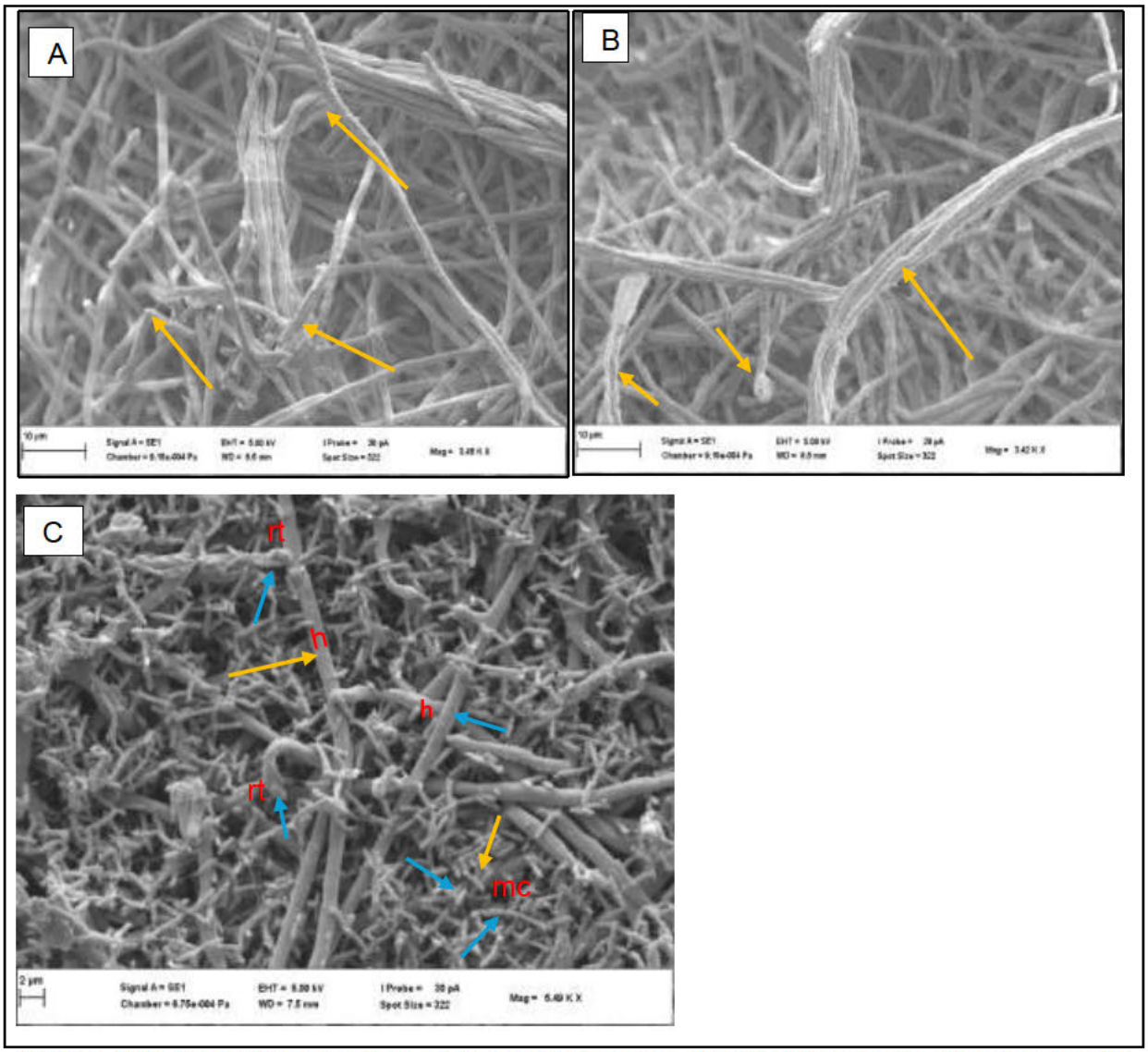

Figure 3.6: Scanning electron microscopy images showing the mode of action by 10 minutes (A) and 15 minutes (B) UV-C treatment and mycelia of *Fusarium solani* (C) grown on potato dextrose agar after 7 days at 25°C. Yellow arrows show the breakage or distortion of mycelia (A and B) as well as hyphae (h) with round tips (rt) and macronidia (mc) of *F. solani* on PDA plate (C).

The interaction between UV-C and *F. solani* was observed in potato surface under SEM. The deposition of extracellular matrix (EM) was observed on the surface of UV-treated potatoes (Figure 3.7 A and B). Micronidia of *F. solani* was observed in untreated potato (Figure 3.7 C).

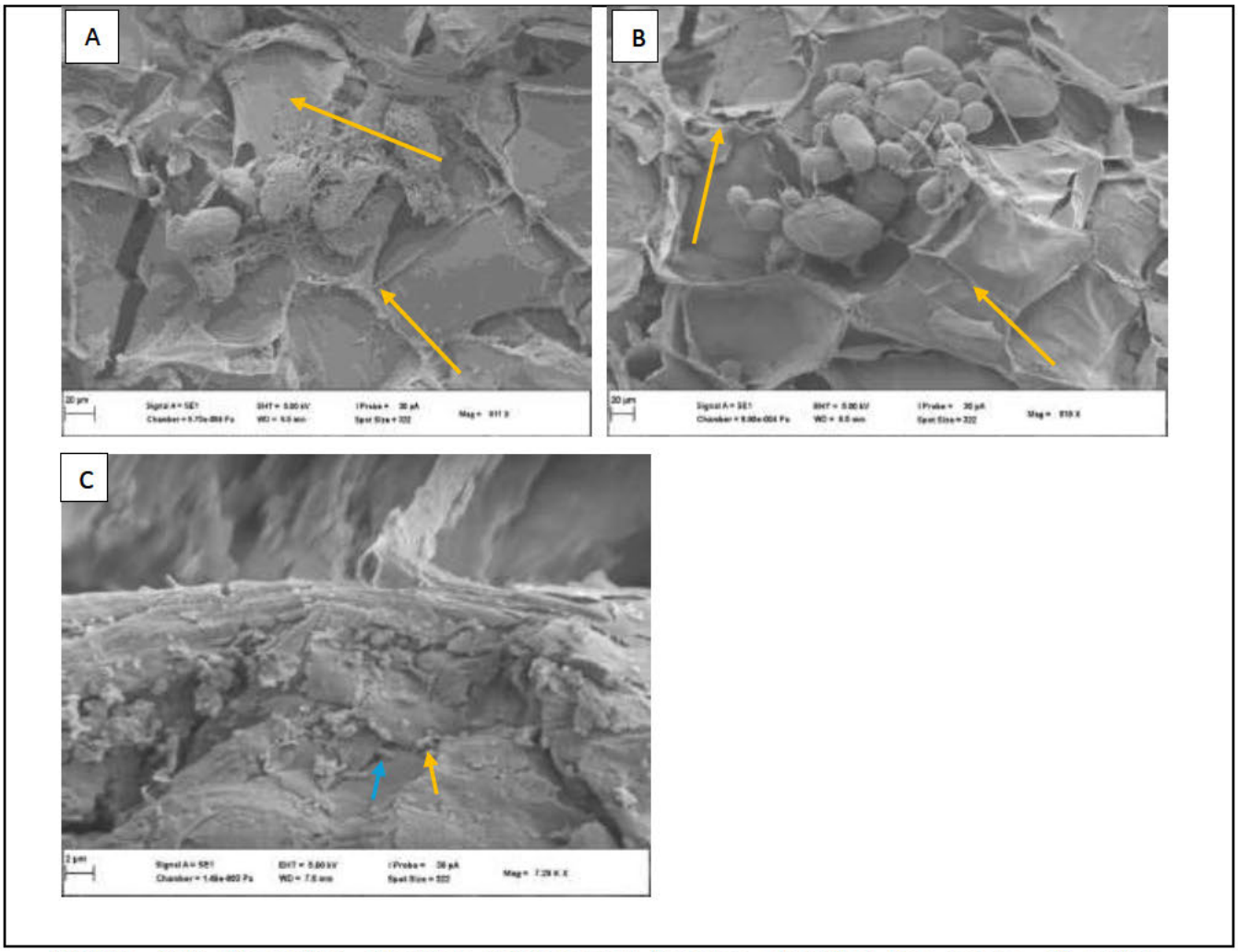

Figure 3.7: Scanning electron microscopy images showing the mode of action between *Fusarium solani* and UV-C irradiation at 10 minutes (A) and 15 minutes (B) as well as the conidia (C) on potato surface after 14 days. Yellow arrows show deposition of extracellular matrix on potato surfaces (A and B) and macronidia of *F. solani* on potato surfaces (C).

3.4 Discussion

Ultraviolet-C (UV-C) irradiation is a cheap, safe, and convenient method for controlling postharvest pathogens during storage (Allende and Artes, 2003). The potential of UV-C irradiation (180-280 nm with maximum at λ = 254 nm) has been studied as an alternative to chemical fungicides to prevent postharvest diseases for horticultural crops (Civello et al, 2006; Khubone and Mditshwa, 2017; Gumede et al., 2018). The current study revealed that UV-C irradiation exhibited significant inhibitory effects against *F. solani* both *in vitro* and *in vivo* (Figure 3.4 (A and B) and 3.5 B and C).

In vivo studies indicated that UV-C treatment of potatoes for 10 minutes and 15 minutes suppressed the incidence of dry rot. The secondary infection by soft rot bacteria was also observed in untreated potatoes. The disease incidence incited by *F. solani* was reduced as the exposure time of potatoes to UV-C treatment was increased. These results are consistent with those obtained from the study of George *et al.* (2015), which reported that UV-C treatment of wavelength 250 nm for 15 minutes minimized fruit decay, preserved sensory attributes, delayed ripening, and prolonged shelf-life of 'Chokanan' mangoes stored at 4°C for fifteen days. Similarly, González-Aguilar *et al*. (2001) demonstrated that UV-C irradiation (8220 mW m^{-2} for 10 minutes) suppressed decay caused by microbial growth on 'Tommy Atkins' mango fruits after fifteen days of storage at 5°C.

The capacity of UV-C to reduce disease incidence in treated potatoes could be linked to its ability to activate β-1,3-glucanase and glucanohydrolase which are pathogenesis related proteins involved in disease resistance. UV-C may have elicited reactions in the tissue that inhibits the penetration and invasion of the fruit by the pathogen (Charles et al., 2009). UV treatment can induce phytoalexin accumulation to concentration inhibitory to the pathogen. In tomatoes, UV-C treatment was reported to induce accumulation of rishitin in UV-treated tomato, therefore increasing its resistance against *B. cinerea* (Charles et al., 2008).

Even though 5 minutes of UV-C treatment could not inhibit *F. solani* in the current study, the study of Romero et al. (2017) reported a successful inhibition of *E. coli* and *L. innocua* growth in 'Tommy Atkins' mango under a similar treatment duration. The inconsistency between these results may be because fungi have a more complex cell structure than bacteria and are much more resistant to germicidal UV-C (Yin et al., 2013). Therefore, for fungal cells to be degraded, the exposure time of the pathogen to UV-C treatment should be increased.

The scanning electron micrographs revealed the morphological changes in the mycelia and conidia of *F. solani* treated with UV-C irradiation. The changes are characterized by shrinkage, breakage, and distortion of mycelia with rough surfaces in UV-C treated samples. In comparison, normal growth of hyphae, thick with smooth surfaces, was observed in the control treatment. The spore production was also increased in untreated potatoes.

The deposition of plant extracellular matrix was observed in UV-C treated potato surfaces. Extracellular matrix act as a physical barrier to prohibit invasion of crop by microbial organisms such as fungi and bacteria (Chivasa et al., 2005). The extracellular matrix of plants gets involved in pathogen-induced defence responses in various ways. For instance, it reinforces the cell wall to hinder penetration of fungal pathogens that employ both mechanical force and digestion to initiate entry. This mechanism involves deposition of lignin and callose (Chivasa et al., 2005; Meyer et al., 2009; Chivasa et al., 2009) secretion of structural proteins (Smith et al., 2015), and oxidative crosslinking of existing proline-rich and hydroxyproline-rich glycoproteins (Otte and Barz, 2000). Overall, these cell wall modifications increase resistance of cells to digestion by microbial proteins (Roberts et al., 2001; Haywood et al., 2002; Seifert et al., 2010).

The mode of action also includes secretion of proteins and biochemical compounds with antimicrobial activity into the extracellular matrix. For example, extracellular hydrogen peroxide (H_2O_2) accumulates around invading pathogenic bacterial cells in the intercellular space (Orozco-Cárdenas et al., 2001). It has been reported that it directly kills the pathogen propagules and stops the invasion (Walters et al., 2003). Furthermore, enzymes capable of degrading fungal cell walls, such as chitinases and b-1,3-glucanases (Ricci, 2012), are released into the extracellular matrix. These enzymes can enhance fungal disease resistance to transgenic plants overexpressing these proteins (Velasquez, 2002). In this study, UV-C treatment showed a potential in inhibiting *Fusarium* dry rot in potatoes.

3.5 Conclusion

In this study, UV-C irradiation has proven to have antifungal properties against postharvest *F. solani* in potatoes. The mycelial growth of *F. solani* and dry rot disease incidence was significantly reduced by UV-C treatment. Therefore, UV-C irradiation can be recommended as an alternative to synthetic fungicides in order to reduce environmental risks and increase the marketable value of potatoes. Application of UV-C may have some commercial challenges, including expensive costs making it difficult for small-scale farmers to adopt. It also requires significant energy, increasing operational costs. Even though UV-C irradiation has provided successful control against several pathogens, its application may have some commercial challenges,

including expensive costs making it difficult for small-scale farmers to adopt. It also requires significant energy, increasing operational costs (Koutchma et al.,2019). Further studies should incestigate the mode of action of UV-C irradiation against postharvest pathogens. The effect of UV-C on pathogenesis-related enzymes and proteins also warrants further investigation.

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Chapter 4

Fungicidal effect of moringa leaf extracts on postharvest *Fusarium solani* of potatoes

Abstract

Fusarium solani, the causal agent of dry rot, significantly affects potato production and causes enormous economic losses during storage. This study evaluated the efficacy of *Moringa oleifera* leaf extracts (MLE) against *F. solani* in *vitro* and *in vivo*. Different concentrations of MLE 1%, MLE 1.5%, MLE 2%, MLE 2.5%, and MLE 3% were evaluated for their efficacy against *F. solani in vitro*. The findings demonstrated that MLE 1.5%, MLE 2.5%, and MLE 3% inhibited the mycelial growth of *F. solani* by ≥ 50%. MLE 2.5% exhibited the highest percentage (100%) of mycelial inhibition. MLE 2% was the least effective in inhibiting (12.14%) the mycelia growth of *F. solani*. The treatments that showed great performance *in vitro* were selected and further screened *in vivo*. For the *in vivo* evaluations, 'Sifra' potatoes were treated with moringa and inoculated with *F. solani,* and incubated at 25°C, 95% relative humidity. The incidence of dry rot was reduced in potatoes treated with moringa leaf extracts. The scanning electron micrographs showed mycelia distortion in samples treated with moringa and the disruption of *F. solani* spores on the treated potatoes. Furthermore, it indicated the formation of biofilms in moringa-treated potatoes. Moringa leaf extracts can be used as an environmental-friendly method for maintaining the quality and extending the shelf-life of potatoes.

Keywords: Postharvest quality, Tuber, Disease, antioxidant activity, shelf life

4.1. Introduction

Potato (*Solanum tuberosum* L.) is one of the most consumed vegetable crops worldwide. It serves as a food source and income in the world's developing countries (Singh et al., 2020). Potatoes have the potential to significantly improve food security due to their high production per unit area and time compared to other crops (Tolessa, 2018). Potatoes are highly sensitive to water and heat stress, thus, the climate change linked to rising temperatures are a serious threat to the potato industry (Mthembu et al., 2022). Climate change causes an increase in carbon dioxide levels and global air temperature, therefore reducing potato yields (Pinhero et al., 2009). Even though

weather extremes may contribute to the loss of potato production, biotic factors, including viruses, bacteria, and fungi of plant diseases, remain the major limiting factor of potato yields (Ranjan et al., 2021).

Among the diseases, fungal dry rot caused by *Fusarium solani* causes a significant loss in storage. *F. solani* cannot penetrate tubers directly, therefore, the infection is mainly through wounds made during harvest and transportation (Fiers et al., 2012). *Fusarium* dry rot can reduce crop establishment by affecting the developing potato sprouts and causing crop losses of up to 25%, while more than 60% of tubers can be infected during storage (Wharton et al., 2007).

The control of *Fusarium* dry rot is essential for achieving improved crop yields and quality of farm produce (Majeed and Muhammad, 2018). *Fusarium* dry rot has been traditionally controlled by chemicals including cymoxanil (cyan acetamide oximes), carbamates (prothiocarb and propamocarb), and Thiabendazole™ (TBZ), as the tubers enter storage (Wharton et al., 2007). Despite the efficacy of some chemicals in controlling diseases, their toxicity endangers the environment (de Chaves et al., 2022). The use of chemicals has also negatively impacted human health. This is due to the prolonged residual effect in the soil (Aktar et al., 2009), from where they eventually get into the food chain, leading to a build-up in the bodies of animals and humans, causing health problems (Aktar et al., 2009).

Therefore, alternative and plant-based control methods which are environmental-friendly and not associated with human and health risks are essential for reducing the use of fungicides. Plant aqueous extracts from different plant species are widely used in organic farming to control pests, weeds, and diseases (Gurjar et al., 2012; Stangarlin et al., 2011; Zaker, 2016). *Moringa oleifera* is a medicinal plant, after intensive research over the years, it is shown to be a safer and more compatible alternative to chemical fungicides (Dwivedi and Neetu, 2012). All parts of the plant, including roots, leaves, stems, flowers, seeds, and essential oils have antifungal properties and are used for medicinal and other purposes (Dwivedi and Enespa, 2012). Chemical analysis of these morphological characteristics indicated that they possess essential minerals, vitamins, proteins, and a wide range of phytochemical compounds that have biological activity and can potentially be used to prevent microbial growth (Arora and Onsare, 2014; Satish et al., 2013).

Moringa leaf extracts have the potential to control fungal diseases while improving the shelf life of fresh produce due to their high antioxidant concentration (Ayirezang et al., 2020). Moringa leaf extracts incorporated with carboxymethyl cellulose (CMC) have been reported to successfully suppress postharvest *C. gloeosporioides* and *A. alternata* in avocado fruits and improve the shelf life of avocado during storage (Tesfay et al., 2017). Goss et al. (2017) reported that *M. oleifera* leaf and seed extracts effectively controlled the growth of *R. solani* and *F. solani in vitro*. Roots, leaves, and pod coat extracts of *Moringa oleifera* significantly reduced radial growth, spore germination, and dry mycelia yield of *F. oxysporum*, *F. solani*, *Alternaria solani*, *A. alternata*, *Rhizoctonia solani*, *Sclerotium rolfsii* (Saccardo, 1911) and *Macrophomina phaseolina* (Tassi.Goid, 1947) (El–Mohamedy and Abdalla, 2014).

Furthermore, a study by Mvumi et al. (2017) showed the germination inhibitory potential of *M. oleifera* leaf chloroform (MLCE) and aqueous extracts (MLAE) against *Alternaria solani*, the causal agent of tomato early blight. Interestingly, El-Mohamedy et al. (2016) showed that field application of moringa extracts improved quality and reduced incidence of early blight in potatoes. Although the effects of moringa extracts on postharvest microbial growth have been extensively studied, there is currently no reported literature on its effect on postharvest disease of potatoes. Therefore, this study evaluated the efficacy of moringa leaf extracts in inhibiting the mycelial growth of *F. solani in vitro* and their effect on the dry rot disease incidence caused by *F. solani* in potatoes.

4.2 Materials and methods

4.2.1 Preparation of moringa aqueous solution

Moringa leaf extract was prepared as described by Tesfay and Magwaza (2017), with a few amendments. Briefly, different amounts of moringa (10g, 15g, 20g, 25g & 30g) leaf powder were weighed, and each was mixed with 100 ml of 70% ethanol to prepare concentrations (MLE 1%, MLE 1.5%, MLE 2%, MLE 2.5% and MLE 3%), respectively. The samples were put in a rotary shaker overnight to mix thoroughly. Thereafter, they were filtered into a clean conical flask using a cheesecloth. A volume of 10 ml of each extract was transferred into Eppendorf tubes and evaporated for 24 hours in the

Genevac (Genevac® EZ 2.3; Ipswich, UK) at 40 °C. The crude extract was suspended with 5ml of distilled water and kept in cold storage for potato treatment.

4.2.2. *In vitro* evaluation of the fungicidal activity of moringa leaf extracts

In vitro effect of moringa leaf extracts against *Fusarium solani* was assessed. Briefly, PDA media was autoclaved at 121°C for 15 minutes and allowed to cool. A volume of 9 ml of PDA was supplemented with 1 ml of each MLE concentration (MLE 1%, MLE 1.5%, MLE 2%, MLE 2.5%, and MLE 3%) and transferred into petri dishes. The plates were allowed to solidify on a laminar flow overnight. Mycelial plugs (3mm x 3mm) were cut from the edges of a pure culture plate of *F. solani* using a flame-sterilized scalpel and placed at the centre of the treated- PDA plates. For the control set, PDA plates were only inoculated with a mycelial plug of *F. solani*. Three replicates per treatment were used. All the plates were incubated at 25 ℃ for seven days. The mycelial growth of *F. solani* was observed and measured at 3-, 5-, and 7 days post-inoculation (dpi). The mycelial growth inhibition was calculated using the following formula:

% Inhibition$= \frac{\mathrm{MGC}-\mathrm{MGT}}{\mathrm{MGC}} x\ 100$, where MGC is the average diameter of mycelia growth in control sample and MGT is the average diameter of mycelia growth in treated sample.

4.2.3 *In vivo* evaluation of the fungicidal activity of plant extract

'Sifra' potatoes were surface sterilized with 70% ethanol for 1 min, rinsed in distilled water for 1 min, and dried at room temperature. Wounded potatoes were inoculated with different concentrations of moringa leaf extracts and were left for 24 hours at room temperature. *F. solani* conidial suspension of concentration 1x10^4 spores/ml was inoculated into the same treated wounds. For the control treatments, some potatoes were only inoculated with the pathogen. There were three replicates, with four potatoes per replicate. The treated potatoes were incubated at 25°C for 21 days at 95% relative humidity (RH). The disease incidence was evaluated every seven days until the last day of the incubation period using the following formula:

$$\%\ \text{disease incidence} = \frac{\text{The number of infected wounds in a treated potato sample}}{\text{total number of wounds in a treated potato}}\ 100$$

4.2.4 Scanning electron microscopy analysis of the interaction between *F. solani* and moringa leaf extracts *in vitro* and *in vivo*

After evaluations, MLE 2.5% and MLE 3% were selected as best-performing treatments and examined for their action mode against *F. solani* under scanning electron microscopy (SEM). Mycelial plugs of *F. solani* (3 mm x 3 mm) were excised and inoculated at the centre PDA plates supplemented with MLE 2.5% and MLE 3%. The plates were incubated for 7 days at 25°C. For *the in vivo* trial, potatoes were sterilized with 70% ethanol, wounded, and inoculated with MLE 2.5% and MLE 3%. Thereafter, the potatoes were inoculated with $1x10^4$ conidial/ml suspension. For control treatments, PDA plates and potatoes were only inoculated with *F. solani*. The inoculated potatoes were incubated at 25 °C for 14 days. The inhibition of mycelial growth and sporulation of *F. solani in vitro* and *in vivo*, respectively, *was* observed under SEM (Zeiss EVO LS15, Carl Zeiss NTS Ltd., Germany) at the Microscopy and Microanalysis Unit, University of KwaZulu-Natal, Pietermaritzburg. Samples were excised from inoculated PDA plates and potatoes, fixed for 3 hours in 3% buffered glutaraldehyde, and double-washed with 0.05M sodium cacodylate buffer for 5 minutes.

The samples were then dehydrated in 2 mL aliquots of 10%, 30%, 50%, and 70% ethanol for 10 minutes each. The samples were rinsed three times with 100% ethanol for 10 minutes each to complete the dehydration process. Thereafter, the samples were put in the Quorum K850 critical drying point dryer (CPD) basket with 100% ethanol. The ethanol was replaced with liquid carbon dioxide (CO_2) during CPD. The liquid CO_2 was heated and pressurized to the critical point where it transformed into a gas without damaging the samples due to surface tension, leaving the samples dry and undamaged. The dried samples were mounted onto SEM stubs using double-sided black tape. The sample stubs were transferred to the Quorum Q150R ES sputter coater and coated twice with gold and palladium so they could be conductive to the electron beam. After drying, the samples were viewed under the Zeiss EVO LS15 SEM.

4.2.4 Statistical analysis

The experiment was laid out in a completely randomized design. The data of mycelial growth and disease incidence was collected and subjected to analysis of variance

(ANOVA) using GenStat® 23rd edition. Duncan's Multiple Range Test (DMRT) was performed to test the significance of the mean differences obtained among the treatments at the 5% level of significance.

4.3 Results

4.3.1 *In vitro* screening of moringa leaf extract against *F. solani*

The results showed that there were significant differences ($p < 0.05$) between the moringa leaf extracts and the control treatment at day 7 post-inoculation (Table 4.1). MLE 1.5%, MLE 2.5% and MLE 3% notably inhibited the mycelia of *F. solani* by ≥ 50%. Initially, mycelia growth was observed in MLE 2.5% treated plates, but it totally disappeared after day 7 of inoculation, exhibiting 100% inhibition (Figure 4.1A). MLE 3% was the second most effective in inhibiting the mycelia growth of *F. solani* (Figure 4.1 B). The antifungal activity of moringa leaf extracts was also observed in MLE 3% (Figure 4.1 B).

Table 4.1: *In vitro* effect of ethanolic moringa leaf extracts on inhibition of mycelial growth of *F. solani* at day 7 of post-inoculation at 25 °C. n = 3

Moringa leaf extract	**Mycelial growth (mm)**	**Inhibition (%)**
	7 dpi	**7dpi**
MLE 2.5%	0.00 a	100
MLE 3%	9.33 b	88.29
MLE 1.5%	19.67 c	75.31
MLE 1%	73.33 d	7.96
MLE 2%	70.00 d	12.14
Control	79.67 d	
P-value	<0.001	
LSD	9.28	
CV%	12	
SED	4.26	

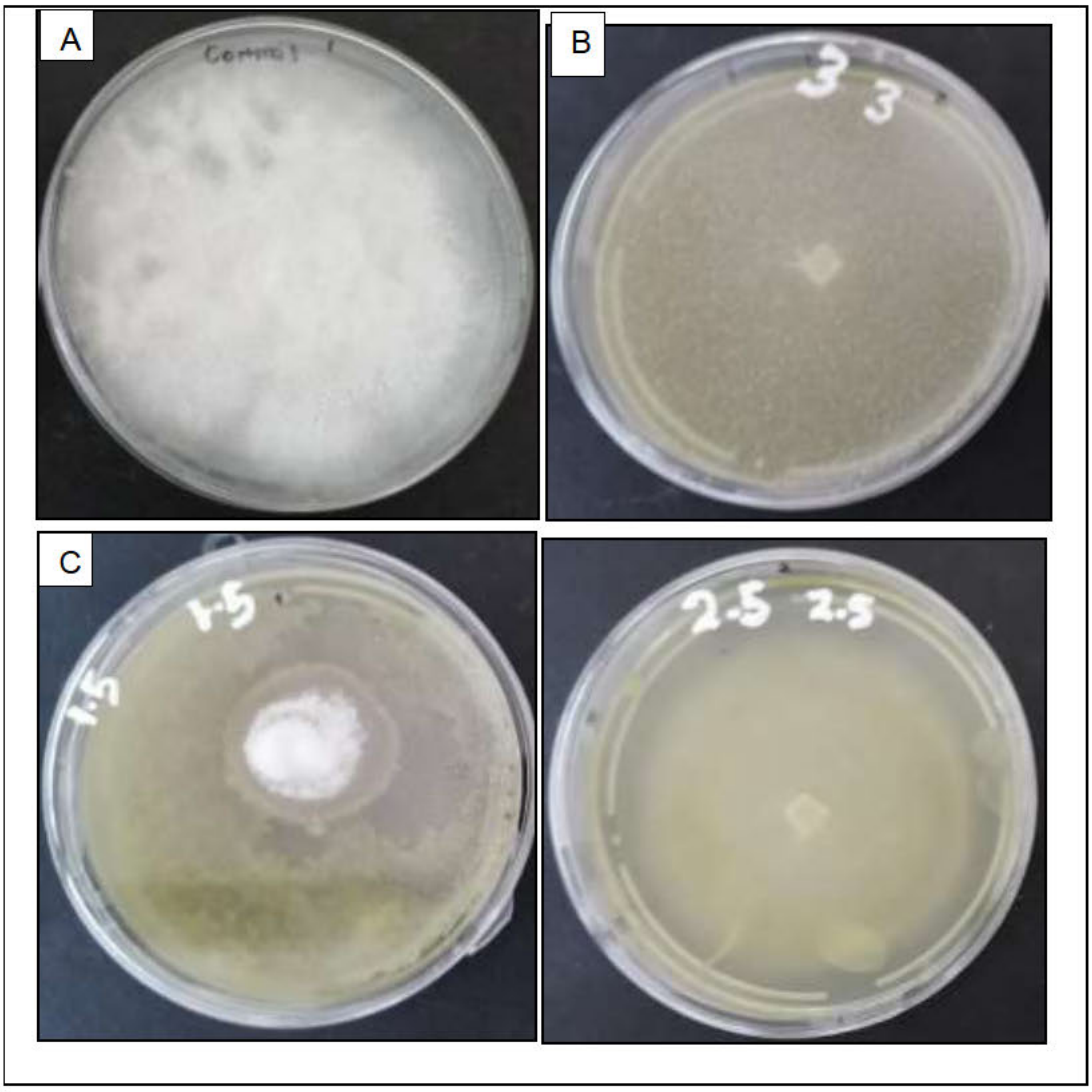

Figure 4.1: The effect of moringa leaf extracts on the mycelial growth of *Fusarium solani* on potato dextrose agar (A) after 7 days of inoculation at 25 °C; MLE 1.5% (A), MLE 2.5(B), and MLE 3 %(C).

4.3.2 Effect of moringa leaf extracts on *Fusarium* dry rot of potatoes

There was a significant difference ($p < 0.05$) between MLE 2.5%, MLE 3%, and control treatment at day 21 post-inoculation (Figure 4.2). Potatoes treated with MLE 3% exhibited the lowest disease incidence (14.58 %) compared to the other treatments. MLE 3% and MLE 2.5% were the first and second most effective treatments in reducing dry rot incidence in potatoes (Figure 4.2). The disease incidence of dry rot decreased with an increase in the concentration of moringa leaf extracts. Control potatoes exhibited a disease incidence of 96%. The secondary infections were observed in untreated potato (Figure 4.3 D).

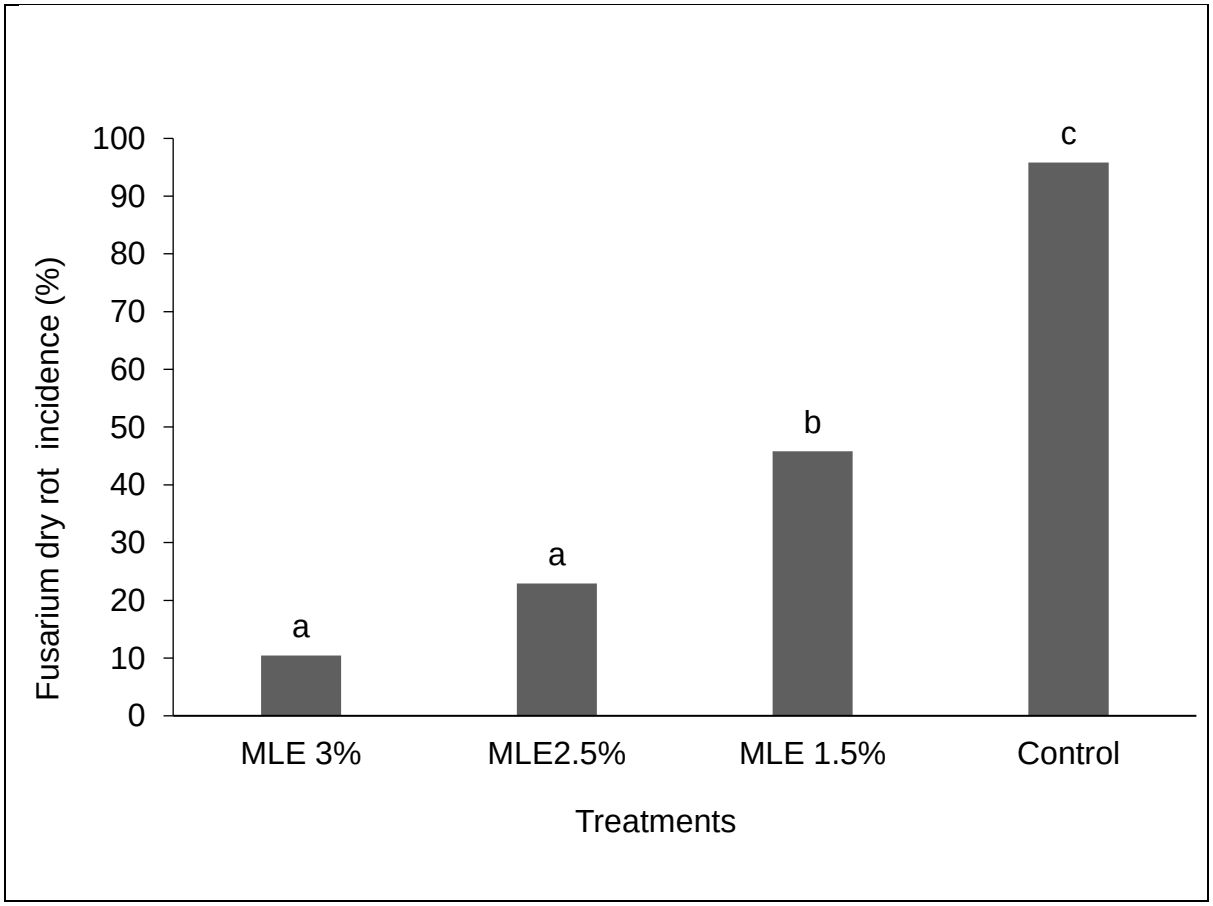

Figure 4.2: The effect of different moringa leaf extracts concentrations on the dry rot disease incidence (%) on 'Sifra' potatoes incubated at 25°C, 95% RH. According to Duncan's Least Significance Difference Test, means followed by the same letter in each bar are not significantly different at $p \leq 0.05$.

Figure 4.3: Disease incidence of *Fusarium* dry rot on untreated (A), MLE 1.5 (B), MLE 2,5 (C) and MLE 3% (D) treated 'Sifra' potatoes incubated at 25°C, 95% RH at day 21 post-inoculation.

4.3.3 Scanning electron microscopy observation of the interaction between *F. solani* and moringa leaf extract

The mode of action by moringa leaf extracts against the mycelia of *F. solani* was examined under scanning electron microscopy. The findings demonstrated that the growth of hyphae treated with MLE 2.5% and MLE 3% was remarkably inhibited, as indicated by mycelial curling, sparsity, and distortion (Figure 4.4 A and B). Control treatment had normal growth of hyphae and spore germination (Figure 4.4 C).

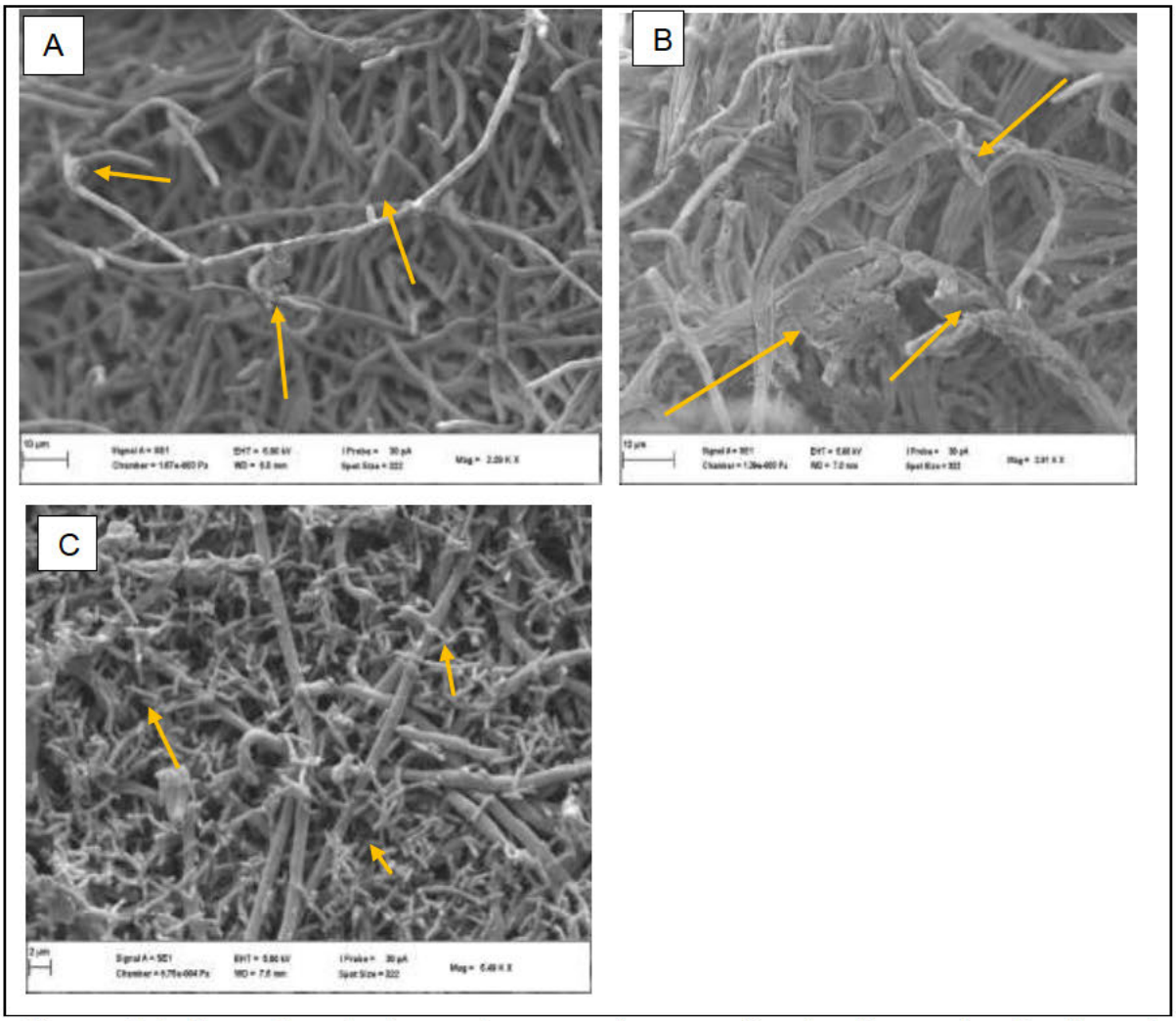

Figure 4.4: Scanning electron microscopy images showing the mode of action of moringa leaf extracts [MLE 2.5% (A), MLE 3% (B)] against mycelia of *F. solani* (C) grown on potato dextrose agar after 7 days at 25°C. Yellow arrows show the breakage or distorting of mycelia (A and B) and mycelia and spores of *F. solani* on PDA plate (C).

The interaction between moringa leaf extracts and *F. solani* was observed on the potato surface. Colonization of the inner surface of potato wounds by biofilms was observed in samples treated with moringa leaf extracts (Figure 4.5 A and B). Biofilms attached to the mycelia and caused clear shrinkage and breakage (Figure 4.5 B). On the other hand, abundant *F. solani* spores were observed on the surface of the untreated potatoes (Figure 4.5 C).

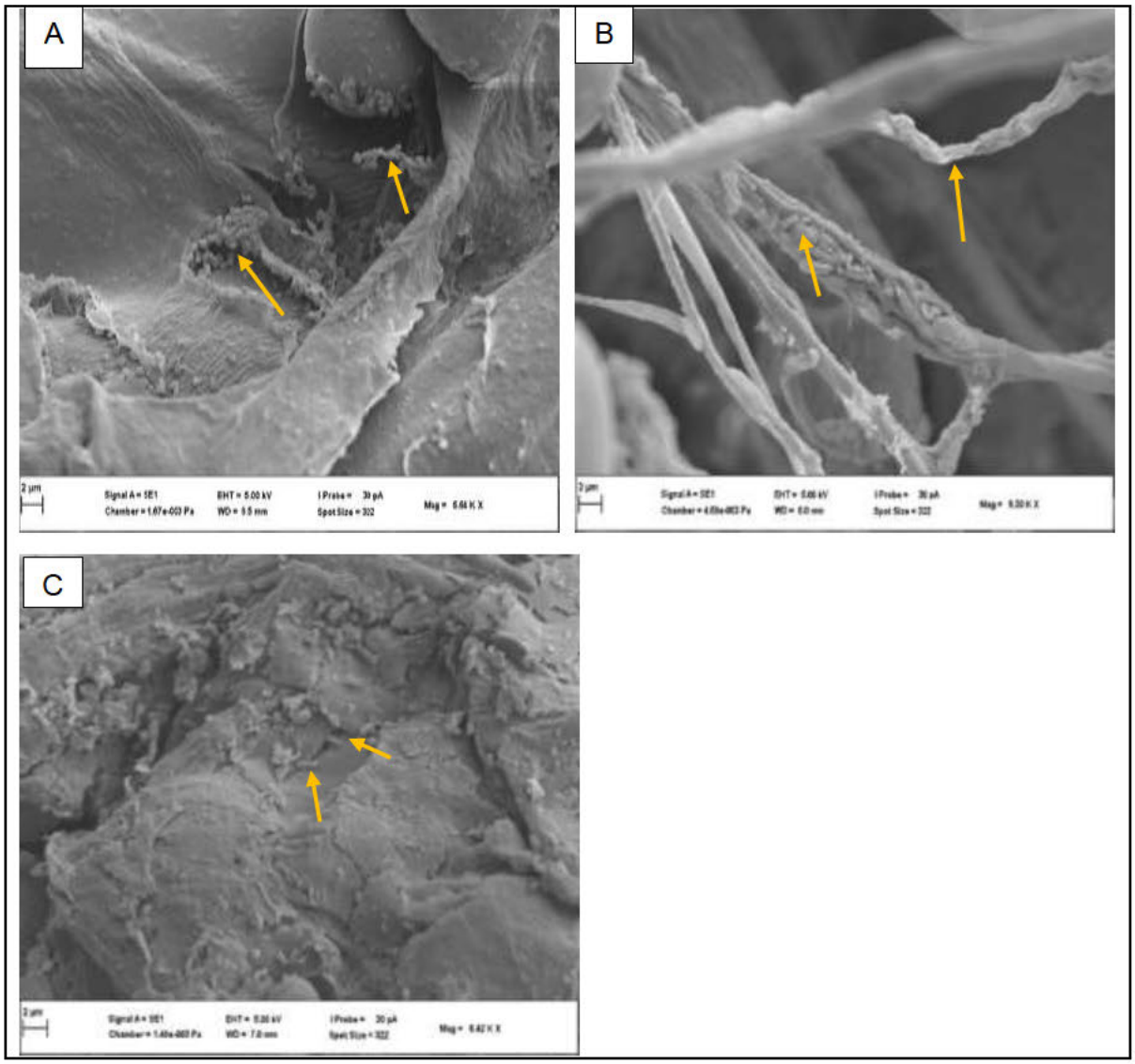

Figure 4.5: Scanning electron microscopy images showing the interaction between *F. solani*, MLE 2.5% (A), MLE 3% (B), and the conidia on potato surface (C) after 14 days. Yellow arrows show biofilms bound on the surface of treated potatoes causing the shrinkage and breakage of the mycelia, as well as conidia spores of *F. solani* on potato surfaces (C).

4.4 Discussion

The use of natural plant extracts as plant disease control agents has been extensively studied since they are safer for the environment, humans, and animals. Fungal pathogens are less likely to develop resistance to these natural pesticides (Shuping and Eloff, 2017). Moringa extracts have the potential to control fungal diseases while improving the shelf life of crops due to their high antioxidant concentration (Kator et al., 2019). Moringa can be used as a control strategy for plant diseases in organic

systems due to its various bioactive ingredients, which have different functions against pathogenic infection within the plant (Abd El-Hack et al., 2018). The current study investigated the antifungal effects of moringa leaf extract against *Fusarium solani* of potatoes. The findings showed that there was a significant difference between MLE 2.5%, MLE 3%, and control treatments *in vitro* and *in vivo*.

The *in vitro* results indicated that MLE 2.5% and MLE 3% were the most effective in inhibiting the mycelial growth of *F. solani*. Dry rot incidence was also reduced in potatoes treated with MLE 2.5% and MLE 3%. It was observed that the antifungal activity of moringa leaf extracts was increased at higher concentrations. These results corroborate with the recent report by Malevu (2022), which evaluated the effect of the same concentrations of moringa leaf extracts in controlling *Botrytis cinerea* both *in vitro* and *in vivo*. Similarly, El-Mohamedy and Abdalla (2014) indicated that *M. oleifera* extracts had antifungal activity against *F. oxysporum*, *F. solani*, *A. solani*, *A. alternata*, *R. solani*, *S. rolfsii* and *M. phaseolina in vitro*. The increased concentrations of *M. oleifera* reduced radial growth and mycelia of these pathogens extracts. The efficacy of MLE to suppress microbial growth could be attributed to its rich and rare combination of zeatin, quercetin, b-sitosterol, caffeoylquinic acid, and kaempferol, which have antifungal and antibacterial activities (Nikkon, 2003; Anjorin et al., 2010; Ashfaq et al., 2012).

The scanning electron micrographs showed morphological changes in the mycelia of samples treated with moringa leaf extracts indicated by thin, sparse, and distorted mycelia. The formation of biofilms, binded on the mycelia was observed in the wounds of potato surfaces treated with moringa. Moringa-treated samples also had a reduced number of *F. solani* spores. In comparison, control samples exhibited average mycelia growth and had abundant spores of *F. solani*. Biofilms are microbial communities that live and grow on surfaces and can consist of a single species or represent multi-species consortia (Costa-Orlandi et al. 2017). Even though biofilms are mainly formed to promote the growth and survival of microbial organisms (Danhorn et al., 2007; Bogino et al., 2013), in some crops, the formation of biofilms protects against postharvest diseases. For instance, biofilm formation in apple wounds was reported to prevent blue mould caused by *Penicillium expansum* (Scherm et al. 2003; Ortu et al. 2005). In the current study, biofilm formation in potato wounds reduced the development of *Fusarium* dry rot.

Plant extracts form biofilm especially in phyllo and carposphere (in wounds) as a mode of action against pathogens (Freimoser et al., 2019). Biofilm formation is an effective strategy utilized by biological control agents to compete for space. The formation of biofilms begins with the attachment of individual cells to a surface and usually involves cell wall modifications, secretion of an extracellular matrix, and hyphae formation (Cavalheiro and Teixeira 2018).

Moringa leaf contains abundant natural antioxidant compounds, including ascorbic acid, carotenoids, tocopherol, and phenols. These compounds denature enzymes that hinder amino acids involved in the spore germination of the pathogen (Cushnie and Lamb, 2005). A thermostable chitin-binding protein (Mo-CBP3) isolated from *M. oleifera* seed extract was reported to inhibit the mycelial growth and spore germination of *F. solani*, *F. oxysporum*, *Colletotrichum musae*, and *C. gloeosporioides* at the highest concentration (Gifoni et al., 2012). The findings of the current study have similarly demonstrated the antifungal properties of *M. oleifera* extracts and their efficacy to reduce dry rot on potatoes.

4.5 Conclusion

The results obtained from this study indicated that moringa leaf extracts have antifungal effects against *Fusarium solani* during storage. Therefore, moringa leaf extracts can be used as a cheap, environmentally-friendly, and reliable method to replace synthetic fungicides in controlling postharvest decay of potatoes. Although moringa leaf extracts have been extensively evaluated, this is one of the first studies to look at their effect on postharvest diseases of potatoes. These findings have shown that the efficacy of moringa extracts is linked to its ability to distort the mycelia and formation biofilms. However, future studies to focus on mechanisms involved in the formation of biofilms especially on potato wounds are required.

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Chapter 5

Integrated control of postharvest *Fusarium solani* of potatoes using UV-C irradiation and moringa leaf extracts

Abstract

Potato, one of the mostly consumed crops globally, is greatly affected by postharvest diseases, including *Fusarium solani*. This study evaluated the combined effect of UV-C and moringa leaf extracts against *F. solani in vitro* and *in vivo*. For *in vitro* effects, potato dextrose agar plates supplemented with moringa concentrations, MLE 1.5%, MLE 2.5%, and MLE 3%, were inoculated with mycelia plugs of *F. solani* and further exposed to UV-C treatment for 15 minutes. The plates were incubated at 25°C for seven days. The combination of 15 min+ MLE 2.5%, 15 min+ MLE 1.5%, 10 min + MLE 2.5%, and 10 min + MLE 3% inhibited mycelia by more than 50%. The highest percentage inhibition (100%) of mycelia of *F. solani* was observed in the samples treated with MLE 2.5% and exposed to UV-C for 15 minutes. For the *in vivo* effects, 'Sifra' potatoes were treated with UV-C and moringa and further inoculated with the spore suspension of *F*. *solani*. Treated potatoes were incubated at 25°C , 95% relative humidity. The findings revealed that potatoes exposed to UV-C for 15 minutes and treated with MLE 3% had the lowest disease incidence (8.33%). The scanning electron micrographs showed abnormal, shrinkage, disruption, aggregation and reduced hyphae length of mycelia in UV- and moringa-treated samples. Furthermore, it indicated the formation of biofilms in UV and moringa-treated surfaces of potato wounds. The findings revealed that the combined treatments enhanced efficacy compared to the single application of either treatment. UV-C can be integrated with moringa and be used to control the dry rot of potatoes, thus improving and extending their postharvest life.

Keywords: Potatoes, *Fusarium solani*, *Moringa oleifera*, Integrated control, Biofilms.

5.1 Introduction

Potatoes are one of the most consumed starch crops globally because they are cheap and a source of essential nutrients, including carbohydrates and starch. They contribute a lot to the reduction of food shortage globally (Birch et al., 2012). Microbial diseases and insects are the major limiting factors affecting potato production (Ranjan

et al., 2021). Apart from field diseases, postharvest diseases cause significant economic losses in the quality and quantity of potatoes during transport, storage, and marketing (Prusky, 2011). *Fusarium* solani is the causal agent of dry rot and is one of the main fungal pathogens that affect potatoes postharvest (Lucas, 2017). This pathogen survives from one season to the next in infected tubers, decaying plant tissue, or in the soil as chlamydospores (Powelson and Rowe, 2008). The development of dry rot is accelerated at a high relative humidity of 70% and 15-20°C (Chen et al., 2020). Control of *Fusarium* dry rot has been mainly achieved through chemical fungicides, including Maxim MZ, Tops MZ, Thiabendazole, and Moncoat MZ (Hay et al., 2019).

However, the continuous application of these fungicides causes *Fusarium* species to develop resistance towards them (Bojanowski et al.,2013). Chemical fungicides may also pose a health risk to humans upon consumption due to chemical residues on treated vegetable potatoes (Aktar et al., 2009). The loss of effectiveness of some chemicals due to the development of resistant strains of pathogens and environmental risks associated with the use of synthetic chemical fungicides raises the urgent need to develop non-chemical control strategies for *Fusarium* dry rot in potatoes. UV-C irradiation has been recognized for its germicidal properties and is commonly used for sterilization, mainly in places where microbial diseases are a major concern (Guerrero-Beltrán et al., 2004).

The effects of UV radiation are not only associated with its germicidal effects but also with physical changes in fruits and vegetables (Maharaj et al., 1999; Nigro et al., 2000). UV-C irradiation has been reported to control dry rot and soft rot in potatoes successfully (Ranganna et al., 1997; Jakubowski, 2019). The potential of combining UV-C irradiation with other postharvest control methods has been investigated with promising results. For instance, Romanazzi et al. (2006) evaluated the effect of combined chitosan and UV-C against grey mould in table grapes. These treatments were synergistic in reducing grey mold incidence and severity in table grapes compared to either single treatment. UV-C irradiation and heat treatment reduced decay and delayed fruit softening and ripening (Pan et al., 2004). Furthermore, Terao et al. (2015) reported that UV-C radiation at 2 kJ/m^2 and hot water treatment inhibited the spore germination of *F. pallidoroseum* and reduced the incidence of dry rot in ‘Galia’ melon.

Moringa oleifera is a medicinal plant with antioxidant and anti-inflammatory properties against diseases and can be used to replace synthetic fungicides (Dwivedi and Neetu, 2012). All parts of the plant, including leaves, seeds, and stems, consist of essential minerals, vitamins, proteins, and a wide range of phytochemical compounds that have biological activity. Thus, moringa plant extracts can be used to prevent the effects of microorganisms (Satish et al., 2013; Arora and Onsare, 2014). Bambalele et al. (2021) reported that the combined effect of moringa leaf extract-carboxymethyl cellulose and gaseous ozone enhanced the antioxidants of mango fruit during storage. Combining these treatments decreased the accumulation of sugars, delaying ripening in mango fruit and increasing the resistance of mango fruit to postharvest diseases.

There is limited literature available on the effect of UV-C treatments when integrated with moringa leaf extracts. Therefore, there is a need to investigate the synergistic effect of UV-C and moringa leaf extracts on postharvest diseases of potatoes. Thus this study evaluated the *in vitro* and *in vivo* effects of integrating UV-C and moringa leaf extracts against *F. solani* of potato.

5.2 Materials and methods

5.2.1 Effect of integrating UV-C irradiation and moringa leaf extracts against *F. solani in vitro*

UV-C and moringa leaf extract treatments, which successfully inhibited the pathogen *in vitro* as single treatments, were selected and integrated to investigate their combined effect against *F. solani*. Potato dextrose agar (PDA) was prepared and autoclaved at 121 °C for 15 minutes. A volume of 9 mL of PDA media was supplemented with 1 ml of MLE 1.5%, MLE 2.5%, and MLE 3% and poured into Petri dishes to solidify. Once the media was solidified, the plates were exposed to UV-C treatment for 10- and 15 minutes at 10 cm. After that, the mycelial plugs of *F. solani* (3 mm x 3mm) were excised from the edges of the growing fungi and inoculated at the centre of the treated plates. For control, *F. solani* was inoculated into fresh PDA plates. The plates were properly sealed with a parafilm and incubated at 25℃. Mycelial growth was examined and measured 3, 5, and 7 days after inoculation. The percentage inhibition of mycelia was calculated using the following formula:

% Inhibition$= \frac{\text{MGC} - \text{MGT}}{\text{MGC}} x\ 100$, where MGC is the average diameter of mycelia growth in control sample and MGT is the average diameter of mycelia growth in treated sample.

5.2.2 Effect of integrating UV-C irradiation and moringa leaf extracts against *F. solani in vivo*

'Sifra' potatoes were purchased at Pick n' Pay, Hayfields, Pietermaritzburg, South Africa. Potatoes were surface sterilized with 70% ethanol for 1 minute, rinsed in distilled water for 1 minute, and dried at room temperature. Wounded potatoes were treated with UV-C for 10- and 15 minutes at 10 cm. After that, moringa leaf extracts were inoculated into the same treated wounds of potatoes and left for 24 hours at room temperature. The potatoes were inoculated with *F. solani conidial* spore suspension of 1×10^4 spores/ml. For the control set, some potatoes were only inoculated with the pathogen suspension. Inoculated potatoes were incubated at 25°C storage with 95% relative humidity for 21 days. There were three replicates per treatment; each replicate had four potatoes. The disease incidence was evaluated every seven days until the last day of the incubation period using the following formula:

$$\%\ \text{disease incidence} = \frac{\text{The number of infected wounds in a treated potato sample}}{\text{total number of wounds in a treated potato}} \text{x } 100$$

5.2.3 Scanning electron microscopy analysis of the interaction of *F. solani* and integrated UV-C and moringa leaf extracts

The treatments 15 minutes + MLE 2.5% and 15 minutes + MLE 3% were selected as best-performing treatments and examined for their action mode against *F. solani* under scanning electron microscopy (SEM). PDA media was amended with concentrations of MLE 2.5%, and MLE 3%, and the plates were left in laminar flow to solidify. The treated plates were exposed to UV-C treatment for 10- and 15 minutes and inoculated with mycelial plugs (3mm x 3mm) of *F. solani*. The plates were incubated for seven days at 25 ℃. For *in vivo* trials, potatoes were sterilized with 70% ethanol, wounded, and exposed to UV-C treatment for 10- and 15 minutes at 10 cm. Subsequently, the potatoes were inoculated with MLE 2.5% and MLE 3%. After 24 hours, the potatoes

were inoculated with *F. solani conidial* spore suspension of 1×10^4 spores/ml concentration. For the control set, PDA plates and potatoes were only inoculated with the pathogen. The inoculated potatoes were incubated at 25 °C for 14 days.

The inhibition of mycelial growth and sporulation of *F. solani was* observed under SEM (Zeiss EVO LS15, Carl Zeiss NTS Ltd., Germany) at the Microscopy and Microanalysis Unit of the University of KwaZulu-Natal, Pietermaritzburg. Samples were excised from inoculated PDA plates and potatoes, fixed for 3 hours in 3% buffered glutaraldehyde, and double-washed with 0.05M sodium cacodylate buffer for 5 minutes. The samples were then dehydrated in 2 mL aliquots of 10%, 30%, 50%, and 70% ethanol for 10 minutes each. The samples were rinsed three times with 100% ethanol for 10 minutes each to complete the dehydration process. Thereafter, the samples were put in the Quorum K850 critical drying point dryer (CPD) basket with 100% ethanol.

The ethanol was replaced with liquid carbon dioxide (CO_2) during CPD. The liquid CO2 was heated and pressurized to the critical point where it transformed into a gas without damaging the samples due to surface tension, leaving the samples dry and undamaged. The dried samples were mounted onto SEM stubs using double-sided black tape. The sample stubs were transferred to the Quorum Q150R ES sputter coater and coated twice with gold and palladium so they could be conductive to the electron beam. After drying, the samples were viewed under the Zeiss EVO LS15 SEM.

5.2.4 Statistical analysis

The data of mycelial growth and disease incidence was collected and subjected to analysis of variance (ANOVA) using GenStat® 23rd edition. Duncan's Multiple Range Test (DMRT) was performed to test the significance of the means differences obtained among the treatments at the 5% significance level.

5.3 Results

5.3.1 Effect of integrating UV-C irradiation and moringa leaf extracts against *F. solani in vitro*

There is a significant difference between all the treatments ($p \leq 0.05$). Most concentrations of moringa leaf extracts inhibited the mycelial growth of *F. solani* at 15

minutes of exposure to UV-C. The combination of UV-C (15 minutes) and MLE 2.5% showed the highest percentage (100%) of mycelia inhibition (Figure 5.1), compared to the combination of UV-C (15 minutes) and MLE 3%, which exhibited the lowest percentage (32.37%) inhibition. There was a formation of biofilm surrounding the mycelia of *F. solani* in UV-C (15 minutes+MLE 1.25%- treated PDA plate (Figure 5.1B).

Table 5.1: Inhibition of mycelial growth of *F. solani* by MLE 1.5%, MLE 2.5%, and MLE 3% integrated with UV-C for 10 and 15 minutes.

Integrated treatments	**Mycelial growth (mm)**	**Inhibition (%)**
15 min + MLE 2.5%	0.00 a	100
15 min + MLE 1.5%	5 a	93.78
10 min + MLE 2.5%	12.00 b	85.06
10 min + MLE 3%	34.67 c	56.84
10 min + MLE 1.5%	45.33 d	43.57
15 min + MLE 3%	54.33e	32.37
Control	80.33 f	
P-value	<0.001	
LSD	6.63	
CV%	11.4	
SED	3.091	

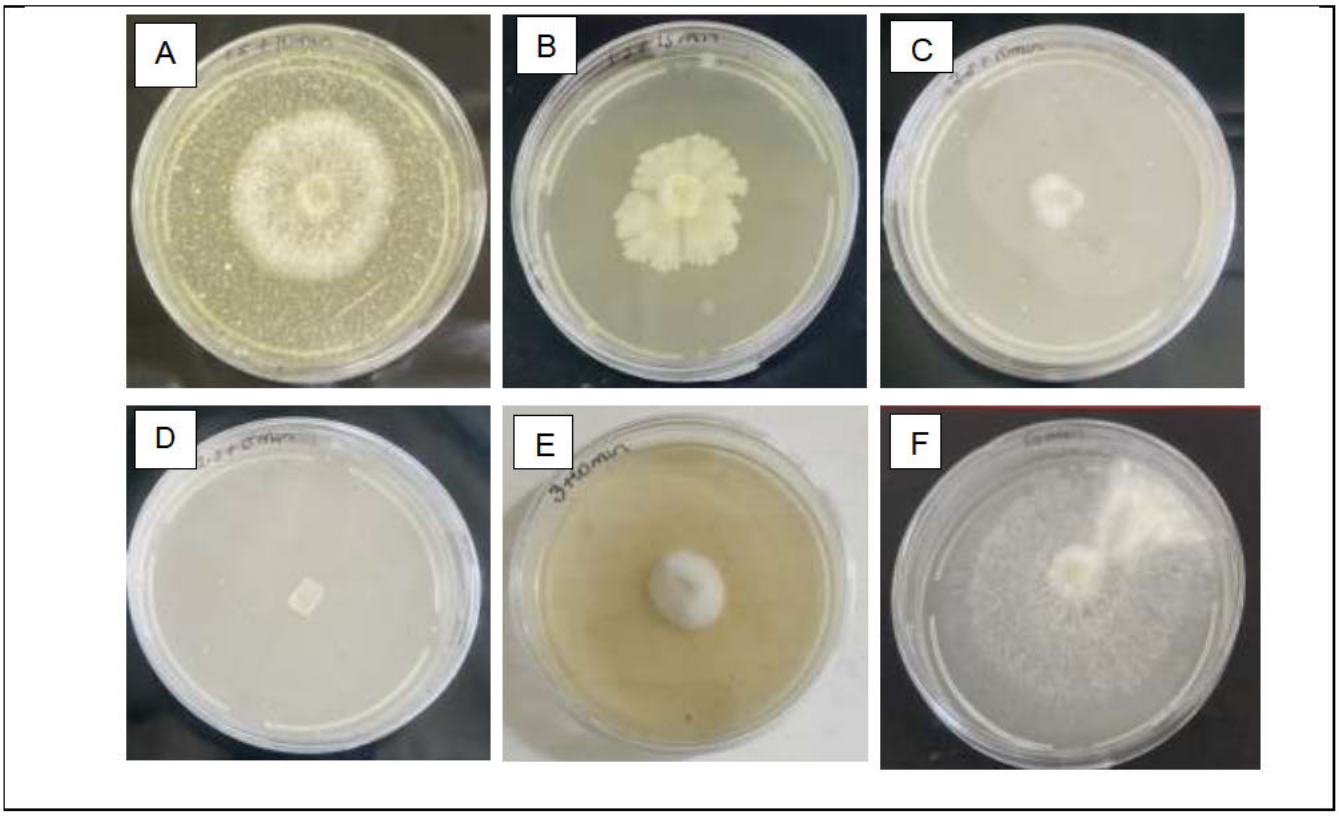

Figure 5.1: Mycelial growth of *F. solani* on the PDA media treated with MLE 1.5% + UV-10 and 15 minutes (A and B), MLE 2.5% + UV-10 and 15 minutes (C and D), MLE 3% + UV-10 minutes (E) and control (F) at 25 ℃ after 7 days of inoculation.

5.3.2 Effect of integrating UV-C irradiation and moringa leaf extracts against *F. solani in vivo*

All the treatments reduced the incidence of dry rot by <50%. UV-C (15 minutes) + MLE 3% was the most effective treatment in reducing dry rot in potatoes. There is a significant difference between the combined treatments UV-C (15 minutes) + MLE 2.5%, UV-C (15 minutes) + MLE 3%, and control. Potatoes exposed to UV-C for 15 minutes and treated with MLE 3% had the lowest disease incidence (8.33%). Untreated (control) potatoes had a dry rot incidence of 97.92% (Figure 5.2).

Figure 5.2: Disease incidence of *Fusarium* dry rot on untreated (A), UV-15 minutes + MLE 2.5% (B), UV-15 minutes + MLE 3% (C), treated 'Sifra' potatoes incubated at 25°C, 95% RH at day 21 post-inoculation.

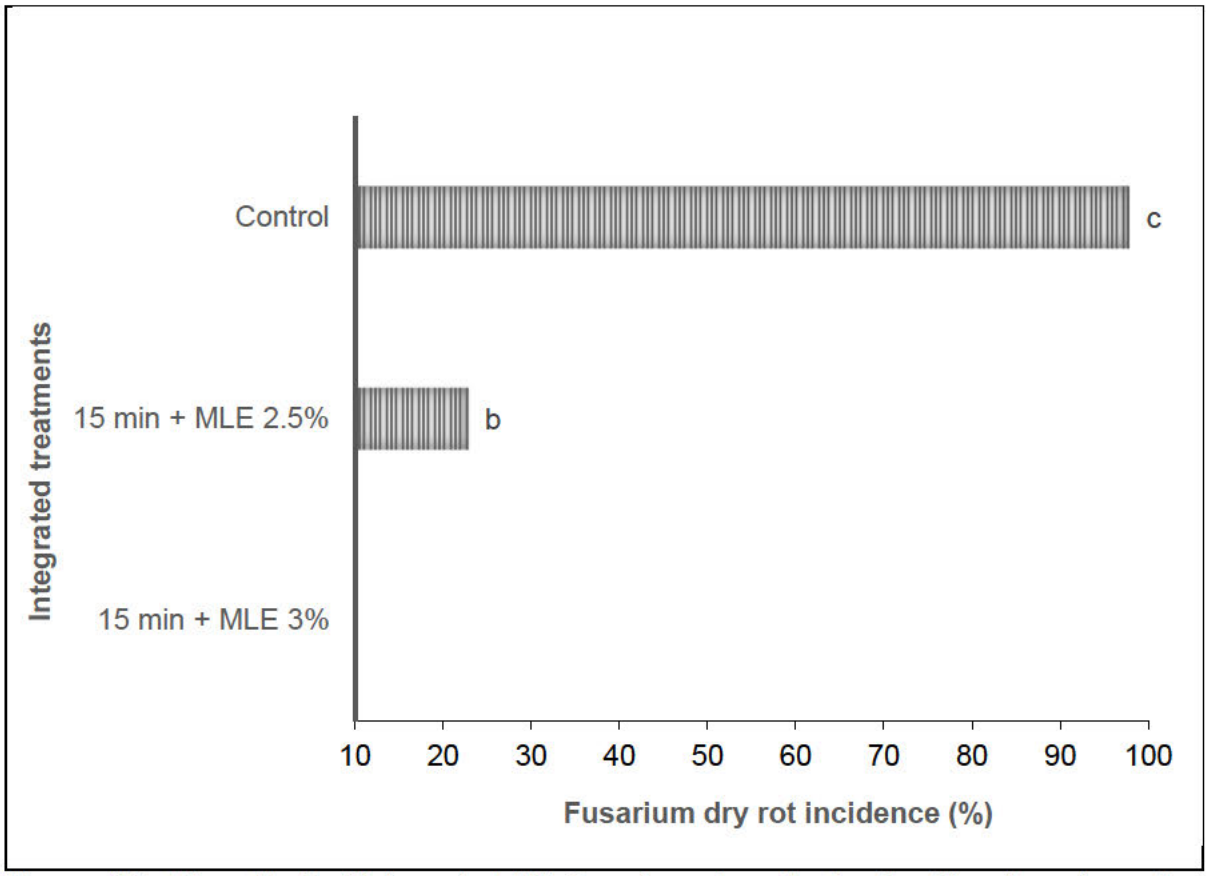

Figure 5.3: The effect of integrated UV-C and moringa leaf extract treatments on the dry rot disease incidence (%) on 'Sifra' potatoes incubated at 25°C, 95% RH. According to Duncan's Least Significance Difference Test, means followed by the same letter in each bar are not significantly different at $p \leq 0.05$.

5.3.3 Scanning electron microscopy observation of the interaction between *F. solani* and combined UV-C and moringa leaf extract *in vitro*

The scanning electron microscopy showed irregular and thin mycelia (Figure 5.4 A) and mycelia broken into many pieces (Figure 5.4 B). Control had average, abundant mycelia (Figure 5.4 C).

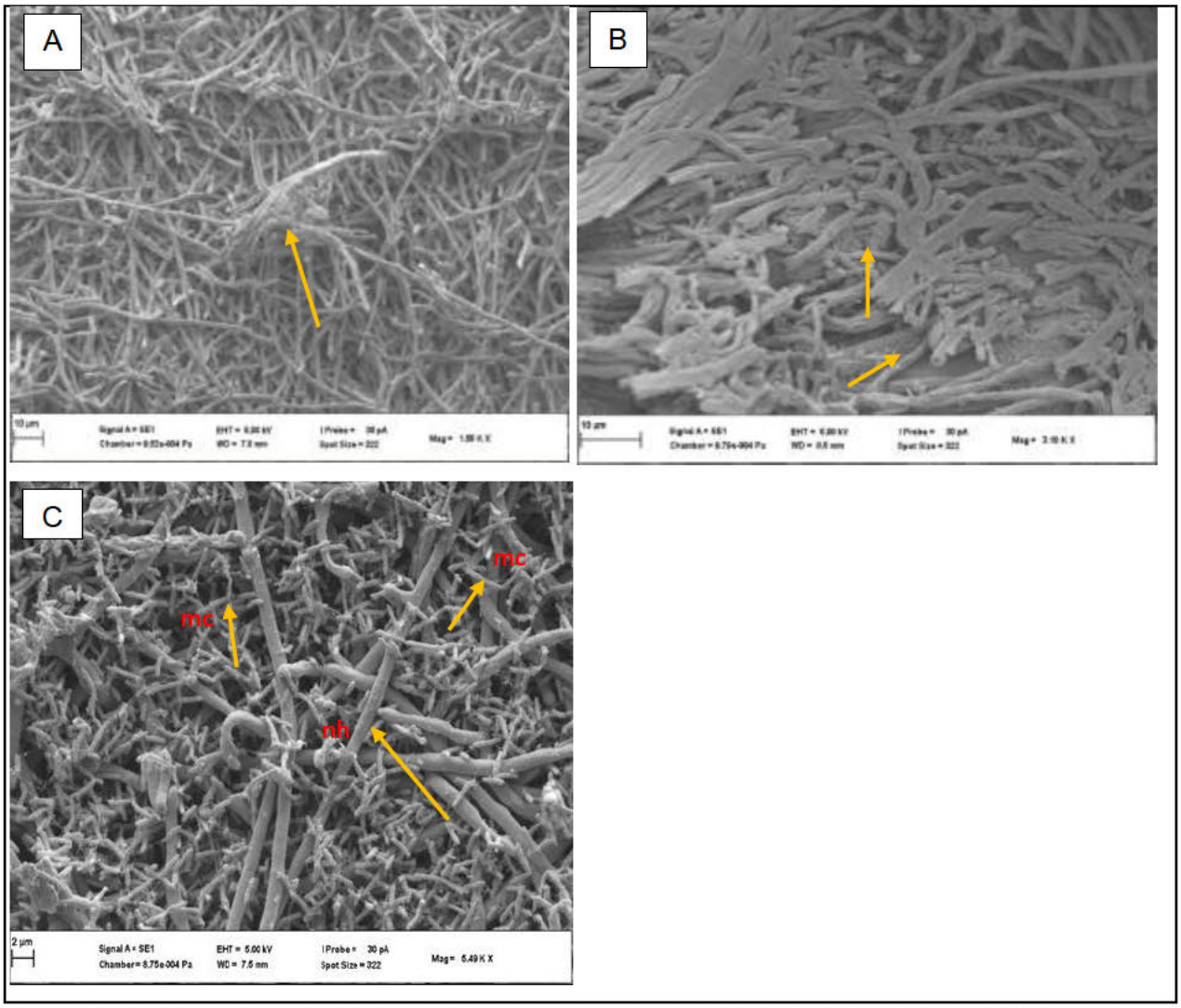

Figure 5.4: Scanning electron microscopy images showing the mode of action of MLE 2.5% + UV-15 minutes (A), MLE 3% + UV-15 minutes (B), and mycelia of *F. solani* (C) grown on potato dextrose agar after 7 days at 25°C. Yellow arrows indicate disruprion and aggregation of hyphae in UV and moringa- treated samples (A and B) as well as normal hyphae (nm) and micronidia in an untreated sample (C).

5.3.4 Scanning electron microscopy observation of the interaction between *F. solani* and combined UV-C and moringa leaf extract *in vivo*

The scanning electron microscopy images indicated the mode of action between the combined treatments, UV-C (15 minutes) + MLE 2.5%, U-VC (15 minutes) + MLE 3%, and *F. solani*. The arrows indicate the formation of biofilms (bf) attached to the mycelia and conidia (co) in UV-C and moringa treated surface of the potato wound (Figure 5.5 A and B) and micronidia of *F. solani* on untreated surface (Figure 5.5 C).

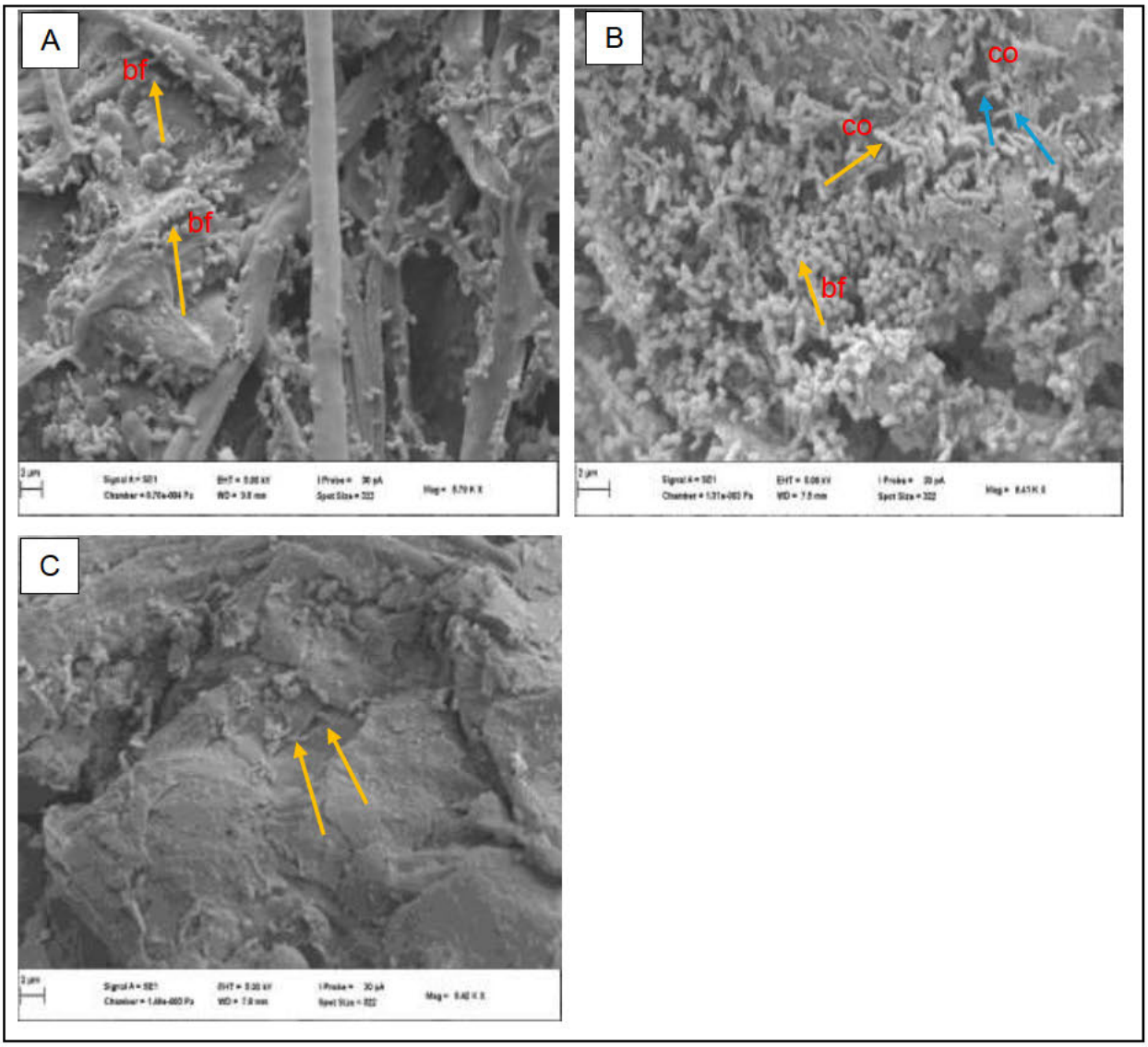

Figure 5.5: Scanning electron microscopy images showing the mode of action of MLE 2.5% + UV-15 minutes (A) and MLE 3% + UV-15 minutes (B) on potato surface.

5.4 Discussion

Fusarium dry rot is one of potatoes' most devastating postharvest diseases worldwide (Lucas, 2017). UV-C irradiation is a convenient method for postharvest control of microbial pathogens and has shown the potential against soft rot and dry rot in potatoes (Ranganna et al., 1997; Rocha et al., 2015; Jakubowski,2019). *M. oleifera* is a medicinal plant and has become a safer and more compatible alternative to chemical fungicides (Dwivedi and Neetu, 2012). It has exhibited antifungal effects against *F. solani* and *F. oxysporum*, the causal agents of potato dry rot (Goss et al., 2017; Mncube et al., 2019; Mncube et al., 2022).

The combined effect of UV-C and moringa leaf extracts against *Fusarium* dry rot was investigated in this study. The findings indicated that combining these treatments suppressed the incidence of dry rot in potatoes. The highest percentage inhibition of the mycelia of *F. solani* was observed in the samples treated with MLE 2.5% and exposed to UV-C for 15 minutes. UV-C treatment of potatoes for 15 minutes reduced the incidence of disease at both MLE 2.5% and MLE 3%. However, the lowest disease incidence was observed on potatoes exposed to UV-C for 15 minutes and treated with MLE 3%. These treatments exhibited synergistic effects against *F. solani* compared to either individual treatment.

The scanning electron micrographs showed morphological changes in the structure of mycelia in treated samples. These changes are characterized by abnormal growth, shrinkage, disruption and aggregation in mycelia.The production of spores was also lowered in samples treated with UV-C and moringa leaf extracts (Figure 5.4 A and B). In contrast, control (untreated) samples showed normal growth of mycelia. The spore germination was also observed in the mycelia of untreated samples (Figure 5.4 C). The formation of biofilms, bound on the mycelia, was observed in the wounds of potato surfaces treated with UV-C and moringa (Figure 5.5 A and B). Biofilm formation, especially in phyllo and carposphere (in wounds) by plant extracts, is considered an important mode of action by biological controls (Freimoser et al., 2019).

In the current study, moringa leaf extracts used this mode of action to compete for nutrients with *F. solani in vitro* and *in vivo*. For the successful formation of biofilms, individual cells of the biological control agent attach to a surface and modify cell walls through secretion of extracellular matrix and hyphae formation (Cavalheiro and Teixeira, 2018). The formed biofilms serve as barriers between the host lesion surface and the phytopathogen (Carmona-Hernandez et al., 2019). Vero et al. (2013) reported that the biofilm formation by *Leucosporidium scottii* (Fell and Phaff 1970) successfully colonized host tissues and provided a biological control against blue and grey mould on apple fruit. This yeast was also reported to have the potential to inhibit brown rot on apple fruit but formed pseudohyphae on peach fruit and caused fruit decay (Giobbe et al., 2007).

The combination of UV-C and moringa leaf extracts effectively controlled potatoes' dry rot. The reduction of the incidence of dry rot could also be attributed to defense-related

enzymes, -1,3-Glucanase (GLU), peroxide (POD), ammonia-lyase (PAL), and chitinase (CHI) activated by UV-C irradiation. These enzymes degrade pathogen cell walls and inhibit pathogen growth and development (Buraphaka et al., 2024).

The antifungal activity of moringa leaf extract may be due to the presence of lipophilic compounds that bind to the cytoplasmic membrane and inhibit the growth of filamentous fungi through membrane permeabilization (Huang et al., 2000). The findings of the current study revealed that the integration of UV-C and moringa leaf extracts increased their efficacy in reducing dry rot of potatoes compared to the single application of either treatment.

5.5 Conclusion

The combination of UV-C and moringa leaf extracts enhanced the benefits of applying each treatment individually. They could be useful in controlling the dry rot of potatoes, thus improving and extending their postharvest life. These findings provide new information on the ability of moringa to form biofilms to reduce the development of dry rot in potatoes. However, further study on the mechanism involved in the formation of biofilms is required.

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Chapter 6

Thesis overview

6.1 Introduction

Potato is globally the third most important non-grain food crop following rice and wheat (FAO, 2016). In South Africa, potatoes are mainly grown under warm conditions, with frequent occurrences of climatic challenges such as heat, hail, and frost (van der Waals et al. 2016). According to STATISTA (2023), South Africa had a total production of potatoes of 2,528.95 million metric tons in 2022. However, the production of potatoes is negatively affected by fungal, bacterial and viral diseases (Ranjan et al., 2021).

Fusarium solani, a causal agent for dry rot, affects the growth of potato sprouts and cause crop losses up to 25%, while more than 60% of tuber losses occur during storage (Tiwari et al., 2020). The infection of *F. solani* is mostly through wounds inflicted during harvest and transportation (Fiers et al., 2012). *F. solani* mainly affects members of the *Solanaceae* family which includes tomato, tobacco, eggplant, chilli pepper, woody nightshade and buffalo bur (Rasul et al., 2019). This pathogen also affects the cucurbit family, including melon, cucumber, and pumpkin (Romberg and Davis, 2007). Extensive research has been conducted to develop control strategies for *F. solani* (Zape et al., 2014; Soltanzadeh et al., 2013; Goss et al., 2017).

6.2 Research objectives and major findings

This study aimed to evaluate the effect of integrating UV-C and moringa leaf extracts to control *F. solani* of 'Sifra' potatoes *in vitro* and *in vivo*. The objectives of this study were to:

- Evaluate the *in vitro* and *in vivo* effect of UV-C irradiation on *F. solani.*
- Evaluate the *in vitro* and *in vivo* effect of moringa leaf extracts on *F. solani*.
- Determine the mode of action of UV-C irradiation and moringa leaf extracts against *F. solani* of potato.
- Evaluate the effect of integrating UV-C irradiation and moringa leaf extracts to control *F. solani in vitro* and *in vivo*.

Below are the major findings of the study:

Chapter 3: *In vitro* and *in vivo* effects of UV-C irradiation against *Fusarium solani* of potato.

- UV-C treatment for 10 and 15 minutes at a 10 cm distance successfully suppressed *F. solani* both in *vitro* and *in vivo*.
- Sifra potatoes treated with UV-C for 15 minutes had a lowest disease incidence (22%).
- The efficacy of UV-C increased with treatment duration.
- The SEM images showed shrinked, broken and distorted mycelia with rough surfaces in UV-C treated samples.
- Damaged spores of *F. solani* were observed in UV-treated potato surfaces.
- It is hypothesized that UV-C activated chitinases and b-1,3-glucanases enzymes which are released into extracellular matrix. These enzymes are responsible for degradation of fungal cell walls.
- It could be argued that the deposition of extracellular matrix acted as a physical barrier to prohibit invasion of the host by the fungi.
- UV-C irradiation exhibited antifungal properties that inhibit the growth of *F. solani in vitro* and dry rot disease incidence in potatoes.

Chapter 4: *In vitro* and *in vivo* screening of moringa leaf extract against *Fusarium solani* of potato.

- Concentrations of moringa (MLE 1.5%, MLE 2.5%, and MLE 3%) inhibited the mycelial growth of *F. solani in vitro*.
- MLE 2.5% and MLE 3% suppressed the incidence of dry rot in ‘Sifra’ potatoes.
- The effectiveness of moringa leaf extracts against *F. solani* increased with concentration.
- The SEM images showed thin, sparse, and distorted mycelia in samples treated with moringa leaf extracts.
- Based on SEM analysis, moringa used biofilm formation as a strategy to compete for nutrients in order to reduce the disease incidence.

- Overall, the efficacy of moringa extracts is linked to its ability to distort the mycelia and formation biofilms.

Chapter 5: Integrated control of UV-C irradiation and moringa leaf extracts against *F. solani in vitro* and *in vivo*

- The combination of UV-15 min+ MLE 2.5%, UV-15 min+ MLE 1.5%, UV-10 min + MLE 2.5%, and UV-10 min + MLE 3% provided a successful inhibition of the mycelia of *F. solani*.
- Integrating 10 minutes and 15 minutes UV-C treatment with MLE 3% suppressed the disease incidence in potatoes.
- The combined treatments enhanced efficacy compared to the single application of either treatment.
- The SEM images showed abnormal growth, shrinkage, disruption, aggregation, reduced hyphae length and diameter as well as pore formation in mycelia in samples treated with UV-C and moringa extracts
- The production of spores was lowered in samples treated with UV-C and moringa leaf extracts

6.3 Recommendations and conclusion

Postharvest fungal diseases are primarily controlled through postharvest fungicides. However, chemical fungicides have negative impacts on human health through consumption of commodities with chemicals residues. These risks associated with the use of fungicides motivate the need to develop novel and non-chemical methods for the potato industry. UV-C irradiation provides a successful control against range of postharvest diseases (Cia et al., 2007; Terao et al., 2015; Jakubowski, 2019). The effectiveness of UV-C in controlling postharvest diseases is linked with its ability to activate chitinases and b-1,3-glucanases enzymes that are responsible for degradation of fungal walls. Several studies have shown that moringa leaf extracts have antifungal properties against microbial diseases (Goss et al 2017; Nkazela et al., 2019; Kubheka et al., 2020). These attributes affect consumers, since they evaluate the visual appearance, colour, taste and texture.

In this study, UV-C and moringa leaf extracts exhibited antifungal effects on *Fusarium* dry rot of potato by providing 100% inhibition of the mycelia *in vitro* and lowering the disease incidence to 8.33%. Thus, UV-C and moringa leaf extracts can be recommended in potato industry for disease control and enhanced quality to increase market value. However, further studies should evaluate on the impact of the combined UV-C and MLE treatments on nutritional quality and sensory attributes of potatoes including texture, appearance and flavour are required. This study is crucial since consumers evaluate the visual appearance, to determine the freshness and acceptability of the product. Nutritional quality is also valued by consumers as it directly affects their health and well-being (Barrett et al., 2010).

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Appendices

Appendix 1: *In vitro* effect of UV-C irradiation on *Fusarium solani* at 15 cm distance after 7 days at 25°C.

Treatments	Mycelial growth (mm)	Inhibition (%)
10 minutes	59.67 a	26.94
5 minutes	65.00 ab	20.41
15 minutes	68.67 b	15.92
Control	81.67 c	
P-value	<0.001	
LSD	8.10	
CV%	6.3	
SED	3.51	

Appendix 2: *In vitro* effect of UV-C irradiation on *Fusarium solani* at 20 cm distance after 7 days at 25°C.

Treatments	Mycelial growth (mm)	Inhibition (%
5 minutes	62.00 a	24.08
10 minutes	67.67 ab	17.14
15 minutes	72.33 b	11.4
Control	81.67 c	
P-value	<0.001	
LSD	7.47	
CV%	5.6	
SED	3.24	