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**The effect of biocontrol agents and plant extracts on postharvest quality of ‘Kent’
mangoes**

By

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DISSERTATION SUMMARY

Mango is a tropical fruit grown in all parts of the world, hence is economically important. This fruit is beneficial as it has medicinal properties helpful to human health. Mango fruit is climacteric with a high respiration rate, leading to rapid ripening, and limiting the shelf life. This also limits the import and export, whilst the fruit is under duress, there are also fungal pathogens that infect this fruit both pre and postharvest. Anthracnose is one of the diseases affecting mangoes which is caused by the fungal pathogen *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. The susceptibility of mango fruit to fungal diseases affects the total yield and profits of the producers. Synthetic fungicides have been used to control the infection caused by fungal pathogens. However, excessive use of fungicides has a negative impact on human health and is hazardous to the environment. Notably, fungal pathogens develop resistance to fungicides over time. The chemicals such as prochloraz have also been used as a treatment in cold storage to prolong the shelf life of mangoes, and the risk of consumers ingesting these chemicals is reported. Contrary to fungicides, this study evaluated the efficacy of biocontrol agents and plant extracts against *C. gloeosporioides* and in prolonging the shelf life of mangoes.

During the *in vitro* studies, 150 yeast and 150 *Bacillus* strains were isolated from different plant leaf materials and screened against *C. gloeosporioides*. A dual assay was conducted, and potato dextrose agar (PDA) was inoculated with the fungal pathogen and biocontrol agents and then stored at 28°C for 7 days. The best two *Bacillus* isolates (SL and SI) and 1 yeast isolate (Ba) that inhibited the pathogen by more than 65% and were selected from a dual culture assay in secondary screening and identified using BLAST. These isolates were further used as potential biocontrol agents against the pathogen *in vivo* on ‘Kent’ mango fruits. The BLAST results identified isolate Ba as *Meyerozyma guilliermondii*, isolates SL and Ss as *Burkholderia contaminans*. These isolates were overall the best treatments with mycelial growth inhibition of 67.91%, 70.00%, and 74.04%, respectively. *M. guilliermondii* and two *B. contaminans* inhibited anthracnose disease incidence on ‘Kent’ mango fruit by 50% compared to the control treatment (0%) after 7 days at 25°C. The scanning electron microscopy (SEM) images showed breakage and shrinkage of *C. gloeosporioides* mycelia *in vitro*, and there was little damage to mycelia and no spore germination on mango fruit. The different plant extracts, *Aloe vera*, *Tetradenia riparia*, *Pelargonium sidoides*, and *Moringa oleifera* were prepared and screened against *C. gloeosporioides*. PDA was amended with different concentrations of the plant extracts (1%, 1.5%, 2%, 2.5%, and 3%) then inoculated with the pathogen, and stored at 28°C

for 7 days. *P. sidoides* was selected as the best-performing plant extract at 2.5% and 3% concentrations with 100% mycelial growth inhibition *in vitro*. Anthracnose disease incidence was significantly reduced by *P. sidoides* at 3% and 2.5% concentrations ($\leq 50\%$ and $\leq 75\%$, respectively) compared to the control fruit with 100% infection. The SEM interaction of 2.5% and 3% of *P. sidoides* with *C. gloeosporioides* *in vitro*; and mango fruit treated with 2.5% and 3% of *P. sidoides* and the pathogen had a limited number of spores and thin mycelia in comparison to the control. Furthermore, this study evaluated the individual and combined effect of *B. contaminans*, *P. sidoides*, and carboxymethyl cellulose (CMC) edible coating on mango fruits at 10°C for 21 days, followed by 7 days at 25°C. Untreated fruit had the highest mass loss (18.53%) compared to all treatments (CMC, BCA, BCA+PE and PE) which had 15.3%, 14.64%, 14.18% and 14.04%, respectively. Mango fruit coated with PE had DPPH scavenging activity at 51.11 $\mu\text{M TE/g DM}$, whereas CMC treated fruit had 29.66 $\mu\text{M TE/g DM}$, even lower than untreated fruit which had 36.52 $\mu\text{M TE/g DM}$. Phenolic content was recorded at 116.4 $\mu\text{g GEA/g DM}$ on PE treated fruit compared to 95.1 $\mu\text{g GEA/g DM}$ for the control fruit. The concentration of AA was 51.95 mg/g on PE treated mango compared to 24.61 mg/g on the control fruit. This study showed that *B. contaminans*, *P. sidoides*, and CMC edible coating can be exploited as postharvest treatments to extend the shelf life of mango fruit.

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.

DECLARATION

I, Siphokazi Anele Radebe, 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 persons' data, pictures, graphs or other information unless specifically acknowledged as being sourced from other persons.
- IV. This thesis does not contain other persons' work unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
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Prof A. Mditshwa (Co-supervisor)

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DEDICATION

Anele, you did it!

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Chapter 1

General introduction

1.1 Background

Mango (*Mangifera indica* L.) is one of the most economically important crops grown worldwide (Mitra, 2014). This is a horticultural crop grown in many countries for its commercial value (Jenny *et al.*, 2019). The cultivation of mango in India is dated back to 4000 to 6000 years (Tharanathan *et al.*, 2006). Major growing countries include India, Pakistan, Bangladesh, Thailand, and the Philippines, South Africa, and Nigeria (Sanders *et al.*, 2000; Awa *et al.*, 2012; Yadav and Pandey, 2016). Mango fruit is an important source of macro- and micronutrients such as minerals, vitamins, phytochemicals, polyphenols, and carotenoids (Lamilla *et al.*, 2021). It is sold mainly as fresh fruit, and processed products such as beverages, juice, jam, pickle, dried fruit, and jelly (Jin *et al.*, 2019).

Mango is a climacteric fruit; thus, it is harvested at the green, matured stage and continues to ripen even after being detached from the tree (Hmnam *et al.*, 2021). Due to its high perishability, domestic and export markets of mango are limited as it ripens faster at ambient temperature, taking about 5-9 days (Singh *et al.*, 2013). Mango has a high postharvest loss, mostly due to moisture loss through transpiration, which has a major contribution to the total fruit mass loss (Bambalele *et al.*, 2021). It is important to note that mango is sold by mass and this factor contributes to the appearance, colour, and texture of the fruit which is affected greatly by moisture loss (Subramanian *et al.*, 2018). Loss of mass in fresh fruits is a major concern as it affects the shelf life and overall acceptability of fruits by consumers (Prasenjit *et al.*, 2021).

The production, domestic, and export markets of mango are currently under threat due to postharvest diseases (Subramanian, *et al.*, 2018). Postharvest diseases, caused by many fungi, bacteria, and viruses, are the major constraints during the postharvest handling chain. Notably, stem-end rot and anthracnose, caused by *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl and *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc respectively, are the most common postharvest fungal diseases threatening the profitability of the mango industry (Arauz, 2000).

The postharvest losses caused by anthracnose on mango are estimated to be up to 38%, which may rise to 100% if proper management strategies are not in place (Khanzada, *et al.*, 2018).

Several fungicides have been used to control postharvest fruit diseases. These include prochloraz, thiabendazole, and fludioxonil (Tesfay, *et al.*, 2017), thiophanate-methyl and other components of benzimidazoles and dithiocarbamates group (Oliveira, *et al.*, 2018). The effectiveness of the fungicides is now declining, which indicates that the pathogen has developed resistance to these management techniques (Oliveira, *et al.*, 2018). According to Kuo (2001), in a study that investigated the effect of prochloraz in controlling anthracnose on mango, increasing the dosage about five times higher than the average baseline led to a slight shift on the efficacy. Depicting that an increase in the dosage of the chemical did not increase the effectivity (Kuo, 2001). The harmful effects of chemical treatments on both the consumer and environmental health calls for eco-friendly and innovative postharvest management measures (Chillet, *et al.*, 2019).

1.2 Problem statement

Mango industry worldwide has reported an increase in losses caused by fungal diseases and the perishability of mango fruit leading to a limited shelf life (Dinh, 2002). This is linked to the production of ethylene that leads to rapid ripening whilst limiting water retention due to transpiration (Bapat *et al.*, 2010). Therefore, leading to the production of mango fruit with low quality and quantity (Dhall, 2013). Exposing fruits to cold storage has been considered the most effective method for prolonging the shelf life of fresh produce by delaying rapid ripening (Barman *et al.*, 2014). However, it has proven to expose the fruits to chilling injury (Thakur *et al.*, 2017). Tropical fruits such as mango are sensitive to extremely low temperatures lower than 10°C as those induce physiological and molecular changes, compromising the fruit quality (Sivankalyani, *et al.*, 2017).

Postharvest management of fruits and vegetables is affected by many factors, including fungal diseases and methods used storage (Baloch and Bibi, 2012). Anthracnose caused by *C. gloeosporioides* is one of the diseases causing great loss of mango (Arauz, 2000). Fungicides effectively eradicate the infection of fungal diseases postharvest, including anthracnose (Thakor, 2019). However, the pathogen resistance to fungicides such as prochloraz, thiabendazole, and fludioxonil thiophanate-methyl and other components of benzimidazoles and dithiocarbamates group has exposed the industry to a decline in best quality fruit export and overall production, also may pose health threats to consumers (Kumar *et al.*, 2007; Oliveira *et al.*, 2018). Some isolates of *C. gloeosporioides* were reported to be resistant to mancozeb and copper oxychloride, which is attributed to the extensive use of fungicides (Kumar *et al.*, 2007). These

losses are not only on mango but also on other alternate hosts such as papaya, guava, and avocado (Uddin *et al.*, 2018). The perishable nature, diseases, pest infestation, and susceptibility of mango fruits to chilling injury when stored at low temperatures are the limiting factors for the international commercial transportation of the fruit hence the exploration of alternative environmentally safe biofungicides.

1.3 Research motivation

Production of mango has been increasing worldwide, because of the consumption for health benefits (Chay *et al.*, 2019). However, this is compromised by diseases, weather conditions, and other factors involved in the whole production, with most losses occurring from the fungal pathogens caused by *C. gloeosporioides* both pre and postharvest (South African Fruit flow, 2020; Schneider *et al.*, 2013). Furthermore, postharvest losses documented are also caused by moisture loss through respiration, negatively impacting the fruit quality, and leading to tremendous financial losses since the price of the fruit is determined by mass (Léchaudel and Joas 2007).

Contrary to fungicides, biocontrol agents and edible coatings with antifungal properties have emerged as an alternative method to protect fruit from the infection of pathogenic fungi (Prasenjit *et al.*, 2021). Biocontrol agents are safe, and natural and can be used to control pathogens. Bacteria and antagonistic yeast are some of the biocontrol agents used against fungal pathogens (Huang *et al.*, 2020; Navarro-Herrera and Ortiz-Moreno 2020). Biocontrol agents are recognized as the promising group due to their strong antagonistic activity, high viability, and broad inhibitory spectrum (Shafi *et al.*, 2017). This is through several mechanisms such as competition, mycoparasitism, production of antibiotics, and the induction of defence responses (Bautista-Rosales *et al.*, 2014).

Edible coatings are nontoxic, tasteless, and nonallergic (Jongsri *et al.*, 2017). They have a protective effect as they have functional compounds such as antimicrobials, antioxidants, and anti-browning agents (Ochoa-Velasco *et al.*, 2021). These inhibit microbial decay and prevent physical deterioration in fruits during storage (Panahirad *et al.*, 2021). They are applied as a thin layer providing the fruit with a barrier that restricts movement of moisture, oxygen, and solutes. Retaining fruit moisture has the greatest effect on fruit mass. The potential of an edible coating is to maintain the quality and extend the shelf-life of fresh fruits (Prasenjit *et al.*, 2021).

These control methods offer a great potential in controlling the infection by the fungal pathogen *C. gloeosporioides* and extending the shelf life of mango fruits.

1.4 Aim and objectives.

The aim of this study was to assess the potential of biocontrol agents (yeast and bacteria) and plant extracts on the postharvest quality of 'Kent' mango fruit.

The specific objectives of this study were to:

- i. Investigate the *in vitro* and *in vivo* effect of biological control agents against *Colletotrichum gloeosporioides* of 'Kent' mango.
- ii. Investigate the *in vitro* and *in vivo* effect of plant extracts against *Colletotrichum gloeosporioides* of 'Kent' mango.
- iii. Evaluate the effect of biocontrol agents, plant extract and carboxymethyl cellulose edible coating on antioxidant activity of 'Kent' mango.

1.5 Dissertation structure

The dissertation has six chapters, and the details of each chapter are described as follows: Chapter 1 is the general introduction of the dissertation; Chapter 2 is the literature review focusing on the production of mango, anthracnose, and control strategies; Chapter 3 reports on the isolation of antagonistic microorganisms from different plant parts and testing their inhibitory effect against *C. gloeosporioides* *in vitro* and *in vivo*; Chapter 4 was on the antifungal effect of different plant extracts against *C. gloeosporioides* *in vitro* and *in vivo*; Chapter 5 evaluated the effect of biocontrol agents, and plant extracts and carboxymethyl cellulose edible coating on antioxidant activity of 'Kent' mango; Chapter 6 summarises the major findings of the study and their implications.

1.6 References

Arauz, L.F., 2000. Mango anthracnose: Economic impact and current options for integrated management. Plant Disease, 84(6), pp.600-611.

- Baloch, M.K. and Bibi, F., 2012. Effect of harvesting and storage conditions on the postharvest quality and shelf life of mango (*Mangifera indica* L.) fruit. South African Journal of Botany, 83, pp.109-116.
- Bambalele, N.L., Mditshwa, A., Magwaza, L.S. and Tesfay, S.Z., 2021. The effect of gaseous Ozone and moringa leaf-carboxymethyl cellulose edible coating on antioxidant activity and biochemical properties of 'Keitt' mango fruit. Coatings, 11(11), p.1406.
- Bapat, V.A., Trivedi, P.K., Ghosh, A., Sane, V.A., Ganapathi, T.R. and Nath, P., 2010. Ripening of fleshy fruit: molecular insight and the role of ethylene. Biotechnology Advances, 28(1), pp.94-107
- Barman, K., Asrey, R., Pal, R.K., Jha, S.K., and Bhatia, K., 2014. Post-harvest nitric oxide treatment reduces chilling injury and enhances the shelf-life of mango (*Mangifera indica* L.) fruit during low-temperature storage. The Journal of Horticultural Science and Biotechnology, 89(3), pp.253-260.
- Bautista-Rosales, P.U., Calderon-Santoyo, M., Servín-Villegas, R., Ochoa-Álvarez, N.A., Vázquez-Juárez, R. and Ragazzo-Sánchez, J.A., 2014. Biocontrol action mechanisms of *Cryptococcus laurentii* on *Colletotrichum gloeosporioides* of mango. Crop Protection, 65, pp.194-201.
- Awa, O.C., Samuel, O., Oworu, O.O. and Sosanya, O., 2012. First report of fruit anthracnose in mango caused by *Colletotrichum gloeosporioides* in Southwestern Nigeria. International Journal of Scientific & Technology Research, 1(4), pp.30-34.
- Sanders, G.M., Korsten, L. and Wehner, F.C., 2000. Survey of fungicide sensitivity in *Colletotrichum gloeosporioides* from different avocado and mango production areas in South Africa. European Journal of Plant Pathology, 106, pp.745-752.
- Chay, K.G., Workeneh, A. and Shifera, B., 2019. A review on production and marketing of mango fruit. World Journal of Agriculture and Soil Science, 2(2), pp.1-7.
- Chillet, M., Minier, J., Hoarau, M. and Meile, J.C., 2019. Potential use of thymol to control anthracnose development in mango. European Journal of Plant Pathology, 155(3), pp.943-952.

- Dhall, R.K., 2013. Ethylene in post-harvest quality management of horticultural crops: A review. *Research & Reviews: A Journal of Crop Science and Technology*, 2(2), pp.9-24.
- Dinh, S.Q., 2002. Post-harvest loss of mango due to anthracnose and its infection biology and resistance of mango to the disease (pp. 2-70). Kasetsart University.
- Hmmam, I.; Zaid, N.;Mamdouh, B.; Abdallatif, A.;Abd-Elfattah, M.; Ali, M., 2021. Storage Behavior of “Seddik” Mango Fruit Coated with CMC and Guar Gum-Based Silver Nanoparticles. *Horticulturae* 7, 44. horticulturae7030044
- <https://www.namc.co.za/wp-content/uploads/2020/08/South-African-Fruit-flow-report-June-2020-Issue38.pdf>. Retrieved on 20 August 2022.
- Huang, H., Tian, C., Huang, Y. and Huang, H., 2020. Biological control of poplar anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. *Egyptian Journal of Biological Pest Control*, 30(1), pp.1-9.
- Jin, J., Jin, Q., Akoh, C.C. and Wang, X., 2019. Mango kernel fat fractions as potential healthy food ingredients: A review. *Critical Reviews in Food Science and Nutrition*, 59(11), pp.1794-1801.
- Jongsri, P., Rojsitthisak, P., Wangsomboondee, T. and Seraypheap, K., 2017. Influence of chitosan coating combined with spermidine on anthracnose disease and qualities of ‘Nam Dok Mai’ mango after harvest. *Scientia Horticulturae*, 224, pp.180-187.
- Khanzada, M.A., Ansari, A., Rajput, M.A., Maitlo, S., Rajput, A.Q. and Ujjan, A.A., 2018. Effect of different abiotic factors on the growth and sporulation of *Colletotrichum gloeosporioides* causing anthracnose of mango. *Plant Protection*, 2(1), pp.23-30.
- Kumar, A.S., Reddy, N.E., Reddy, K.H. and Devi, M.C., 2007. Evaluation of fungicidal resistance among *Colletotrichum gloeosporioides* isolates causing mango anthracnose in Agri Export Zone of Andhra Pradesh, India. *Plant Pathology Bulletin*, 16(3), pp.157-160.
- Kuo, K.C., 2001. Sensitivity of mango anthracnose pathogen, *Colletotrichum gloeosporioides*, to the fungicide prochloraz in Taiwan. *Proceedings of the National Science Council, Republic of China. Part B, Life Sciences*, 25(3), pp.174-179.

- Lamilla, C.P., Vaudagna, S.R., Alzamora, S.M., Mozgovej, M. and Rodriguez, A., 2021. Effect of the high-pressure assisted-infusion processing on nutritional and antioxidant properties of mango cubes. *Innovative Food Science and Emerging Technologies*, 71, p.102725.
- Léchaudel, M. and Joas, J., 2007. An overview of preharvest factors influencing mango fruit growth, quality, and postharvest behaviour. *Brazilian Journal of Plant Physiology*, 19, pp.287-298.
- Mitra, S.K., 2014, August. Mango production in the world–present situation and future prospect. In *XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): IV 1111* (pp. 287-296).
- Navarro-Herrera, Y.Y. and Ortíz-Moreno, M.L., 2020. Yeast strains with antagonist activity against *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. and their phenotypic characterization. *Egyptian Journal of Biological Pest Control*, 30(1), pp.1-8.
- Ochoa-Velasco, C.E., Pérez-Pérez, J.C., Varillas-Torres, J.M., Navarro-Cruz, A.R., Hernández-Carranza, P., Munguía-Pérez, R., Cid-Pérez, T.S. and Avila-Sosa, R., 2021. Starch edible films/coatings added with carvacrol and thymol: *In vitro* and *in vivo* evaluation against *Colletotrichum gloeosporioides*. *Foods*, 10(1), p.175.
- Oliveira, P.D.L., de Oliveira, K.Á.R., dos Santos Vieira, W.A., Câmara, M.P.S. and de Souza, E.L., 2018. Control of anthracnose caused by *Colletotrichum* species in guava, mango and papaya using synergistic combinations of chitosan and *Cymbopogon citratus* (DC ex Nees) Stapf. essential oil. *International Journal of Food Microbiology*, 266, pp.87-94.
- Panahirad, S., Dadpour, M., Peighambaroust, S.H., Soltanzadeh, M., Gullón, B., Alirezalu, K. and Lorenzo, J.M., 2021. Applications of carboxymethyl cellulose-and pectin-based active edible coatings in preservation of fruits and vegetables: A review. *Trends in Food Science & Technology*, 110, pp.663-673.
- Prasenjiti, K., Nayan, K., and Sutanu, M., 2021. A critical review on use of edible coating to enhance shelf life of mango. *British Journal of Applied Science and Technology*, Past ISSN: 2231-0843, NLM ID: 101664541

- Schneider, D., Stern, R.A., Love, C. and Noy, M., 2013, June. Factors affecting'Omer'and'Maya'mango production in Israeli orchards. In X International Mango Symposium 1075 (pp. 95-102).
- Shafi, J., Tian, H. and Ji, M., 2017. *Bacillus* species as versatile weapons for plant pathogens: a review. *Biotechnology and Biotechnological Equipment*, 31(3), pp.446-459.
- Sivankalyani, V., Maoz, I., Feygenberg, O., Maurer, D. and Alkan, N., 2017. Chilling stress upregulates α -linolenic acid-oxidation pathway and induces volatiles of C6 and C9 aldehydes in mango fruit. *Journal of Agricultural and Food Chemistry*, 65(3), pp.632-638.
- Subramanian, K.S., Sekar, C., Meneka, S.S. and Prakash, L.V., 2018. Reducing postharvest losses in mango in South Asia. Chede Cooperative Union, pp.1-4
- Tesfay, S.Z., Magwaza, L.S., Mditshwa, A. and Mbili, N., 2017, September. Carboxyl methylcellulose (CMC) incorporated with moringa leaf and seed extracts as new postharvest organic edible coating for avocado (*Persea americana* Mill.) fruit. In VII International Conference on Managing Quality in Chains (MQUIC2017) and II International Symposium on Ornamentals in 1201 (pp. 161-168).
- Uddin, M., Shefat, S., Afroz, M. and Moon, N., 2018. Management of anthracnose disease of mango caused by *Colletotrichum gloeosporioides*: A review. *Acta Scientific Agriculture*, 2(10), pp.169-177.
- Jenny, F., Sultana, N., Islam, M., Khandaker, M.M. and Bhuiyan, M.A.B., 2019. A review on anthracnose of mango caused by *Colletotrichum gloeosporioides*. *Bangladesh Journal Plant Pathology*, 35(1), pp.65-74.
- Singh, Z., Singh, R.K., Sane, V.A. and Nath, P., 2013. Mango-postharvest biology and biotechnology. *Critical Reviews in Plant Sciences*, 32(4), pp.217-236.
- Thakor, N.J., 2019. Indian mango-production and export scenario. *Peach*, 18(107), pp.0-12.
- Tharanathan, R.N., Yashoda, H.M. and Prabha, T.N., 2006. Mango (*Mangifera indica* L.), "The king of fruits"-An overview. *Food Reviews International*, 22(2), pp.95-123.

Yadav, A.S. and Pandey, D.C., 2016. Geographical perspectives of mango production in India. Imperial Journal of Interdisciplinary Research, 2(4), pp.257-265.

Thakur, J.P., Gothwal, P.P. and Singh, I., 2017. Post-harvest treatments for extension of mango fruit var. Dashehari (*Mangifera indica*, L.). International Journal of Food Science and Nutrition, 2(4), pp.156-162.

Chapter 2

Literature review

2.1 Introduction

Mango (*Mangifera indica* L.) is one of the most commonly grown and consumed fruits in subtropical and tropical regions (Rajwana *et al.*, 2011). *Mangifera* genus contains about 49 species with *Mangifera indica* L. as the most important species (Tharanathan *et al.*, 2006). The most prevalent are ‘Tommy Atkins’, ‘Haden’, ‘Ataulfo’, ‘Kent’, ‘Keitt’ and ‘Alphonso’ (Ntsoane *et al.*, 2019). It has been widely grown in Asia for its edible qualities and has spread worldwide (Arauz, 2000). In South Africa, mango production is around 93870 tons per season. The main producing province is Limpopo with the highest production followed by Mpumalanga, KwaZulu Natal and Western Cape (South African Fruit flow, 2020). Mango peel has a high concentration of polyphenolic properties and has been used as traditional medicine in some regions such as India, Cuba, and Australia (Umamahesh *et al.*, 2020; Núñez Sellés *et al.*, 2002)

Mango tends to degrade rapidly postharvest, especially at ambient temperature where most fungal pathogens grow faster (Khanzada *et al.*, 2018; Jenny *et al.*, 2019). Storage under controlled temperature is required to prevent post-harvest losses, also creating unfavourable conditions for fungal pathogens is considered one of the cheapest control strategies against the growth of fungal pathogens (Arampath and Dekker, 2019). Ambient temperature accelerates the ripening process and reduces shelf life (Himmam *et al.*, 2021). However, prolonged shelf life is compulsory commercially to maximise profits. During the peak season, harvested mango is stored in storages with controlled temperatures to reduce ripening and the growth of fungal diseases such as stem-end rot (SER) and anthracnose (Arampath and Dekker, 2019).

The experiment of storage under controlled temperatures on mango fruit was conducted by Yasunaga *et al.* (2018), using 15°C, 25°C, and 35°C during storage and transportation of mango fruits. This study reported that a decrease in temperature from >10°C caused chilling injury on mango fruits. Whereas temperatures from 15-20°C maintained fruit quality during the export duration of about 2-3 weeks. This indicates that for long time storage, mango fruits can be stored at temperatures between 15-20°C.

Favourable temperatures for *C. gloeosporioides* were also investigated by (Khanzada *et al.*, 2018). The rapid growth of mycelia for this pathogen was observed at temperatures greater than 20°C. This shows the efficacy of storage under controlled temperatures. *Lasiodiplodia theobromae* had the maximum growth at 25-30°C, little to no growth at too low and high temperatures (Vijay *et al.*, 2021). From both experiments, a conclusion can be drawn that at low temperatures shelf life and quality of mango are maximised whilst compromising the growth and sporulation of some fungal pathogens.

Relative humidity is one of the important factors in maintaining the quality of mango and minimizing postharvest losses by reducing respiration (Hussen, 2021). For most fruits and vegetables, the optimum relative humidity ranges from 80-95%, these include loquat fruit, mango, cucumber, eggplant, strawberry, and raspberry (Kahramanoğlu, 2020; Hussen 2021; Shoji *et al.*, 2022). Controlled relative humidity during storage is essential for reducing mass loss caused by deterioration and the severity of the chilling injury (Shoji *et al.*, 2022). Relative humidity is also important for the growth and sporulation of fungal pathogens (Kulkarni, 2019). Moist environments usually favour the growth of most fungal diseases such as SER and anthracnose (Uddin *et al.*, 2018), hence sanitation of the storage and crates is one of the cheapest methods in reducing the inoculum (Lee *et al.*, 2004), also controlling storage temperature while creating unfavorable conditions for the growth of fungal pathogens (Khanzada *et al.*, 2018).

Mango has a high postharvest loss, mostly due to moisture loss through transpiration, which has a major contribution on the total fruit mass loss (Bambalele *et al.*, 2021). It is important to note that mango is sold by weight and this factor contributes to the appearance, colour, and texture of the fruit which is affected greatly by moisture loss (Subramanian *et al.*, 2018). Loss of weight of fruits is an important issue for increasing shelf life and overall acceptability of fruits (Prasenjit *et al.*, 2021).

2.2 Anthracnose

The mango industry remains uncertain of production and profits due to the fungal pathogens that negatively affects the production, yield, and profits generated. Amongst the diseases, anthracnose of mango caused by the pathogen *C. gloeosporioides* is one of the major plant pathogens with a wide host range.

2.2.1 Pathogen taxonomy and morphology

C. gloeosporioides is the causal agent of anthracnose, from the family *Glomerellaceae*, genus *Colletotrichum*, and *gloeosporioides* as the species (Ajay Kumar, 2014). Spores are produced asexually by *C. gloeosporioides*. This fungal pathogen produces conidial spores of a cylindrical shape with a variation in dimension, as observed by different studies, with an average of about 5-7 μm width and 20 μm length (Ajay Kumar, 2014). This great variation in size and shape of the conidia of *C. gloeosporioides* is associated with the host from which the pathogen is isolated and its area of origin (Nelson, 2008). The mycelium of *C. gloeosporioides* varies, may grow to be white, peach to light yellowish on a petri dish with PDA (Mu,*et al.*, 2021).

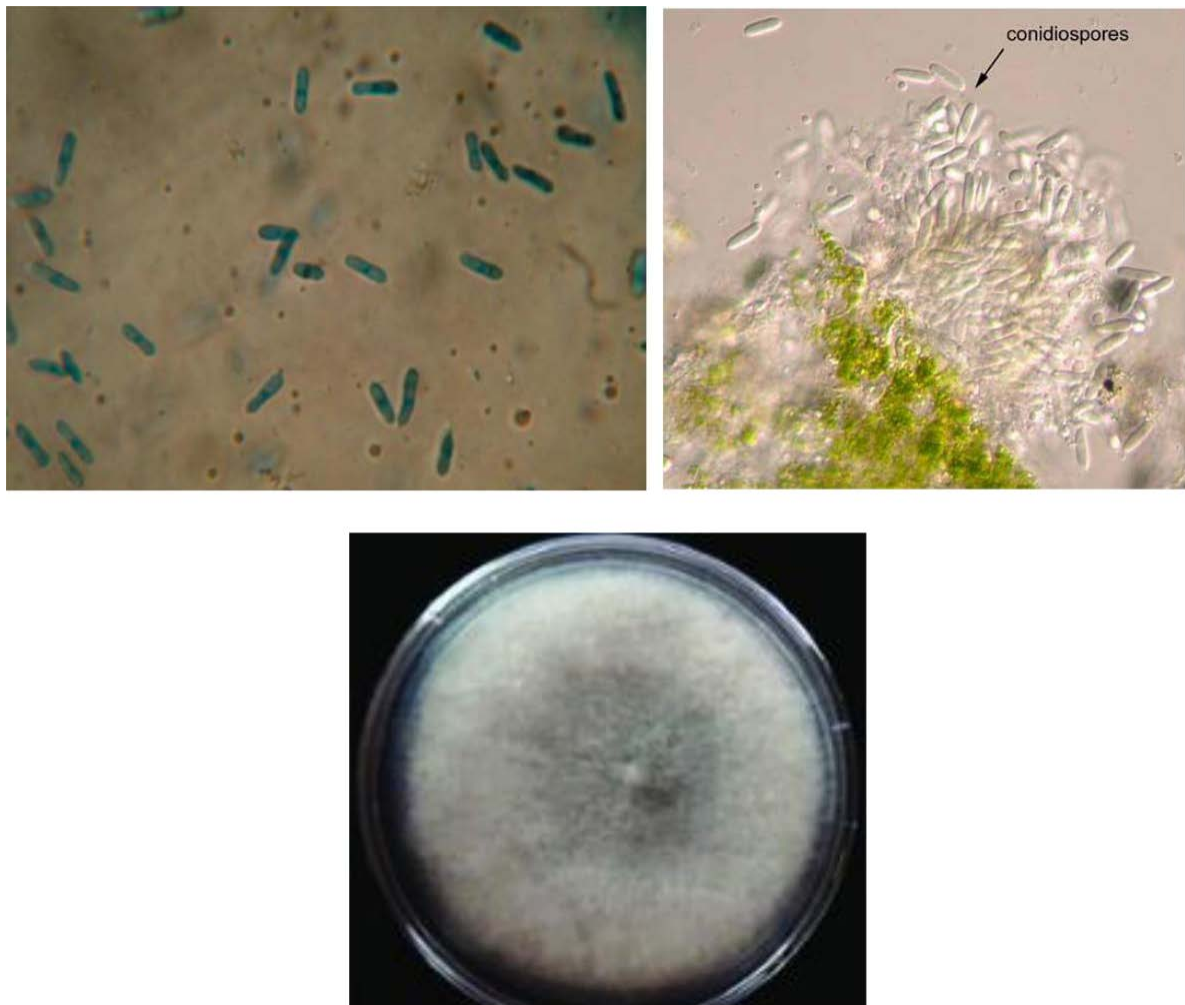


Figure 2.1: Conidia of *Colletotrichum gloeosporioides* (Nelson, 2008; Ajay Kumar, 2014; Mu,*et al.*, 2021).

2.2.2 Economic importance

Collectotrichum gloeosporioides is a fungal pathogen causing anthracnose on many fruits and vegetables postharvest. This was discovered in the early years, 1867 (Dutta, 1958). Anthracnose has a wide host range, including banana, mango, citrus fruits, pepper, chillies, guava, papaya, and avocado (Alahakoon and Brown, 1994; Sakinah *et al.*, 2014; Sharma and Kulshrestha, 2015; Florida and China, 2016). This fungal pathogen causes devastating losses on horticultural produce, affecting yield and production. The study reporting first incidence of anthracnose by Awa *et al.* (2012), in Nigeria was conducted and 231 fungal pathogens were isolated from mango trees. 96 isolates were *C. gloeosporioides* and they also reported that sixty percent of mango trees surveyed were found infected with anthracnose and over 34% of fruits produced on those trees were found severely infected. A report on severe epidemics in Thailand resulted in 80% loss of chillies (Ren *et al.*, 2020). These losses negatively impact production of many fruits and vegetables.

2.2.3 Epidemiology, infection process and disease cycle

The life cycle of *C. gloeosporioides* that causes anthracnose in mango is illustrated in Figure 2.2 (Uddin *et al.*, 2018). The major source of inoculum is the infected plant material. Anthracnose is favoured by wet, humid, warm conditions and spread by infected seeds, rain splash, and moist winds. When the rain splashes on the infected plants and spreads the inoculum to both leaves and fruits (Uddin *et al.*, 2018) it often results in fruit drop and fruit rot.

The fungus primarily invades injured or weakened tissues of plants and produces various specialized structures during the infection process (Arauz, 2000). These specialized structures, conidia, acervulli, and appressoria are formed during the interaction between the host and pathogen. *C. gloeosporioides* colonises injured plant tissue, and forms number of acervuli and conidia (Arauz, 2000). The infected leaves are identified by lesions that later spread to developing fruits ready to be harvested. Conidia can be spread over relatively short distances.

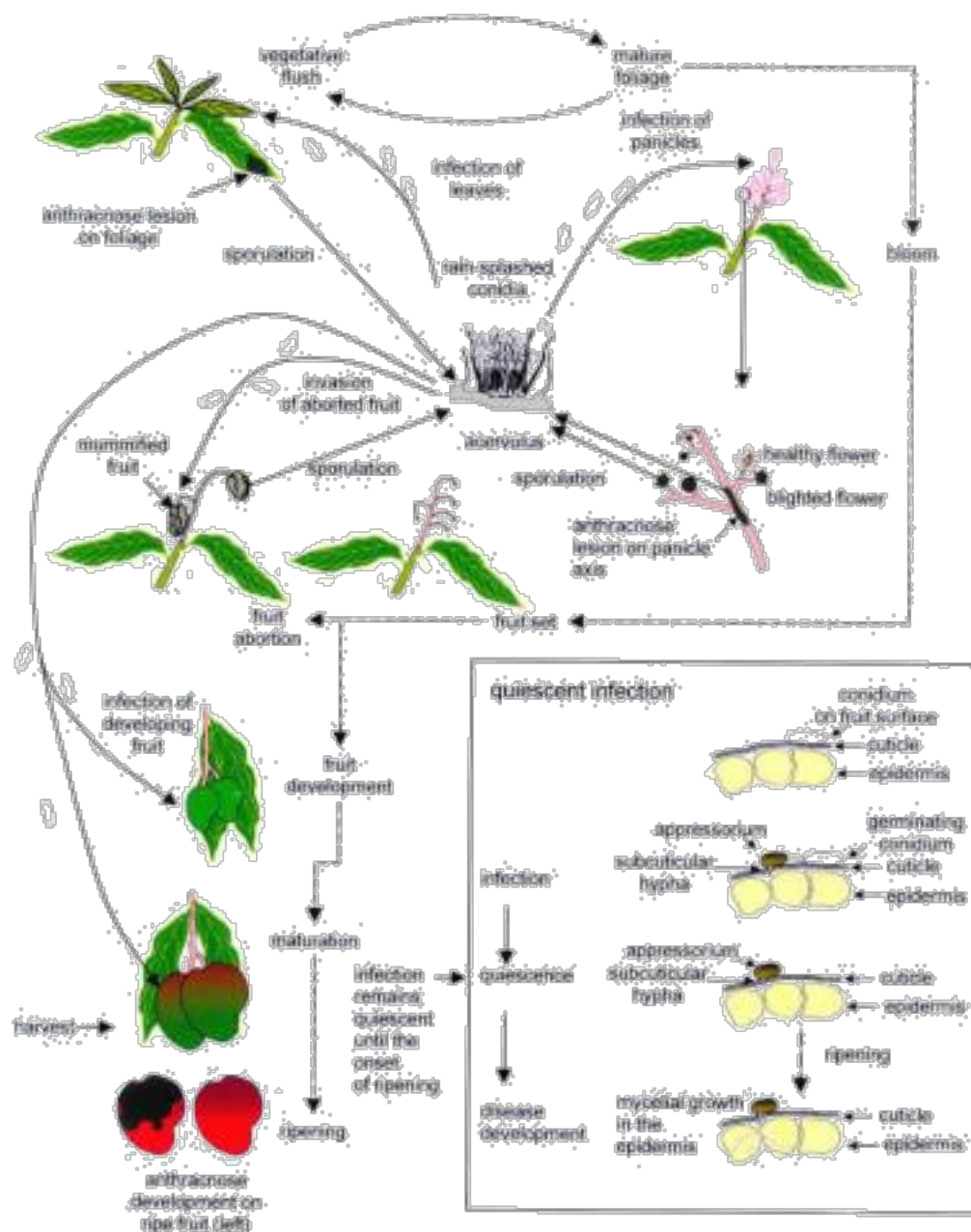


Figure 2.2: Anthracnose disease cycle on mango with solid lines and dotted lines representing mango phenology (Arauz, 2000).

2.2.4 Symptoms

Anthracnose originates from infections such as bruises on fruits (Figure 2.3 A, B, C, and D) (Arauz, 2000). The fungus causes lesions on both young and old leaves of mango preharvest leading to the observed dead tissues which spread towards the main veins (Arauz 2000) (Figure 2.3A). Postharvest handling exposes the fruit to wounds caused by puncturing, and the infection begins on the wounds and then spreads to the whole fruit. Figure 2.3 B shows the watery spots, and sunken black lesions on fruit (Troncoso-Rojas and Tiznado-Hernandez,

2014). Irregular dark brown to black lesions that later cause fruit hardening is indicated in Figure 2.3C (Sharma, and Kulshrestha, 2015). Severe symptoms of anthracnose are linear necrotic regions lending an alligator skin effect, that leads to cracking of the epidermis (Hossain 2017).

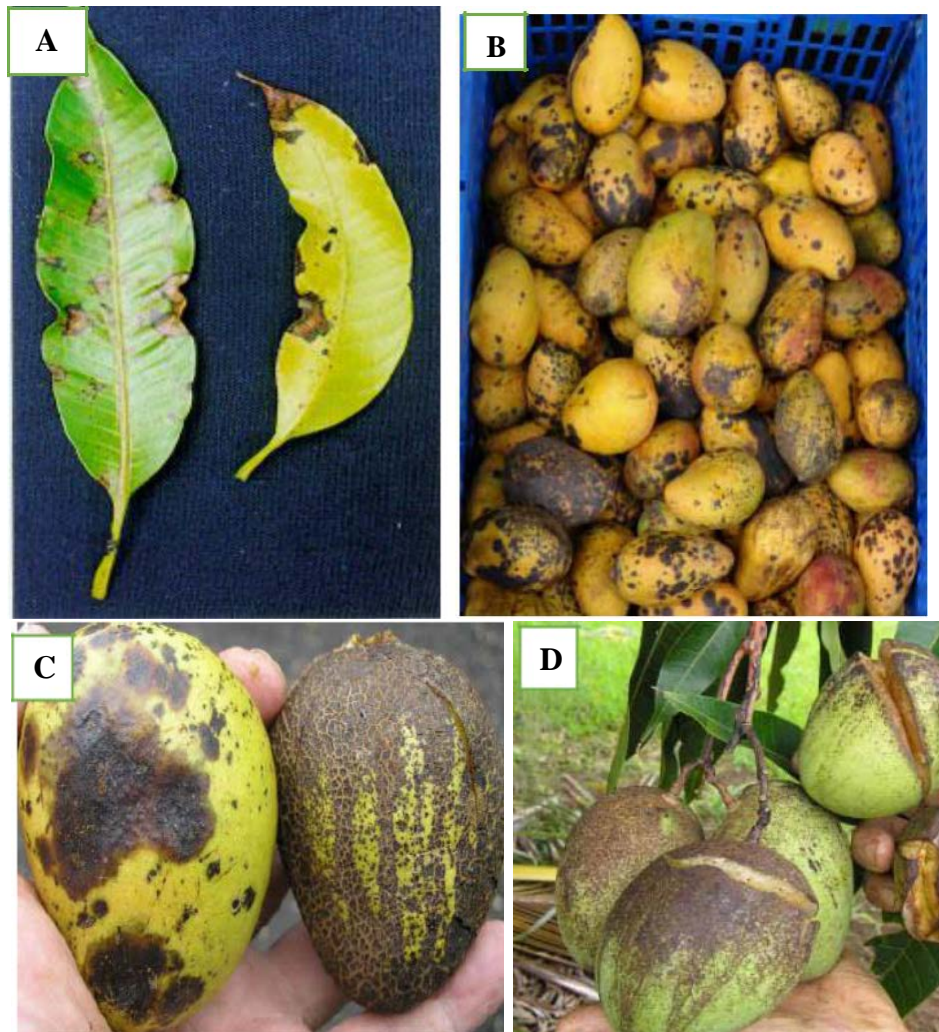


Figure 2.3: Pre and postharvest infection and symptoms caused by anthracnose on mango (Paudel *et al.*, 2022).

2.3 Management strategies of anthracnose

There are many diseases caused by fungi, and most of them have negative impacts on the production of many fruit crops and vegetables (Diskin, *et al.*, 2019). There are disease management strategies that are currently being used for controlling fungal diseases (Ons *et al.*, 2020). Preventative and curative actions are continuously used to prevent infection caused by fungal pathogens and to reduce and manage the inoculum strength and contamination by

mycotoxins, respectively (Nunes, 2012). These management strategies include chemical, physical, and biological control.

2.3.1 Chemical treatments

The use of fungicides has been at its peak in the past few years and has successfully controlled fungal diseases on different crops. Mancozeb, prochloraz, thiabendazole, and fludioxonil are registered fungicides that have been used continuously in controlling fungal diseases of mango (Tesfay, *et al.*, 2017; Banya, *et al.*, 2020). The excessive use of the fungicides is slowly leading to the loss of efficacy against the fungal pathogens, and the development of fungicide resistance (Banya, *et al.*, 2020; Jeyaraj, *et al.*, 2023).

As reviewed by Saxena *et al.* (2016), azoxystrobin has been reported to increase shelf life and the quality of plants including controlling the anthracnose caused by the pathogen *C. gloeosporioides*. However, azoxystrobin is among many fungicides that pose some serious health and safety hazards when ingested, as these environmentally unfriendly fungicides are toxic and are not for human consumption (Saxena *et al.*, 2016). The toxicity and harmful effects that come with synthetic fungicides can cause bodily harm, hospitalisation, or death (Tao *et al.*, 2020). These are the undesirable effects of using chemicals that may also affect the income of the farmers and other environmental concerns (Banya *et al.*, 2020).

2.3.1.1 Prochloraz

Prochloraz is an imidazole fungicide that has been reported to successfully control many fungal diseases for many decades in most parts of the world (Vinggaard *et al.*, 2006). This was used to control infections and reduce inoculum while maximizing production. This fungicide has been discovered to have unfavourable toxicological properties (Shimshoni *et al.*, 2020), leading to the use of the fungicide being discontinued in most parts of the world. The use of this fungicide against fungal pathogens was banned globally in 2021 (Mokgalapa *et al.*, 2022).

2.3.1.2 Thiabendazole

Thiabendazole is a benzimidazole fungicide that is systemic. This fungicide has the advantage of preventing fungal infections compared to the most contact fungicides that control and

eliminate mycelial growth (Feng *et al.*, 2018). However, fungicide resistance by pathogens is increasing. Resistance to methyl benzimidazole carbamate (MBC) fungicides is mainly associated with changes in amino acid sequence caused by mutations in codons 6, 50, 167, 198, 200, and 240 of the β -tubulin gene (Mora-Aguilera *et al.*, 2021). More than 115 species of pathogenic fungi are reported to be resistant to benzimidazole products (Mora-Aguilera *et al.*, 2021). This indicates the reduced effectiveness of the fungicides.

2.3.1.3 Fludioxonil

This fungicide was tested because of its potential and effectiveness in inhibiting the decay postharvest, comparing fludioxonil with prochloraz (Shimshoni *et al.*, 2020). It was reported that fludioxonil is suitable for replacing the discontinued prochloraz. This was based on the experiment of both chemicals against stem-end rot of avocado caused by *L. theobromae* (Shimshoni, *et al.*, 2020). López-Velázquez, *et al.* (2022) reported the use of other chemical against a fludioxonil-resistant strain of *C. gloeosporioides*, this declares fludioxonil as one of the fungicides that are not eligible to be used against some of the major fungal pathogens affecting mango production, and it needs to be attended to.

2.3.1.4 Mancozeb

Mancozeb is a non-systemic fungicide that has been used to control fungal diseases of fruits and vegetables (Carniel *et al.*, 2019). Previous research was conducted using different fungicides, and mancozeb had the highest percentage inhibition against the mycelial growth of *C. gloeosporioides* (Satapathy and Beura, 2019). This fungicide has been reported to be harmful to humans hence there are reports of it being banned as a fungicide (Saha *et al.*, 2022). Bayoumi (2022) reported harmful effects of toxic fungicides on human health which lead to congenital malformation, neurological, respiratory, dermal, and reproductive effects. This may also lead to death due to intoxication.

2.3.2 Biological control

The use of fungicides is declining due to concerns surrounding their safety. Most fungicides are being banned as they pose a threat and negative impacts both on the environment and human health (Bayoumi, 2022), also most fungal pathogens are gradually

increasing resistance to these fungicides (Mora-Aguilera *et al.*, 2021). Hence, the intense search for safer ways of controlling fungal pathogens without compromising the quality and quantity of the production. There are currently ongoing studies on the use of more environmentally friendly, and safer control measures (Moodley, *et al.*, 2021). Biological control agents (BCAs) are now the main focus alternative to toxic fungicides. This includes the application of other microorganisms to control the harmful fungal pathogens without harming the host (Mbili, 2012). BCAs have a large habitat, as they are present in soil, water, roots, and on plant surfaces, and can be isolated from these sources (Zhang, *et al.*, 2019). Yeast, fungi, and bacteria are some of the explored BCAs in controlling fungal diseases (Moodley, 2021).

Biocontrol agents have different mechanisms of action, such as the production of antimicrobial substances, induced systemic resistance, parasitism, signal interference, nutrient and space competition (Figure 2.4) (Chen *et al.*, 2016). These mechanisms enable the effectiveness of these microorganisms against pathogenic fungi.

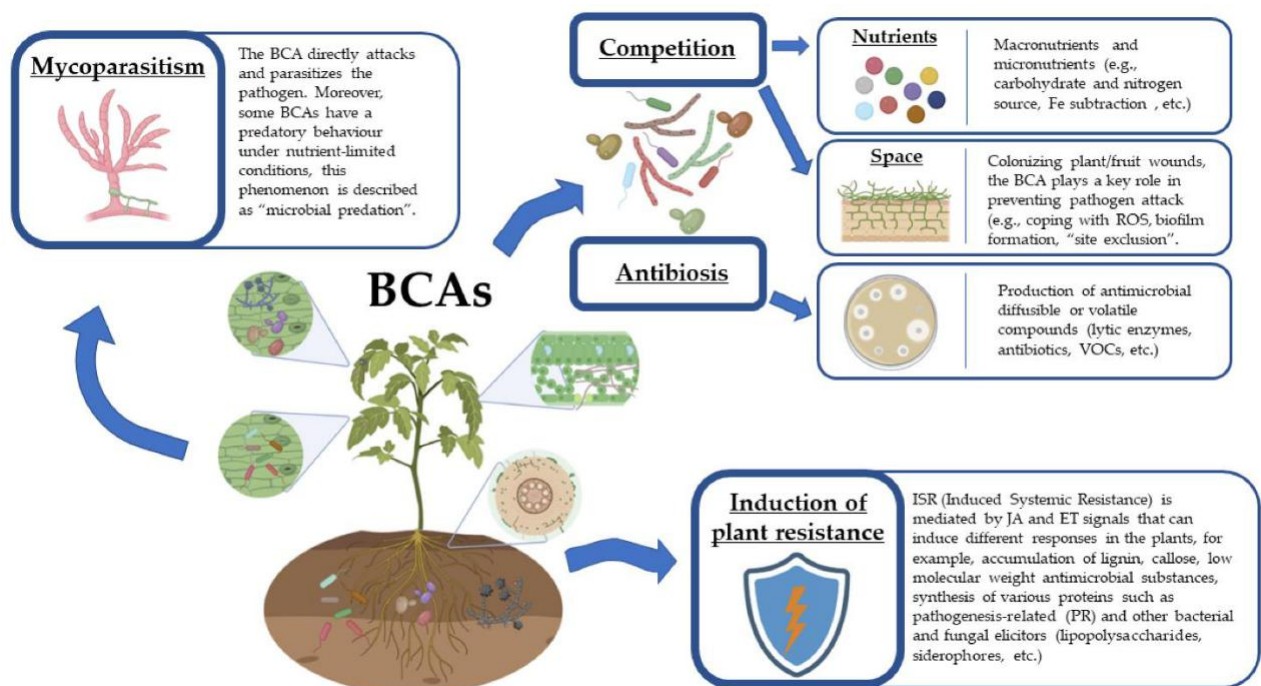


Figure 2.4: Mechanisms of action by biological control agents against plant diseases caused by pathogenic fungi (Palmieri *et al.*, 2022).

Mycoparasitism is a direct interaction or attack where the biological control agent parasitizes the fungal pathogen. This reduces the inoculum, sporulation, and further growth of the pathogen, whereas antibiosis inhibits the growth or kills another microorganism, by making

use of the volatile antimicrobial compounds produced (Palmieri *et al.*, 2022). Competing for nutrients and space is also one of the strategies used in the reduction of the inoculum, this is based on the rapid growth of BCA's and occupation against pathogenic fungi. Microorganisms may also be used to induce plant resistance by enhancing their defence capacity, reducing the susceptibility of plants against pathogenic fungi, and using induced systemic resistance (ISR) (Moodley *et al.*, 2021). Scientists have been exploring these mechanisms to report on the efficacy of the use of BCAs (Dwiastuti *et al.*, 2021).

2.3.2.1 Yeasts

Natural yeasts explored as biocontrol agents, including *Candida oleophila*, *Aureobasidium pullulans*, *Metschnikowia fructicola*, *Cryptococcus albidus*, *Saccharomyces cerevisiae*, have been registered and may be applied as biocontrol agents against fungal diseases (Freimoser *et al.*, 2019). Yeast is one of the antagonistic micro-organisms that is non-pathogenic, with a simple nutritional requirement. The use of yeast is advantageous as it colonises a wide range of substrates, the ability to adapt to the fruits containing high sugar concentrations for a long period (Konsue, *et al.*, 2020).

2.3.2.2 *Bacillus* spp.

Bacteria are also used in controlling many fungal diseases on fruits and *Bacillus subtilis*, *B. amyloliquefaciens* and *B. velezensis* are among the registered antagonistic bacteria reported. *Bacillus* species demonstrate great antimicrobial activity and hence are used against fungal diseases in vegetables (Chen *et al.*, 2016; Di Canito *et al.*, 2021; Kim *et al.*, 2021). *Bacillus* spp. consist of a diverse secondary metabolites and the ability to produce a variety of structurally different antagonistic substances, these species are also heat tolerant (Zhang, *et al.*, 2019).

2.3.3 Physical control

2.3.3.1 Hot Water Treatment (HWT)

Treatment with hot water at the post-harvest stage controls most fungal diseases, when spores are located on the surface of fruits and vegetables. This method is linked to the reduction of pathogen spore germination and mycelial growth and plant or fruit tissues respond using their defence by secreting enzymes to alleviate the damage caused by the stress (Siddiqui and Ali, 2014).

According to Bambalele *et al.* (2021a) HWT maintains the colour and the appearance of the fruit peel. Dutra *et al.* (2018) reported that dipping passion fruits in hot water for 5 min at 47°C showed the most efficacy. Temperatures of 42.5°C and 45°C have been reported to reduce post-harvest rot caused by fungal pathogens such as *C. gloeosporioides*, *Cladosporium sp.*, *Phomopsis sp.*, *Fusarium spp.* and *Rhizopus spp.* (Dutra *et al.*, 2018).

Hot-water treatment is effective directly against pathogens and on fruit physiology, delaying biochemical ripening by reducing ethylene secretion, senescence and reducing the respiratory rate for an extended shelf-life (Dutra *et al.*, 2018). It may also be integrated with other edible coatings such as CMC, phosphite, 1-MCP, and chitosan (1-methylcyclopropene) (Khalil, *et al.*, 2022). However, there are factors affecting mango fruits negatively, such as maturity stage, cultivars, exposure time, and temperature. Small and immature fruits are easily damaged as they are less heat tolerant, this may result in mechanical damage and fruits with poor quality. Prolonged exposure to high temperatures for a long time can cause heat-induced injury, and weight loss leading to rapid fruit decay (Bambalele *et al.*, 2021a).

2.3.4 Plant extracts

The use of synthetic fungicides is decreasing as fungal pathogens such as *L. theobromae*, *C. gloeosporioides*, and *Alternaria alternata* are slowly becoming resistant to their effectivity (Mora-Aguilera *et al.*, 2021). Hence the dosage used is gradually increasing, posing a great threat to human health because the maximum residue limit (MRL) must be adhered to (Arauz, 2000). Plant extracts are among the control measures that are considered safe, natural, biodegradable, bio-efficacious, economically, and environmentally safe. These are currently used to control and manage infection by phytopathogens (Baka and Mousa, 2020). Plant extracts are used mostly for their antimicrobial and antifungal activities (Alvindia and Mangoba, 2020). The reports from the research conducted on plant extracts in controlling anthracnose have shown the efficacy and the potential of *Moringa oleifera* plant extract (Alvindia *et al.*, 2022).

2.3.4.1 *Pelargonium sidoides*

Pelargonium has many species that have been reported as natural products for medicinal properties, as they have become an important part of the healthcare department for many people (Aboobaker *et al.*, 2019). The antimicrobial potential of *P. sidoides* was tested by Aboobaker *et al.* (2019) and showed the ability as the endophytic fungi against *Pseudomonas aeruginosa*

and *Staphylococcus aureus*. However, this natural extract is effectively used in fever infections and neutralises some bacteria and viruses (Jekabsone *et al.*, 2019). There is not enough research and reports on *Pelargonium* in controlling the fungal pathogens.

2.3.4.2 *Tetradenia riparia*

Tetradenia riparia is one of the indigenous herbaceous plants recognized for its medicinal properties. It originated in Central, Eastern, and Southern Africa and has now spread to most parts of the world (Shimira, 2022). This plant is effective against human diseases such as malaria, dental abscesses, headache, and worm infections (Van Puyvelde *et al.*, 2018), mouth ulcers, toothache, cough, colds, and flu (Shimira, 2022). There is little to no literature cited currently on *Tetradenia riparia* as a plant extract against fungal pathogens.

2.3.4.3 *Aloe vera*

Aloe vera is a well-known medicinal plant that is usually used for many therapeutic purposes (Danish *et al.*, 2020). This plant is naturally composed of many useful compounds and is used for its antifungal and antibacterial activity against different strains of fungal pathogens and bacteria. It has been reported to have a positive effect on burning wounds, as it has anti-inflammatory and immunomodulatory effects (Choi, and Chung 2003). The active compounds reported in this plant are saponins, sugars, enzymes, vitamins, aloesin, aloemodin, aloin, acemannan aloemannan, aloeride, methylchromones, flavonoids, naftoquinones, sterols, minerals, anthraquinones, amino acids, lignin and salicylic acid and other different compounds including fat-soluble and water-soluble vitamins, enzymes, minerals, simple/complex sugars, organic acid and phenolic compounds (Danish, *et al.*, 2020).

Aloe vera was used as a coating gel in mango fruits by Shah and Hashmi, (2020) and influenced the storage and shelf life of mango by minimising the decay, reduced weight loss, ethylene production, and respiration rate. This had a positive effect on fruit quality parameters such as titratable acidity (TA), total soluble solids (TSS), fruit firmness, ascorbic acid, and peel colour as they were also retained by the treatment (Shah and Hashmi, 2020). Not much research has been done on the effect of *A. vera* in controlling fungal pathogens, however the research done by Kankamol *et al.*, 2021 on the effect of *A. vera* against *C. gloeosporioides* showed that the

increase in *A. vera* concentration also increased the effectivity, resulting in a reduced mycelial growth of the pathogen.

2.3.4.4 *Moringa oleifera*

Moringa oleifera is considered one of the safest plants to be used as plant extracts against pathogenic fungi (Alvindia and Mangoba, 2020). This plant extract prohibits the growth of post-harvest diseases because of its antimicrobial activity (Zakawa *et al.*, 2020). It is also used because it is a good source of phytochemical compounds with potential use in pharmaceuticals, biodiesel, and functional food applications (Alvindia and Mangoba, 2020). The efficacy of *M. oleifera*, applied with CMC as an edible coating on mango fruit was reported by Bambalele *et al.* (2019) and had the least fruit decay and mycelial growth. *Moringa* has also been identified to comprise natural antioxidants because of the presence of phenolics, carotenoids, flavonoids, and ascorbic acid (Bambalele *et al.*, 2021).

2.4 Conclusion

Mango is a commercial fruit that is economically important. The factors such as high perishability, diseases, pest infestation, and susceptibility to chilling injury must be adhered to maximize the production of fruits with high quality. This then allows the full exploitation of biological control agents and plant extracts as they are reported as safe and cheaper methods of controlling plant diseases. It is also important to note environmental factors such as temperature and relative humidity as they contribute in extending shelf life of mango while controlling the mycelial growth of fungal pathogens. These control strategies are effective as they minimise the use of harmful and toxic chemicals that have a negative impact on both the environment and human health.

2.5 References

- Aboobaker, Z., Viljoen, A., Chen, W., Crous, P.W., Maharaj, V.J. and Van Vuuren, S., 2019. Endophytic fungi isolated from *Pelargonium sidoides* DC: antimicrobial interaction and isolation of a bioactive compound. *South African Journal of Botany*, 122, pp.535-542.
- Alahakoon, P.W. and Brown, A.E., 1994. Host range of *Colletotrichum gloeosporioides* on tropical fruit crops in Sri Lanka. *International Journal of Pest Management*, 40(1), pp.23-26.
- Ajay Kumar, G., 2014. *Colletotrichum gloeosporioides*: biology, pathogenicity, and management in India. *Journal of Plant Physiology and Pathology*, 2(2), pp.2-11.
- Alvindia, D.D.G. and Mangoba, M.A.A., 2020. Biological activities of *Moringa oleifera* Lam. against anthracnose of mango caused by *Colletotrichum gloeosporioides* Penz. *Archives of Phytopathology and Plant Protection*, 53(13-14), pp.659-672.
- Saxena, A., Sarma, B.K. and Singh, H.B., 2016. Effect of azoxystrobin based fungicides in management of chilli and tomato diseases. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 86, pp.283-289.
- Alvindia, D.D.G., Mangoba, M.A.A., Abduzakhurov, J.T. and Santiago, E.F., 2022. Biocidal activities of *Curcuma longa* L. against anthracnose of mango fruits caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. *Archives of Phytopathology and Plant Protection*, 55(5), pp.515-526.
- Ren, L., Wang, S.F., Shi, X.J., Cao, J.Y., Zhou, J.B. and Zhao, X.J., 2020. Characterisation of sensitivity of *Colletotrichum gloeosporioides* and *Colletotrichum capsici*, causing pepper anthracnose, to picoxystrobin. *Journal of Plant Diseases and Protection*, 127, pp.657-666.
- Florida, I. and China, B.P., 2016. First report of anthracnose of carambola caused by *Colletotrichum gloeosporioides* in Northeast India. *Indian Journal of Plant Protection*. 44(4), pp.479-480.
- Sakinah, M.I., Suzianti, I.V. and Latiffah, Z., 2014. Phenotypic and molecular characterization of *Colletotrichum* species associated with anthracnose of banana (*Musa* spp.) in Malaysia. *Genetics and Molecular Research*, 13(2), pp.3627-3637.
- Arampath, P.C. and Dekker, M., 2019. Bulk storage of mango (*Mangifera indica* L.) and pineapple (*Ananas comosus* L.) pulp: effect of pulping and storage temperature on

- phytochemicals and antioxidant activity. *Journal of the Science of Food and Agriculture*, 99(11), pp.5157-5167.
- Arauz, L.F., 2000. Mango anthracnose: Economic impact and current options for integrated management. *Plant Disease*, 84(6), pp.600-611.
- Baka, Z.A. and Mousa, M.M., 2020. *In vitro* and *in vivo* biocontrol activity of extracts prepared from Egyptian indigenous medicinal plants for the management of anthracnose of mango fruits. *Archives of Phytopathology and Plant Protection*, 53(15-16), pp.715-730.
- Bambalele, N., Mditshwa, A., Tesfay, S.Z. and Magwaza, L.S., 2019, November. Moringa leaf extract infused into carboxymethyl cellulose edible coating combined with gaseous ozone as postharvest treatments maintain quality and extend shelf life of mango fruit. In II International Symposium on Moringa 1306 (pp. 225-232).
- Bambalele, N.L., Mditshwa, A., Magwaza, L.S. and Tesfay, S.Z., 2021. The effect of gaseous ozone and moringa leaf-carboxymethyl cellulose edible coating on antioxidant activity and biochemical properties of 'Keitt' mango fruit. *Coatings*, 11(11), p.1406.
- Bambalele, N.L., Mditshwa, A., Magwaza, L.S. and Tesfay, S.Z., 2021. Recent advances on postharvest technologies of mango fruit: A review. *International Journal of Fruit Science*, 21(1), pp.565-586.
- Banya, M., Garg, S. and Meena, N.L., 2020. A review: Chilli anthracnose, its spread and management. *Journal of Pharmacognosy and Phytochemistry*, 9(4), pp.1432-1438.
- Bayoumi, A.E., 2022. Deleterious effects of banned chemical pesticides on human health in developing countries. *Pesticides-Updates on Toxicity, Efficacy and Risk Assessment*, p.169.
- Carniel, L.S.C., Niemeyer, J.C., de Oliveira Filho, L.C.I., Alexandre, D., Gebler, L. and Klauberg-Filho, O., 2019. The fungicide mancozeb affects soil invertebrates in two subtropical Brazilian soils. *Chemosphere*, 232, pp.180-185.
- Chen, J.L., Sun, S.Z., Miao, C.P., Wu, K., Chen, Y.W., Xu, L.H., Guan, H.L. and Zhao, L.X., 2016. Endophytic *Trichoderma gamsii* YIM PH30019: a promising biocontrol agent with hyperosmolar, mycoparasitism, and antagonistic activities of induced volatile organic compounds on root-rot pathogenic fungi of *Panax notoginseng*. *Journal of Ginseng Research*, 40(4), pp.315-324.

- Choi, S. and Chung, M.H., 2003, March. A review on the relationship between *Aloe vera* components and their biologic effects. In *Seminars in integrative medicine* (Vol. 1, No. 1, pp. 53-62). WB Saunders.
- Subramanian, K.S., Sekar, C., Meneka, S.S. and Prakash, L.V., 2018. Reducing postharvest losses in mango in South Asia. Chede Cooperative Union, pp.1-4
- Danish, P., Ali, Q., Hafeez, M.M. and Malik, A., 2020. Antifungal and antibacterial activity of *Aloe vera* plant extract. *Biological and Clinical Sciences Research Journal*, 2020(1).
- Di Canito, A., Mateo-Vargas, M.A., Mazzieri, M., Cantoral, J., Foschino, R., Cordero-Bueso, G. and Vigentini, I., 2021. The role of yeasts as biocontrol agents for pathogenic fungi on postharvest grapes: A review. *Foods*, 10(7), p.1650.
- Diskin, S., Sharir, T., Feygenberg, O., Maurer, D. and Alkan, N., 2019. Fludioxonil - a potential alternative for postharvest disease control in mango fruit. *Crop Protection*, 124, p.104855.
- Dutra, J.B., Blum, L.E.B., Lopes, L.F., Cruz, A.F. and Uesugi, C.H., 2018. Use of hot water, combination of hot water and phosphite, and 1-MCP as post-harvest treatments for passion fruit (*Passiflora edulis* f. *flavicarpa*) reduces anthracnose and does not alter fruit quality. *Horticulture, Environment and Biotechnology*, 59(6), pp.847-856.
- Dwiastuti, M.E., Soesanto, L., Aji, T.G., Devy, N.F. and Hardiyanto, 2021. Biological control strategy for postharvest diseases of citrus, apples, grapes and strawberries fruits and application in Indonesia. *Egyptian Journal of Biological Pest Control*, 31, pp.1-12.
- Feng, J., Hu, Y., Grant, E. and Lu, X., 2018. Determination of thiabendazole in orange juice using an MISPE-SERS chemosensor. *Food Chemistry*, 239, pp.816-822.
- Freimoser, F.M., Rueda-Mejia, M.P., Tilocca, B. and Migheli, Q., 2019. Biocontrol yeasts: mechanisms and applications. *World Journal of Microbiology and Biotechnology*, 35(10), pp.1-19.
- Hmmam, I.; Zaid, N.;Mamdouh, B.; Abdallatif, A.;Abd-Elfattah, M.; Ali, M., 2021. Storage Behavior of “Seddik” Mango Fruit Coated with CMC and Guar Gum-Based Silver Nanoparticles. *Horticulturae* 7, 44. horticulturae7030044

- Hossain, M., 2017. Management of postharvest anthracnose disease of mango caused by *Colletotrichum gloeosporioides* (Doctoral dissertation, Department of Plant Pathology, Sher-e-Bangla Agricultural University).
- Hussen, A., 2021. Impact of temperature and relative humidity in quality and shelf life of mango fruit. *International Journal of Horticulture and Food Science*, 3(1), pp.46-50.
- Tharanathan, R.N., Yashoda, H.M. and Prabha, T.N., 2006. Mango (*Mangifera indica* L.), “The king of fruits” - An overview. *Food Reviews International*, 22(2), pp.95-123.
- Jekabsone, A., Sile, I., Cochis, A., Makrecka-Kuka, M., Laucaityte, G., Makarova, E., Rimondini, L., Bernotiene, R., Raudone, L., Vedlugaite, E. and Baniene, R., 2019. Investigation of antibacterial and antiinflammatory activities of proanthocyanidins from *Pelargonium sidoides* DC root extract. *Nutrients*, 11(11), p.2829.
- Jenny, F., Sultana, N., Islam, M., Khandaker, M.M. and Bhuiyan, M.A.B., 2019. A review on anthracnose of mango caused by *Colletotrichum gloeosporioides*. *Bangladesh Journal Plant Pathology*, 35(1), pp.65-74.
- Jeyaraj, A., Elango, T., Chen, X., Zhuang, J., Wang, Y. and Li, X., 2023. Advances in understanding the mechanism of resistance to anthracnose and induced defence response in tea plants. *Molecular Plant Pathology*, 24(10), pp.1330-1346.
- Lee, S., Costello, M., Kang, g., 2004. Efficacy of chlorine dioxide gas as a sanitizer of lettuce leaves. *Journal of Food Protection* 67(7), pp. 1371-1376.
- Kahramanoğlu, İ., 2020. Preserving postharvest storage quality of fresh loquat fruits by using different biomaterials. *Journal of Food Science and Technology*, 57(8), pp.3004-3012.
- Kankamol, C., Srikam, W. and Chumsiriwong, K., 2021. Antimicrobial activities of *Aloe vera* rind extracts against plant pathogenic bacteria and fungi. *Agriculture and Natural Resources*, 55(5), pp.715-723.
- Khalil, H.A., Abdelkader, M.F., Lo’ay, A.A., El-Ansary, D.O., Shaaban, F.K., Osman, S.O., Shenawy, I.E., Osman, H.E.H., Limam, S.A., Abdein, M.A. and Abdelgawad, Z.A., 2022. The combined effect of hot water treatment and chitosan coating on mango (*Mangifera indica* L. cv. Kent) fruits to control postharvest deterioration and increase fruit quality. *Coatings*, 12(1), p.83.

- Khanzada, M.A., Ansari, A., Rajput, M.A., Maitlo, S., Rajput, A.Q. and Ujjan, A.A., 2018. Effect of different abiotic factors on the growth and sporulation of *Colletotrichum gloeosporioides* causing anthracnose of mango. *Plant Protection*, 2(1), pp.23-30.
- Kim, Y.S., Lee, Y., Cheon, W., Park, J., Kwon, H.T., Balaraju, K., Kim, J., Yoon, Y.J. and Jeon, Y., 2021. Characterization of *Bacillus velezensis* AK-0 as a biocontrol agent against apple bitter rot, caused by *Colletotrichum gloeosporioides*. *Scientific Reports*, 11(1), pp.1-14.
- Konsue, W., Dethoup, T. and Limtong, S., 2020. Biological control of fruit rot and anthracnose of postharvest mango by antagonistic yeasts from economic crops leaves. *Microorganisms*, 8(3), p.317.
- Kulkarni, S., 2019. Epidemiology of greengram (*Vigna radiate*) anthracnose in northern Karnataka. *Journal of Pharmacognosy and Phytochemistry*, 8(4), pp.434-437.
- López-Velázquez, J.G., Ayón-Reyna, L.E., Vega-García, M.O., López-Angulo, G., López-López, M.E., López-Zazueta, B.A. and Delgado-Vargas, F., 2022. Caprylic acid in *Vitex mollis* fruit and its inhibitory activity against a thiabendazole-resistant *Colletotrichum gloeosporioides* strain. *Pest Management Science*, p. 5043-5480
- Mbili, N.C., 2012. Evaluation of integrated control of postharvest grey mould and blue mould of pome fruit using yeast, potassium silicate and hot water treatments. Master of Science dissertation, Department of Plant Pathology, University of KwaZulu-Natal, Pietermaritzburg, South Africa.
- Mokgalapa, N., Akinola, S.A., Shoko, T., Pillai, S.K. and Sivakumar, D., 2022. Chitosan molecular weights affect anthracnose incidence and elicitation of defence-related enzymes in avocado (*Persea americana*) cultivar 'Fuerte'. *International Journal of Food Microbiology*, 366, p.109561.
- Moodley, A.J., 2021. Biological control of head rot and damping-off of sunflower using yeasts, *Bacillus* spp. and *Trichoderma* spp. Master of Science dissertation, Department of Plant Pathology, University of KwaZulu-Natal, Pietermaritzburg, South Africa.
- Dutta, K.S., 1958. Inheritance of resistance to anthracnose in watermelon. Master of Science dissertation, Department of Horticulture University of Dacca, East Pakistan.

- Mora-Aguilera, J.A., Rios-Lopez, E.G., Yanez-Zuniga, M., Rebollar-Alviter, A., Nava-Diaz, C., Leyva-Mir, S.G., Sandoval-Islas, J.S. and Tovar-Pedraza, J.M., 2021. Sensitivity to MBC fungicides and prochloraz of *Colletotrichum gloeosporioides* species complex isolates from mango orchards in Mexico. *Journal of Plant Diseases and Protection*, 128(2), pp.481-491.
- Nelson, S.C., 2008. Mango anthracnose (*Colletotrichum gloeosporioides*). *Plant Disease*, pp.1-8
- Ntsoane, M.L., Zude-Sasse, M., Mahajan, P. and Sivakumar, D., 2019. Quality assessment and postharvest technology of mango: A review of its current status and future perspectives. *Scientia Horticulturae*, 249, pp.77-85.
- Nunes, C.A., 2012. Biological control of postharvest diseases of fruit. *European Journal of Plant Pathology*, 133, pp.181-196.
- Ons, L., Bylemans, D., Thevissen, K. and Cammue, B.P., 2020. Combining biocontrol agents with chemical fungicides for integrated plant fungal disease control. *Microorganisms*, 8(12), p.1930.
- Palmieri, D., Ianiri, G., Del Grosso, C., Barone, G., De Curtis, F., Castoria, R. and Lima, G., 2022. Advances and perspectives in the use of biocontrol agents against fungal plant diseases. *Horticulturae*, 8(7), p.577.
- Paudel, A., Poudel, P. and Yogi, M., 2022. Insights on the mango anthracnose and its anagement. *Journal of Plant Pathology Research*, 4(1), pp.81-90.
- Prasenjit, K., Nayan, K., and Sutanu, M., 2021. A critical review on use of edible coating to enhance shelf life of mango. *British Journal of Applied Science and Technology*, Past ISSN: 2231-0843, NLM ID: 101664541
- Rajwana, I.A., Khan, I.A., Malik, A.U., Saleem, B.A., Khan, A.S., Ziaf, K., Anwar, R. and Amin, M., 2011. Morphological and biochemical markers for varietal characterization and quality assessment of potential indigenous mango (*Mangifera indica*) germplasm. *International Journal of Agriculture and Biology*, 13(2) pp.151-158.
- Saha, S., Chakrabarty, P.K. and Banerjee, K., 2022. Producing crops without mancozeb? Perspectives on recent regulatory dilemmas and ways out. *ACS Agricultural Science and Technology*, 2(2), pp.272-275.

- Satapathy, R. and Beura, S., 2019. Fungicidal management of *Colletotrichum gloeosporioides* (Penz.) causing cashew anthracnose. *International Journal of Chemical Studies*, 7(3), pp.1219-1221.
- Shah, S. and Hashmi, M.S., 2020. Chitosan–*Aloe vera* gel coating delays postharvest decay of mango fruit. *Horticulture, Environment, and Biotechnology*, 61(2), pp.279-289.
- Sharma, M. and Kulshrestha, S., 2015. *Colletotrichum gloeosporioides*: an anthracnose causing pathogen of fruits and vegetables. *Biosciences Biotechnology Research Asia*, 12(2), pp.1233-1246.
- Shimira, F., 2022. *Tetradenia riparia*, an ethnobotanical plant with diverse applications, from antimicrobial to anti-proliferative activity against cancerous cell lines: A systematic review. *Journal of Herbal Medicine*, p.100537.
- Shimshoni, J.A., Bommuraj, V., Chen, Y., Sperling, R., Barel, S., Feygenberg, O., Maurer, D. and Alkan, N., 2020. Postharvest fungicide for avocado fruits: antifungal efficacy and peel to pulp distribution kinetics. *Foods*, 9(2), p.124.
- Shoji, K., Schudel, S., Onwude, D., Shrivastava, C. and Defraeye, T., 2022. Mapping the postharvest life of imported fruits from packhouse to retail stores using physics-based digital twins. *Resources, Conservation and Recycling*, 176, p.105914.
- Siddiqui, Y. and Ali, A., 2014. *Colletotrichum gloeosporioides* (Anthracnose). In *Postharvest Decay*. Academic Press, p. 337-371.
- Tao, H., Bao, Z., Jin, C., Miao, W., Fu, Z. and Jin, Y., 2020. Toxic effects and mechanisms of three commonly used fungicides on the human colon adenocarcinoma cell line Caco-2. *Environmental Pollution*, 263, p.114660.
- Tesfay, S.Z., Magwaza, L.S., Mditshwa, A. and Mbili, N., 2017, September. Carboxyl methylcellulose (CMC) incorporated with moringa leaf and seed extracts as new postharvest organic edible coating for avocado (*Persea americana* Mill.) fruit. In VII International Conference on Managing Quality in Chains (MQUIC2017) and II International Symposium on Ornamentals in 1201 (pp. 161-168).
- Troncoso-Rojas, R. and Tiznado-Hernández, M.E., 2014. *Alternaria alternata* (black rot, black spot). In *Postharvest decay* (pp. 147-187). Academic Press.

- Umamahesh, K., Gandhi, A.D. and Reddy, O.V., 2020. Ethnopharmacological applications of mango (*Mangifera indica* L.) peel-A review. Current Pharmaceutical Biotechnology. Current Pharmaceutical Biotechnology, Volume 21, pp. 1298-1303(6)
- Uddin, M., Shefat, S., Afroz, M. and Moon, N., 2018. Management of anthracnose disease of mango caused by *Colletotrichum gloeosporioides*: A review. Acta Scientific Agriculture, 2(10), pp.169-177.
- Van Puyvelde, L., Liu, M., Veryser, C., De Borggraeve, W.M., Mungarulire, J., Mukazayire, M.J. and Luyten, W., 2018. Active principles of *Tetradenia riparia*. IV. Anthelmintic activity of 8 (14), 15-sandaracopimaradiene-7 α , 18-diol. Journal of Ethnopharmacology, 216, pp.229-232.
- Vijay, S., Renukadevi, P., Mangammal, P. and Krishnamoorthy, S.V., 2021. Effect of media, temperature, and pH on the growth and pycnidial production of *Lasiodiplodia theobromae* (Pat.), causative of black root rot in mulberry. The Pharma Innovation Journal 2021; 10(11): 340-343
- Vinggaard, A.M., Hass, U., Dalgaard, M., Andersen, H.R., Bonefeld-Jørgensen, E., Christiansen, S., Laier, P. and Poulsen, M.E., 2006. Prochloraz: an imidazole fungicide with multiple mechanisms of action. International Journal of Andrology, 29(1), pp.186-192.
- Yasunaga, E., Fukuda, S., Nagle, M. and Spreer, W., 2018. Effect of storage conditions on the postharvest quality changes of fresh mango fruits for export during transportation. Environmental Control in Biology, 56(2), pp.39-44.
- Zakawa, N.N., Oyebanji, E.O., Timon, D. and Batta, K., 2020. Anti-fungal activities of aqueous leaf extracts of *Moringa oleifera* Lam. on *Mangifera indica* L. post-harvest fruit-rot pathogens from some markets in yola north, adamawa state. World Journal of Pharmaceutical Research SJIF Impact Factor 8.084 Volume 9, Issue 6, 1675-1687.
- Zhang, S., Zheng, Q., Xu, B. and Liu, J., 2019. Identification of the fungal pathogens of postharvest disease on peach fruits and the control mechanisms of *Bacillus subtilis* JK-14. Toxins, 11(6), p.322.

Mu, T., Zhang, Z., Liu, R., Liu, S., Li, Z., Zhang, X. and Xia, J., 2021. Morphological and molecular phylogenetic analyses reveal three species of *Colletotrichum* in Shandong province, China. *MycoKeys*, 85, p.57.

Chapter 3

In vitro and in vivo screening of antagonistic biocontrol agents against *Colletotrichum gloeosporioides* of 'Kent' mango

Abstract

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc is well known for causing anthracnose disease in fresh horticultural produce. This leads to tremendous economic losses for the producers and fruit distributors. This study evaluated the biocontrol activity of antagonistic yeasts and *Bacillus* species against *C. gloeosporioides* *in vitro* and *in vivo*. A total of 150 yeast isolates and 150 *Bacillus* isolates were isolated from leaves of different plant materials and screened against the pathogen *in vitro* using a dual assay. From primary screening trial, 15 yeasts and 25 *Bacillus* isolates inhibited the mycelial growth by $\leq 62.50\%$ and $\leq 63.37\%$, respectively, after 7 days at 28°C. The results from secondary screening trial indicated that yeast isolates YBa and two *Bacillus* isolates (BSs and BSl) significantly inhibited the mycelial growth ($p < 0.01$) by 67.91%, 74.04%, and 70.00% respectively compared to the control treatment after 7 days at 28°C. These isolates were further selected for *in vivo* trials and screened against *C. gloeosporioides* and inhibited anthracnose disease incidence by 50% compared to the control treatment after 7 days at 25°C. YBa, BSs and BSl were then identified using BLAST as *Meyerozyma guilliermondii*, *Burkholderia contaminans* and *B. contaminans*, respectively. The interaction between these isolates and *C. gloeosporioides* was observed under scanning electron microscopy (SEM) and exhibited mycelial breakage and shrinkage *in vitro*, and there was little damage to no spore germination on mango fruit. This study proved that biocontrol agents have the potential to control anthracnose of mango.

Keywords: *Colletotrichum gloeosporioides*, anthracnose, biocontrol agents, mango, *Burkholderia contaminans*, *Meyerozyma guilliermondii*.

3.1 Introduction

Mango is a commercial horticultural crop that gained interest because of its health-promoting properties (Masibo and He, 2008). It is a rich source of essential growth elements such as minerals, carbohydrates, vitamins, amino acids, fats in daily diets, and phenols (Rymbai *et al.*, 2013; Jenny *et al.*, 2019). The increased intake of mango fruit is linked with a reduced risk of infection with diseases such as cardiac and cardiovascular disease (Masibo and He, 2009; Sivakumar *et al.*, 2011). The production, domestic, and export markets of mango are

currently under threat because of postharvest diseases as they have a major contribution to reduce fruit quality and quantity (Subramanian *et al.*, 2018).

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc is the causal agent of anthracnose disease (Arauz, 2000). Anthracnose is one of the economically important diseases of mango (Jenny *et al.*, 2019). It is reported to result in fruit losses both qualitatively and quantitatively, estimated to be up to 38%, and may rise to 100% if proper management strategies are not in place (Khanzada, *et al.*, 2018). The use of chemical fungicides remains the main strategy to control anthracnose in mangoes (Monteon Ojeda *et al.*, 2012). Research conducted by Sharma *et al.* (2019) on the effect of propiconazole and azoxystrobin in controlling anthracnose of mango, showed a significantly reduced disease incidence from the treatment with fungicides *in vivo* in comparison to untreated mango fruit. However, the intensive use of chemical fungicides is a threat to human health (Kumar *et al.*, 2007), environment, and may lead to fungicide resistance by pathogenic fungi (Wang *et al.*, 2023). In the past decade, extensive research on evaluating the use of nonchemical and environmentally friendly methods for controlling fungal diseases in many fruits and vegetables has been conducted (Palou *et al.*, 2008; Sharma *et al.*, 2021).

Biological control agents (BCAs) such as antagonistic yeast and bacterial isolates and *Trichoderma* spp. are naturally efficient strains of microorganisms used to reduce or control pathogenic microorganisms (Kefialew and Ayalew, 2008; Raymaekers *et al.*, 2020). These can be isolated from the soil, leaves, stems, and bark of different crops. Biocontrol agents such as *Trichoderma* and *Bacillus* use beneficial organisms such as secondary metabolites to reduce the inoculum by the fungal pathogens (Junaid *et al.*, 2013). Most biological control agents are endophytic and can protect plants from pathogenic fungi by colonizing the internal tissues of the host plants (Hong and Park, 2016). Biocontrol agents are used against fungal pathogens and are promising due to their antimicrobial properties.

Bacillus species have secondary metabolites with biocontrol ability against various phytopathogens such as *C. gloeosporioides* (Kim *et al.*, 2021). There are various species under the genus *Bacillus*, these include *B. subtilis*, *B. amyloliquefaciens* and *B. velezensis*, *B. mojavensis*, and *B. licheniformis* (Alvindia, and Acda, 2015; Etesami, and Alikhani, 2018; Reyes-Estebanez, *et al.*, 2020).

Alvindia and Acda (2015) reported on *B. amyloliquefaciens* controlling the anthracnose of mango *in vitro* and *in vivo*. *B. amyloliquefaciens* suppressed the growth of *C. gloeosporioides*

on petri dishes and had an impact on spore formation on mango fruit surface significantly reducing disease incidence of anthracnose compared to untreated fruit. Recently, Bu *et al.* (2021) used *B. subtilis* to control gray mold of tomatoes caused by *Botrytis cinerea*. The plate confrontation had the inhibition 39.63% and 81.40% on tomato fruit *in vivo*.

Yeast antagonists are also an explored option in treating the pathogenic fungi affecting tropical fruits postharvest. *Meyerozyma caribbica* is one of the yeast isolates that have been reported to inhibit *C. gloeosporioides* on mango (Aguirre-Güitrón, *et al.*, 2022). Iñiguez-Moreno *et al.* (2021) used *M. caribbica* against *C. gloeosporioides* and *Fusarium* spp. on avocado and showed inhibition greater than 78% both *in vitro* and *in vivo*. Sharma *et al.* (2021) and Sudha *et al.* (2021) reported that *Pichia anomala* and *M. caribbica* inhibited *C. gloeosporioides* *in vitro* and *in vivo* by 79.63% and 60%, respectively. The objective of this study was to evaluate the antagonistic effect of biological control agents obtained from different plant materials against anthracnose disease caused by *C. gloeosporioides*.

3.2 Materials and methods

3.2.1 Isolation of *Colletotrichum gloeosporioides*

The pathogen, *Colletotrichum gloeosporioides*, was isolated from ‘Keitt’ mango fruit obtained from Food Lovers fruit and veg supermarket, Scottsville, Pietermaritzburg, South Africa (SA). Fruit showing symptoms of anthracnose were selected for isolation of the fungal pathogen. Infected fruits were surface sterilised with 70% ethanol and rinsed with sterile distilled water 3 times and allowed to dry. Fruit peel showing anthracnose symptoms were cut (1 cm x 1 cm) using a flame-sterilised scalpel. Potato dextrose agar (PDA) from Meck was prepared and poured into sterile Petri dishes (20ml/plate) and allowed to solidify. Pieces of decayed mango fruit were conveyed into freshly prepared PDA plates and were incubated for 7 days at 28°C.

3.2.2 Identification of the isolated *C. gloeosporioides*

After obtaining pure cultures of *C. gloeosporioides*, light microscope (Carl Zeiss, Germany) was used to identify the pure cultures of *C. gloeosporioides*. For microscopic identification, a drop of autoclaved distilled water was placed on a clean glass slide and a thin smear of *C. gloeosporioides* mycelia from 7-day old cultures was placed aseptically on water. The suspension was covered with a cover slip and viewed under the light microscope at 40x

magnification to observe the spores of *C. gloeosporioides*. *C. gloeosporioides* pure culture was then stored on 70% glycerol (v/v) at -80°C for long-term storage.

3.2.3 Isolation of biocontrol agents

Bacillus and yeast strains were isolated from the leaves of 50 plant species collected at Harding, KwaZulu-Natal and Botanical Gardens, University of KwaZulu-Natal, Pietermaritzburg. *Bacillus* and yeast strains were isolated using the technique by (Abraham *et al.*, 2010).

3.2.3.1 Isolation of yeast strains

The leaf samples were cut into pieces and rinsed with autoclaved tap water to wash off the soil and other microorganisms that might be on the leaf surface. Approximately 80g of leaf material of plant species was weighed and placed in a 250 ml Erlenmeyer flask containing 100 ml of double sterilized distilled water. The Erlenmeyer bottles were then placed in a water bath at 130 rotations per minute for 1 hour at 28°C to create a stock solution. A serial dilution was prepared from the stock solution and 1 ml of each dilution was added to 9 ml of double-sterilized distilled water. A volume of 200 µl of each dilution was inoculated on yeast extract peptone dextrose (YEPD) agar plates and incubated at 28°C for 3 days. Pure cultures of yeast isolates were sub-cultured onto freshly prepared potato dextrose agar (PDA) and thereafter stored in 70% glycerol (v/v) at -80°C for long-term storage.

3.2.3.2 Isolation of *Bacillus* species

For *Bacillus spp.* isolation, the same procedure for yeast was followed with a few amendments. The Erlenmeyer bottles containing the leaf material with double sterilized distilled water were then placed in a water bath at 130 rotations per minute for 1 hour at 80°C. A serial dilution was prepared from the stock solution and 1 ml of each dilution was added to 9 ml of double-sterilized distilled water. A volume of 200 µl of each dilution was inoculated on PDA plates and incubated at 28°C for 3 days. Pure cultures of *Bacillus* isolates were obtained by subculturing single colonies onto fresh PDA plates and stored in 70% glycerol.

3.2.4 *In vitro* effect of biocontrol agents against *C. gloeosporioides*

The radial growth inhibition of *C. gloeosporioides* strains by antagonistic yeasts and *Bacillus* isolates was tested according to the modified eclipse screening method described by Perez et al. (2016). The 3-4-day-old yeast cells grown on PDA plates were streaked parallel to each other 15 mm from the edge of the plate of fresh PDA. A single mycelial cube (5x5 mm) of *C. gloeosporioides* cut from the actively growing edge of a 5-day-old culture on PDA was placed

at the centre of the two streaked lines of yeast. The same procedure was adopted for *Bacillus* isolates. PDA plates inoculated with *C. gloeosporioides* only served as a control. The *in vitro* primary screening of 150 yeast isolates and 150 *Bacillus* isolates was performed to determine the degree of inhibition against *C. gloeosporioides*. From the primary screening, 15 yeast isolates and 25 *Bacillus* isolates with % inhibition $\geq 50\%$ were selected for secondary screening, and the same methodology was used. Each bioassay was replicated three times and incubated in the dark at 28°C and the mycelial growth (mm) was recorded at days 3, 5, and 7. The mycelial growth diameter of *C. gloeosporioides* exposed to the yeast and *Bacillus* isolates was measured and the mycelial growth inhibition percentage was calculated in relation to the control by using the following formula:

$$L = \frac{C-T}{C} \times 10$$

where,

L = inhibition of radial mycelial growth (%)

C = average radial growth of the pathogen in control

T = average radial growth of the pathogen in the presence of the antagonists.

A scale was developed that grouped the isolates based on their inhibition range in which Class 1 contained isolates that achieved $\leq 40\%$ inhibition, Class 2 contained isolates that achieved between 41-69% inhibition, and Class 3 contained isolates that achieved $\geq 70\%$ inhibition.

3.2.5 Molecular identification of the best isolates

The molecular identification of the isolated biocontrol agents was done according to Weisburg *et al.*, (1991) and Turner *et al.*, (1999). Genomic DNA was extracted from the biocontrol agents using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, Catalogue No. D6005). The 16S target region was amplified as presented in Table 3.1. The integrity of the PCR amplicons was visualized on a 1% agarose gel (CSL-AG500, Cleaver Scientific Ltd) stained with EZ-vision® Bluelight DNA Dye. The NEB Fast Ladder was used on all gels (N3238) as size standard. The fragments were enzymatically purified using the ExoSAP procedure (NEB M0293L; NEB M0371) and purified amplicons using (Zymo Research, ZR-96 DNA Sequencing Clean-up Kit™, Catalogue No. D4050). Sequenced in the forward and reverse direction (Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000) using the ABI 3730xl Genetic Analyzer (Applied Biosystems, Thermo Fisher

Scientific) for each reaction of every sample. CLC Bio Main Workbench was used to assemble the forward and reverse sequencing reads to form a consensus sequence for each sample. BLASTn analysis (with default parameters) was performed on the NCBI website (Altschul *et al.*, 1997).

Table 3.1: 16S Primers sequences

Name of the Primer	Target	Sequence (5' to 3')
16S-27F	16S rDNA sequence	AGAGTTTGATCMTGGCTCAG
16S-1492R	16S rDNA sequence	CGGTTACCTTGTTACGACTT

3.2.6 *In vivo* effect of yeast and *Bacillus* spp. against *C. gloeosporioides* on mango

Healthy, matured, untreated “Kent” mangoes were procured from Durban Fresh Produce Market and transported to the Plant Pathology Facilities in the University of KwaZulu-Natal, Pietermaritzburg Campus. The fruits were then disinfected with 15% sodium hypochlorite for 1 minute then rinsed 2-3 times with autoclaved distilled water and airdried for 2 hours. Mango fruits were wounded with a sterile cock borer at four points, the wounds estimated to be 5mm deep and 5mm wide. There were three biocontrol isolates (*M. guilliermondii*, and two strains of *Burkholderia contaminans*) that were prepared. The wounds were then inoculated with a volume of 30µl of yeast treatment, other fruits inoculated with *Bacillus* suspension at 1×10^8 ml⁻¹ concentration adjusted using a hemocytometer. The fruits were then dried for 4-5 hours, thereafter the mycelial plug of the pathogen *C. gloeosporioides* was inoculated on the fruit. The control fruits were only inoculated with the mycelial plug of the pathogen. The fruits were then stored at 25°C with a relative humidity of 90-95% for 7 days. The disease incidence (DI) of anthracnose was determined using the following equation:

$$\text{Disease incidence} = \frac{\text{Number of decayed wounds}}{\text{Number of total wounds}} \times 100$$

3.2.7 Scanning electron microscopy (SEM) analysis of the interaction between *C. gloeosporioides* and biocontrol agents *in vitro* and *in vivo*

Biocontrol agents that successfully inhibited the growth of *C. gloeosporioides* in a dual-culture and on mango fruits were grown on freshly prepared PDA petri dish. The mycelial disc of *C. gloeosporioides* (5 mm x 5 mm) was placed at the centre of the petri dish and each side of the petri dish was streaked with the biocontrol agent and incubated at 28°C for 7 days. The inhibition of mycelial growth and sporulation of *C. gloeosporioides* was observed under scanning electron microscopy (SEM). For *in vivo* trials, mango fruits were disinfected with sodium hypochlorite for 1 minute, wounded, and inoculated with $1 \times 10^8 \text{ ml}^{-1}$ of biocontrol agents then kept at 25°C for 7 days. After 7 days, the fruits were viewed under SEM Zeiss EVO LS15 (Carl Zeiss NTS Ltd., Germany) conducted at the Microscopy and Microanalysis Unit, University of KwaZulu-Natal, Pietermaritzburg, South Africa. Samples were cut from inoculated PDA plates and fruit samples and held for 2 hours in fixation of 3% buffered glutaraldehyde and washed twice in 0.05M sodium cacodylate buffer for 5 minutes. The samples were then dehydrated with approximately 2 ml aliquots of 10%; 30%; 50%; 70% and 90% ethanol for 10 minutes per concentration. The samples were rinsed three times with 100% ethanol for 10 minutes to complete the dehydration process. After the dehydration process, samples were placed in the Quorum K850 critical drying point dryer (CPD) basket with 100% ethanol. The ethanol was replaced with liquid carbon dioxide (CO₂) during CPD. The liquid CO₂ was heated and pressurized to the critical point at which the liquid turned into a gas without damaging the samples due to surface tension, leaving the samples dry and undamaged. Using black double-sided tape, the dried samples were carefully mounted onto SEM stubs. The sample stubs were transferred to the Quorum Q150R ES sputter coater. In this step, the samples were coated twice with gold and palladium to make them conductive to the electron beam. After drying, the samples were examined under the Zeiss EVO LS15 SEM.

3.2.8 Statistical analysis

All experiments were set up in a completely randomised design. The data obtained were subjected to an analysis of variance (ANOVA) using GenStat® 20th edition. Significant differences between treatments were determined by using Duncans Multiple Range Test (DMRT) at $P \leq 0.05$.

3.3 Results

3.3.1 Cultural and morphological characterization of the pathogen

The isolated fungal pathogen in PDA grew at 28°C after 7 days of incubation. Mycelial growth was white and fluffy in the petri dish (Figure 3.1). This visual and microscopic observation was aimed at illustrating the pathogen spores and mycelial growth in the petri dish (Figure 3.1). The isolated pathogen was for *in vitro* and *in vivo*, in comparison with the results after the interaction with biological control agents against the pathogen.

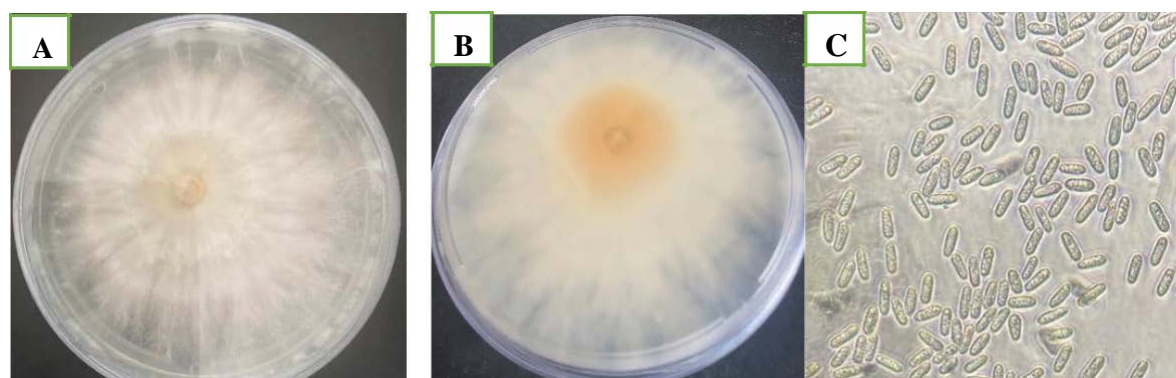


Figure 3.1: The identification of the *Colletotrichum gloeosporioides* culturally and morphologically after 7 days at 28°C. A and B are the Petri dishes showing the mycelia of the pathogen, and C represents pathogen spores under the light microscope (40x).

3.3.2 *In vitro* effect of *Meyerozyma guilliermondii* and *Burkholderia contaminans* against *C. gloeosporioides*

Table 3.2: The grouping of yeast and bacterial isolates according to their average percentage inhibition against *C. gloeosporioides*.

Class	Percentage of average inhibition range	Percentage of <i>Bacillus</i> isolates (%)	Percentage of yeast isolates (%)
1	0-40%	0.00	0.00
2	41-69%	92	100
3	70-100%	8	0.00

Mycelial growth of *C. gloeosporioides* was inhibited by all *Bacillus* and yeast isolates (Appendices 1 and 2, Table 3.1). Eight yeast isolates inhibited the mycelial growth of *C. gloeosporioides* by more than 65% and 2 yeast isolates inhibited the pathogen by 50-55% after 7 days (Table 3.3). Isolate Ba had the highest inhibition (67.91%) of *C. gloeosporioides* mycelial growth compared to other yeast isolates (Figure 3.2, Table 3.3).

Table 3.3: Secondary screening and mean percentage inhibition of *C. gloeosporioides* by selected potential yeast antagonists and their class ratings after 7 days at 28°C. Means with the same letters have no significant difference, according to Duncan's multiple range test ($P \leq 0.05$).

Isolates	Mycelial growth (mm)	% Inhibition	Class
Ba	25.67a	67.91	2
445	26.33a	67.09	2
500	26.67a	66.66	2
EL	26.67a	66.66	2
W	27.00a	66.25	2
TA	27.33a	65.84	2
467	27.67a	65.41	2
Pa	27.67a	65.41	2
Im	28.67a	64.16	2
Ss	29.00a	63.75	2
425	30.00ab	62.50	2
CM	31.00ab	61.25	2
Po	31.00ab	61.25	2
CO	36.00bc	55.00	2
Gm	39.33c	50.84	2
Control	80.00d		1
P-level	<0.001		
CV%	2.0		

Fourteen *Bacillus* isolates inhibited mycelial growth of *C. gloeosporioides* by more than 65% after 7 days (Table 3.4). *Bacillus* isolates SL and Ss had the highest inhibition (70.00% and 74.04%, respectively) of *C. gloeosporioides* mycelial growth after 7 days (Table 3.4, Figure 3.3).

Table 3.4: Secondary screening and mean percentage inhibition of *C. gloeosporioides* by selected potential *Bacillus* antagonists and their class ratings. Means with the same letters have no significant difference, according to Duncan's multiple range test ($P \leq 0.05$).

Isolates	Mycelial growth (mm)	% Inhibition	Class
Ss	21.00a	74.04	3
SL	24.33ab	70.00	3
Ma	25.33bc	68.73	2
Sa	25.33abc	68.73	2
169	26.33bcd	67.49	2
445	27.00bcd	66.67	2
Pa	27.00bcd	66.67	2
415	27.33bcd	66.26	2
ChL	27.33bcd	66.26	2
IBF	27.33bcd	66.26	2
Fe	27.67bcd	65.84	2
Pt	27.67bcd	65.84	2
W	28.00bcd	65.43	2
OF	28.33bcd	65.02	2
498	28.67bcd	64.60	2
419	29.00bcde	64.20	2
Avo	29.33cde	63.79	2
Ba	29.33cde	63.79	2
173	29.67cde	63.37	2
TA	30.00cde	62.96	2
513	30.33de	62.56	2
IBL	30.33de	62.56	2
444	31.00de	61.73	2
188	33.67e	58.43	2
Control	81.00f		1
P-level	<0.001		
L.S.D	4.067		
CV%	8.30		

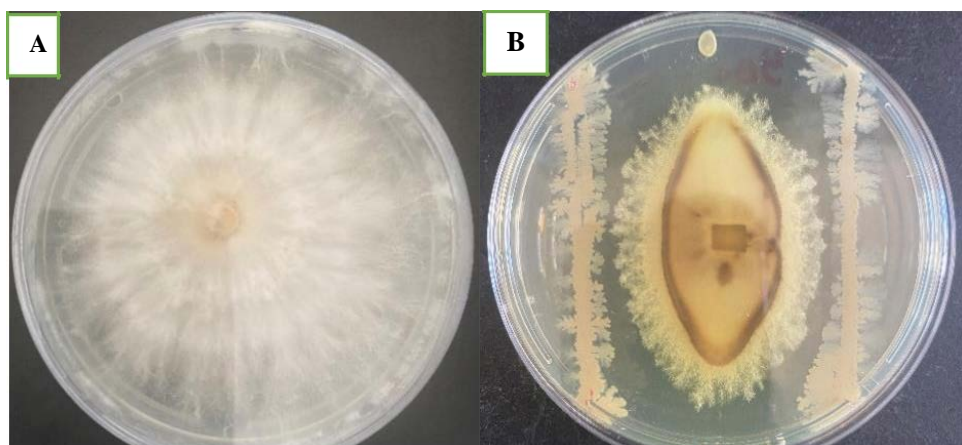


Figure 3.2 *In vitro* inhibitory activity of yeast isolate YBa on potato dextrose agar after 7 days incubation at 28°C. (A) Control plate inoculated only with *C. gloeosporioides*; (B) YBa (*Meyerozyma guilliermondii*) after 7 days.



Figure 3.3: *In vitro* interaction and inhibition of *C. gloeosporioides* with BSS and BSI bacterial isolates on PDA after 7 days incubation at 28°C. (A) *C. gloeosporioides* in the absence of antagonist; (B) BSS (*Burkholderia contaminans*) and (C) BSI (*Burkholderia contaminans*) after 7 days.

3.3.3 Molecular identification of bacterial isolates

The identification of the three best-performing biocontrol agents was done using the primers stated in Table 3.5. These isolates had the highest inhibition percentage against the fungal pathogen *C. gloeosporioides*. BSS and BSI were both identified as two bacterial strains of *Burkholderia contaminans* and the yeast isolate YBa was identified as *Meyerozyma guilliermondii*.

Table 3.5: The identified biological control agents and the primers used.

Isolate name	Species name	Primer	Accession number
BSS	<i>Burkholderia contaminans</i>	16S rDNA	CP090637.1
BSI	<i>Burkholderia contaminans</i>	16S rDNA	CP090637.1
YBa	<i>Meyerozyma guilliermondii</i>	16S rDNA	NR117946.1

3.3.4 *In vivo* effect of *Meyerozyma guilliermondii* and *Burkholderia contaminans* against *C. gloeosporioides*

Fruits were observed for disease incidence and mycelial growth inhibition after 7 days post-inoculation at 25°C (Figure 3.4) during *in vivo* screening. There was less disease incidence of *C. gloeosporioides* on mango fruit treated with both *B. contaminans* isolates and *M. guilliermondii* (Figure 3.4 B, C and D) yeast isolate compared to the control fruit (Figure 3.4 A) which had 100% infection.



Figure 3.4: *In vivo* interaction and inhibition of *C. gloeosporioides* with *Meyerozyma guilliermondii* yeast isolate, *Burkholderia contaminans* isolates respectively on mango fruits after 7 days post inoculation at 25°C. (A) control with the pathogen only (A), *Meyerozyma guilliermondii* yeast isolate (B), and bacterial isolates *Burkholderia contaminans* (C and D), 7 days post-inoculation.

3.3.5 Scanning electron microscope analysis of the interaction between *C. gloeosporioides* and biocontrol agents

Mycelial growth and conidia of *C. gloeosporioides* were observed using the SEM (Figure 3.5 B, C, and D) after 7 days post inoculation at 28°C. The interaction and mode of action of *M. guilliermondii* yeast isolate, and *B. contaminans* isolates against *C. gloeosporioides* changes in the mycelial and hyphal structures of *C. gloeosporioides*. SEM micrographs revealed that the biocontrol agents caused damage to the morphology of both pathogen mycelia and conidia (Figure 3.5 B, C, and D) compared to the control (Figure 3.5 A).

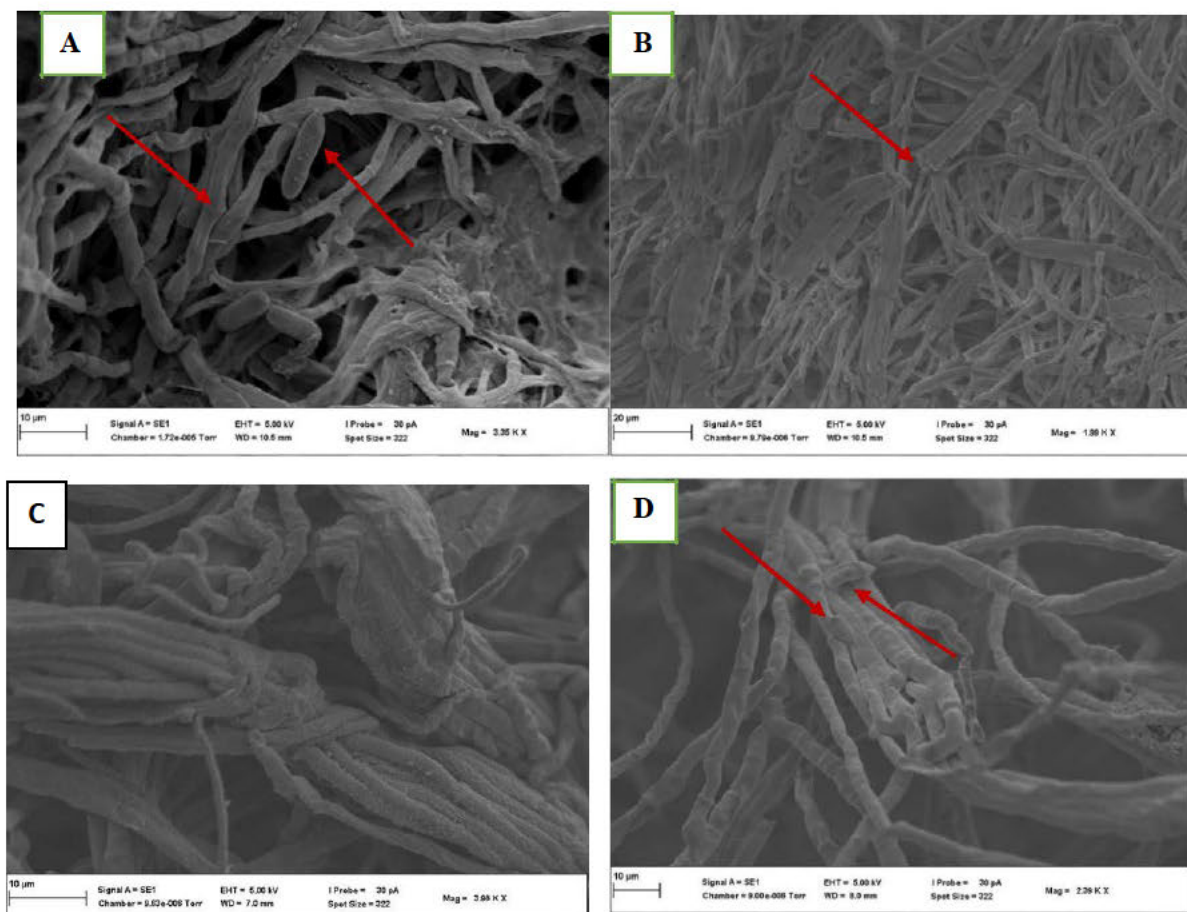


Figure 3.5: Scanning electron microscopy micrographs of the interaction of biocontrol agents against *C. gloeosporioides* on PDA, control with the pathogen only (A), *Meyerozyma guilliermondii* yeast isolate (B), and bacterial isolates *Burkholderia contaminans* (C and D), 7 days post-inoculation. Red arrows indicate the damaged mycelia and spores of *C. gloeosporioides* (B, C and D) and mycelia and spores of *C. gloeosporioides* on PDA (A).

The interaction of bacterial isolates and *C. gloeosporioides* on the surface of mango is shown in Figure 3.6. The spores of the pathogen were abundant on the surface of the mango fruit that was inoculated with the pathogen only (Figure 3.6 A). Fruit treated with the *M. guilliermondii* yeast isolate and *B. contaminans* isolates respectively had limited spores with the interaction of the biocontrol agents with *C. gloeosporioides* as shown in Figure 3.6 B, C, and D.

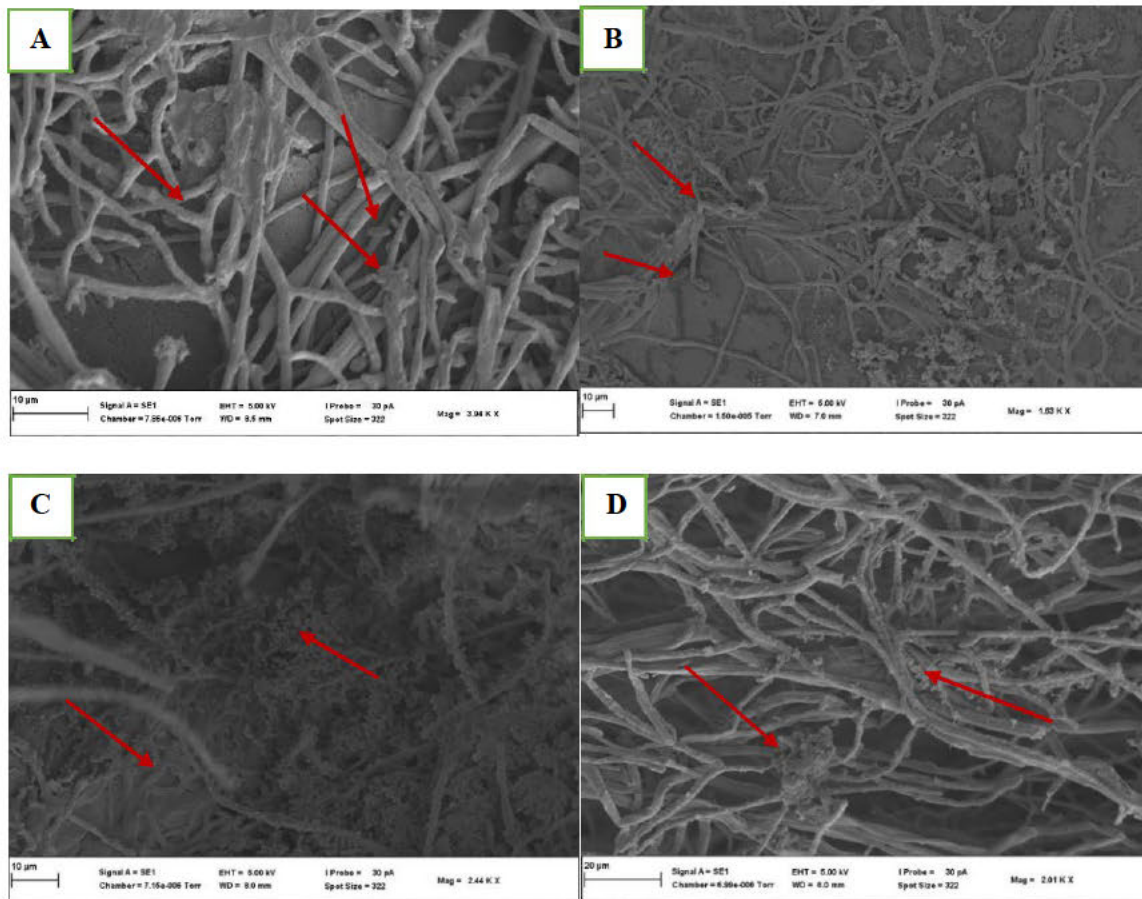


Figure 3.9: Scanning electron microscopy micrographs of the interaction of biocontrol agents against *C. gloeosporioides* on 'Kent' mango fruits, control with the pathogen only (A), *Meyerozyma guilliermondii* yeast isolate (B), and bacterial isolates *Burkholderia contaminans* (C and D), 7 days post inoculation. Red arrows indicate the damaged mycelia and spores of *C. gloeosporioides* (B, C and D) and mycelia and spores of *C. gloeosporioides* on PDA (A).

3.4 Discussion

The use of alternative control measures that are natural predators of the pathogen to successfully suppress fungal diseases was explored by Fenta *et al.* (2023). As biocontrol agents depict potential in protecting horticultural produce during pre- and postharvest against fungal pathogens following the physical and chemical control methods. The findings showed that *M. guilliermondii* yeast isolate inhibited the growth of *C. gloeosporioides* mycelia by >60%, whereas bacterial isolates *B. contaminans* isolates had an inhibition of >70% *in vitro*, in comparison with the non-treated control using a confrontation plate assay as shown in Figure 3.2 and 3.3. This shows that *Bacillus* species used in this study have the potential to be used as a biocontrol agents

against *C. gloeosporioides*. Antagonistic yeast isolates had no significant difference except for the bacterial isolates had a significant difference and displayed the lowest inhibition % (Table 3.3 and 3.4).

B. contaminans isolates had the greatest control of *C. gloeosporioides* compared to *M. guilliermondii* yeast isolate *in vivo*, exhibiting an increased level of disease control on the matured and ripe mango fruit. The average control of *M. guilliermondii* was 63.33% and for *B. contaminans* it was more than 65% 7 days post inoculation. There was a greater suppression of disease development of *C. gloeosporioides* in mango fruits treated than the non-treated control (Figure 3.4). The interaction of the biocontrol agents had a significant effect on the development of mycelial growth. This is in line with the findings from Kim, *et al* (2021) where pre-treatment of the fruits with biocontrol agents and then a fungal suspension suppressed apple bitter rot, compared to post-treatment of the fruits with the cell suspension then the biocontrol agent.

The effect on the morphology of the pathogen mycelia and conidia was observed on the SEM for both plate assay and on the fruit surface. For the treated samples, stunted and lysed mycelia were notably observed (Figure 3.8 B, C, and D). There was normal mycelial growth on the control plate illustrated in Figure 3.8 A. Fruit treated with the biocontrol agents were less damaged on the surface with fewer pathogen sporulation (Figure 3.9 A, B, and C). On the other hand, the control had more spores on the surface compared to the treated fruits (Figure 3.9 A).

Yeast isolates have different inhibitory effects on fungal pathogens (Di Canito *et al.*, 2021) as they colonize different habitats and occur naturally on leaves and fruit surfaces (Palmieri *et al.*, 2022). The antagonistic reaction of yeast isolates has previously been tested and found to have enormous potential in controlling the fungal pathogens affecting different horticultural crops (Di Canito *et al.*, 2021). Yeast has been reported to prevent the formation of the germ tube and appressorium, avoiding the penetration to the host cell wall, by creating a pool of competition for nutrients (González-Gutiérrez *et al.*, 2023). This is a yeast-pathogen interaction, competing for sugars and carbohydrates (González-Gutiérrez, *et al.*, 2023). Bacterial isolates produce antibiotics to control fungal pathogens (Palmeri *et al.*, 2022), while also creating direct competition for the phytopathogenic fungi (Albayrak, 2019). The use of natural antagonists is essential to reduce the use of toxic agricultural inputs for improving the quantity of quality produce.

3.5 Conclusion

Effective management of anthracnose in mangoes during postharvest handling is key in improving the quality and net income of producers. The use of nonchemical methods such as BCAs to control postharvest diseases is likely to open access to lucrative organic markets. This study revealed that *M. guilliermondii* yeast antagonist and *B. contaminans* species reduced anthracnose of mango in comparison to the non-treated control. Notably, there was a slight disease incidence in treated fruit, and this could be linked to the fact that anthracnose is a latent disease. Thus, the disease could have been present but not visible even before the experiment. The potential of combining BCAs with other nonchemical methods to completely suppress anthracnose should be assessed. Moreover, future studies should evaluate the preharvest use of BCAs for an effective control of *C. gloeosporioides*.

3.6 References

- Abraham, A.O., Laing, M.D. and Bower, J.P., 2010. Isolation and *in vivo* screening of yeast and *Bacillus* antagonists for the control of *Penicillium digitatum* of citrus fruit. *Biological Control*, 53(1), pp.32-38.
- Aguirre-Güitrón, L., Calderón-Santoyo, M., Lagarón, J.M., Prieto, C. and Ragazzo-Sánchez, J.A., 2022. Formulation of the biological control yeast *Meyerozyma caribbica* by electrospraying process: effect on postharvest control of anthracnose in mango (*Mangifera indica* L.) and papaya (*Carica papaya* L.). *Journal of the Science of Food and Agriculture*, 102(2), pp.696-706.
- Albayrak, Ç.B., 2019. *Bacillus* species as biocontrol agents for fungal plant pathogens. *Bacilli and Agrobiotechnology: Phytostimulation and Biocontrol: Volume 2*.
- Alvindia, D.G. and Acda, M.A., 2015. The antagonistic effect and mechanisms of *Bacillus amyloliquefaciens* DGA14 against anthracnose in mango cv. 'Carabao'. *Biocontrol Science and Technology*, 25(5), pp.560-572.
- Arauz, L.F., 2000. Mango anthracnose: Economic impact and current options for integrated management. *Plant diseases* 84(6), pp.600-611.

- Bu, S., Munir, S., He, P., Li, Y., Wu, Y., Li, X., Kong, B., He, P. and He, Y., 2021. *Bacillus subtilis* L1-21 as a biocontrol agent for postharvest gray mold of tomato caused by *Botrytis cinerea*. *Biological Control*, 157, p.104568.
- Di Canito, A., Mateo-Vargas, M.A., Mazzieri, M., Cantoral, J., Foschino, R., Cordero-Bueso, G. and Vigentini, I., 2021. The role of yeasts as biocontrol agents for pathogenic fungi on postharvest grapes: A review. *Foods*, 10(7), p.1650.
- Etesami, H. and Alikhani, H.A., 2018. *Bacillus* species as the most promising bacterial biocontrol agents in rhizosphere and endorhiza of plants grown in rotation with each other. *European Journal of Plant Pathology*, 150, pp.497-506.
- Fenta, L., Mekonnen, H. and Kabtimer, N., 2023. The exploitation of microbial antagonists against postharvest plant pathogens. *Microorganisms*, 11(4), p.1044.
- González-Gutiérrez, K.N., Ragazzo-Sánchez, J.A., Barros-Castillo, J.C., Narváez-Zapata, J.A. and Calderón-Santoyo, M., 2023. Yeasts with potential biocontrol of *Colletotrichum gloeosporioides* in avocado (*Persea americana* Mill. cv. Hass) and characterization of *Yamadazyma mexicana* mechanisms. *European Journal of Plant Pathology*, 165(3), pp.525-543.
- Hong, C.E. and Park, J.M., 2016. Endophytic bacteria as biocontrol agents against plant pathogens: current state-of-the-art. *Plant Biotechnology Reports*, 10, pp.353-357.
- Iñiguez-Moreno, M., González-Gutiérrez, K.N., Ragazzo-Sánchez, J.A., Narváez-Zapata, J.A., Sandoval-Contreras, T. and Calderón-Santoyo, M., 2021. Morphological and molecular identification of the causal agents of post-harvest diseases in avocado fruit, and potential biocontrol with *Meyerozyma caribbica*. *Archives of Phytopathology and Plant Protection*, 54(7-8), pp.411-430.
- Jenny, F., Sultana, N., Islam, M., Khandaker, M.M. and Bhuiyan, M.A.B., 2019. A review on anthracnose of mango caused by *Colletotrichum gloeosporioides*. *Bangladesh Journal of Plant Pathology*, 35(1), pp.65-74.
- Junaid, J.M., Dar, N.A., Bhat, T.A., Bhat, A.H. and Bhat, M.A., 2013. Commercial biocontrol agents and their mechanism of action in the management of plant pathogens. *International Journal of Modern Plant and Animal Sciences*, 1(2), pp.39-57.

- Kefialew, Y. and Ayalew, A., 2008. Postharvest biological control of anthracnose (*Colletotrichum gloeosporioides*) on mango (*Mangifera indica*). *Postharvest Biology and Technology*, 50(1), pp.8-11.
- Khanzada, M.A., Ansari, A., Rajput, M.A., Maitlo, S., Rajput, A.Q. and Ujjan, A.A., 2018. Effect of different abiotic factors on the growth and sporulation of *Colletotrichum gloeosporioides* causing anthracnose of mango. *Plant Protection*, 2(1), pp.23-30.
- Kim, Y.S., Lee, Y., Cheon, W., Park, J., Kwon, H.T., Balaraju, K., Kim, J., Yoon, Y.J. and Jeon, Y., 2021. Characterization of *Bacillus velezensis* AK-0 as a biocontrol agent against apple bitter rot, caused by *Colletotrichum gloeosporioides*. *Scientific Reports*, 11(1), p.626
- Kumar, A.S., Reddy, N.E., Reddy, K.H. and Devi, M.C., 2007. Evaluation of fungicidal resistance among *Colletotrichum gloeosporioides* isolates causing mango anthracnose in Agri Export Zone of Andhra Pradesh, India. *Plant Pathology Bulletin*, 16(3), pp.157-160.
- Masibo, M. and He, Q., 2008. Major mango polyphenols and their potential significance to human health. *Comprehensive Reviews in Food Science and Food Safety*, 7(4), pp.309-319.
- Masibo, M. and He, Q., 2009. Mango bioactive compounds and related nutraceutical properties-a review. *Food Reviews International*, 25(4), pp.346-370.
- Monteon Ojeda, A., Mora Aguilera, J.A., Villegas Monter, Á., Nava Diaz, C., Hernández Castro, E., Otero-Colina, G. and Hernández Morales, J., 2012. Temporal analysis and fungicide management strategies to control mango anthracnose epidemics in Guerrero, Mexico. *Tropical Plant Pathology*, 37, pp.375-385.
- Palmieri, D., Ianiri, G., Del Grosso, C., Barone, G., De Curtis, F., Castoria, R. and Lima, G., 2022. Advances and perspectives in the use of biocontrol agents against fungal plant diseases. *Horticulturae*, 8(7), p.577.
- Palou, L., Smilanick, J.L. and Droby, S., 2008. Alternatives to conventional fungicides for the control of citrus postharvest green and blue moulds. *Stewart Postharvest Review*, 2(2), pp.1-16.

- Raymaekers, K., Ponet, L., Holtappels, D., Berckmans, B. and Cammue, B.P., 2020. Screening for novel biocontrol agents applicable in plant disease management—a review. *Biological Control*, 144, p.104240.
- Reyes-Estebanez, M., Sanmartin, P., Camacho-Chab, J.C., Susana, C., Chan-Bacab, M.J., Águila-Ramírez, R.N., Carrillo-Villanueva, F., De la Rosa-Escalante, E., Arteaga-Garma, J.L., Serrano, M. and Ortega-Morales, B.O., 2020. Characterization of a native *Bacillus velezensis*-like strain for the potential biocontrol of tropical fruit pathogens. *Biological Control*, 141, p.104127.
- Rymbai, H., Srivastav, M., Sharma, R.R., Patel, C.R, and Singh, A.K., 2013. Bio-active compounds in mango (*Mangifera indica* L.) and their roles in human health and plant defence—a review. *The Journal of Horticultural Science and Biotechnology*, 88(4), pp.369-379.
- Sharma, A., Sharma, I.M. and Sharma, M., 2019. Efficacy and economics of fungicides for management of mango anthracnose. *Indian Phytopathology*, 72, pp.361-366.
- Sharma, A., Sharma, I.M., Sharma, M., Sharma, K. and Sharma, A., 2021. Effectiveness of fungal, bacterial and yeast antagonists for management of mango anthracnose (*Colletotrichum gloeosporioides*). *Egyptian Journal of Biological Pest Control*, 31(1), pp.1-11.
- Sivakumar, D., Jiang, Y. and Yahia, E.M., 2011. Maintaining mango (*Mangifera indica* L.) fruit quality during the export chain. *Food Research International*, 44(5), pp.1254-1263.
- Sudha, S., Narendrappa, T. and Sivakumar, G., 2021. Management of post-harvest anthracnose disease in mango using promising biocontrol agents. *The Pharma Innovation Journal*, 10(3), pp.210-214.
- Wang, S., Wang, X., He, Q., Lin, H., Chang, H., Liu, Y., Sun, H. and Song, X., 2023. Analysis of the fungicidal efficacy, environmental fate, and safety of the application of a mefentrifluconazole and pyraclostrobin mixture to control mango anthracnose. *Journal of the Science of Food and Agriculture*, 103(1), pp.400-410.
- Subramanian, K.S., Sekar, C., Meneka, S.S. and Prakash, L.V., 2018. Reducing postharvest losses in mango in South Asia. *Chede Cooperative Union*, pp.1-4

Chapter 4

***In vitro* and *in vivo* screening of the antifungal effect of different plant extracts against *Colletotrichum gloeosporioides* of ‘Kent’ mango.**

Abstract

Postharvest diseases are the greatest contributor to losses of horticultural commodities. Anthracnose caused by *Colletotrichum gloeosporioides* is one of the key diseases that cause significant losses. This study aimed to evaluate the efficacy of *Moringa oleifera*, *Tetradenia riparia*, *Pelargonium sidoides*, and *Aloe vera* plant extracts against *C. gloeosporioides* *in vitro* and *in vivo*. *In vitro* antifungal effects of *M. oleifera*, *T. riparia*, *P. sidoides*, and *A. vera* at 1%, 1.5%, 2%, 2.5%, and 3% concentrations were evaluated by amending potato dextrose agar with these extracts, then inoculated with mycelial plugs of *C. gloeosporioides*, and incubated at 28°C for 7 days. The *A. vera* extract did not inhibit the pathogen *in vitro* with 8.62 maximum inhibition percentage. *T. riparia* and *M. oleifera* inhibited the mycelial growth with 90.2% and 49.80% inhibition percentage respectively, whereas *P. sidoides* treated plates had no pathogen mycelial growth compared to control plates. Mango ‘Kent’ fruit were treated with *P. sidoides* concentrations of 2.5% and 3%, and control fruit were inoculated with *C. gloeosporioides* only. Fruits were incubated at 25°C and disease incidence was evaluated after 7 days. Compared to the control and 2.5% treatment, 3% pelargonium significantly reduced the disease incidence of anthracnose with $\leq 50\%$, whereas 2.5% had $\leq 75\%$ and control fruits had 100% infection. The interaction between *P. sidoides* and *C. gloeosporioides* was observed under the scanning electron microscope (SEM). *C. gloeosporioides* treated with *P. sidoides* at 2.5% and 3% had fractured and fewer spores and thin mycelia compared to the pathogen only. Mango fruit inoculated with the pathogen only had visible and healthy spores compared to the fruits treated with *P. sidoides* which had mycelia shrinkage and breakage. *P. sidoides* has antifungal and antimicrobial properties and can be recommended as an alternative, with the potential to control postharvest fungal pathogens and reduce the use of fungicides.

Keywords: *Colletotrichum gloeosporioides*, anthracnose, plant extracts, mango, *Pelargonium sidoides*

4.1 Introduction

Postharvest diseases have a major contribution to losses of horticultural commodities. The main contributing factors are opportunistic fungi, bacteria, and viruses (Matrose *et al.*, 2021). Mango is a commonly produced fruit grown in the subtropical and tropical regions of the world (Arauz 2000). It has health-benefiting attributes such as polyphenol mangiferin which shows great potential in combating degenerative diseases like heart diseases and cancer (Masibo and He 2008; Bayoumi 2022). Mango fruit is climacteric with a high respiration rate, leading to rapid ripening (Bambalele *et al.*, 2021a). That limits the shelf life of mango, and restricts the import and export, whilst the fruit is under duress, there are also fungal pathogens that infect this fruit both pre and postharvest (Tarabih, 2020).

The number of fungal diseases affecting fruits and vegetables continues to increase and this has a devastating effect on economic returns for producers (Atiq *et al.*, 2020). There are various treatments for maintaining the quality of mango fruit. These control strategies include gaseous ozone, edible coatings, plant extracts, and essential oils (Tijjani *et al.*, 2016; Bambalele *et al.*, 2021). Fungicides have been used for a very long time and were effective, however, due to the toxic mycotoxins produced, they are deemed harmful to the environment and consumers. Moreover, fungal pathogens are becoming resistant to fungicides; hence their efficacy is compromised (Banya *et al.*, 2020).

There are natural phytochemicals in plant extracts that can be used as biopesticides (Leng *et al.*, 2011). Moringa leaf extract (MLE) with carboxymethyl cellulose (CMC) has been reported to improve fruit quality and shelf life of avocado fruit (Tesfay and Magwaza 2017). The research conducted by Liamngee *et al.* (2019) on the effect of moringa on tomato fruit also revealed its potential in extending shelf life and preserve the quality of fruit in storage, while reducing the infection by fungal pathogens such as *Aspergillus niger*, *A. flavus*, *A. fumigatus*, and *Penicillium griseofulvum*. Researchers have been exploring the use of plant extracts as they have been shown to possess antimicrobial properties that may be used in controlling microbial diseases postharvest (El Khetabi *et al.*, 2022).

Studies by Mendy *et al.* (2019) reported the efficacy of *Aloe vera* as a plant extract amended in PDA and used as a gel on papaya fruit against *Fusarium* sp., *Lasiodiplodia theobromae*, *A. niger*, and *C. gloeosporioides*. Their findings showed that 15%, 25%, and 50% concentrations of the extracts inhibited the fungal growth *in vitro* and wound infection in papaya fruit. *Pelargonium* spp. are currently used as essential oils in exploring the antimicrobial properties

(El Ouadi *et al.*, 2022). On the other hand, *Tetradenia riparia* is reported to have antimicrobial properties, however, it is currently used for human diseases (Luanda and Ripanda, 2022). The aim of this study was to evaluate the efficacy of *M. oleifera*, *T. riparia*, *P. sidoides*, and *A. vera* in the inhibition of the mycelial growth of *C. gloeosporioides* *in vitro* and their effect on the disease incidence of 'Kent' mango fruit.

4.2 Materials and methods

4.2.1 Preparation, and storage of plant extracts

A. vera, *P. sidoides*, *T. riparia*, and *M. oleifera* were extracted as described by Tesfay *et al.* (2017). Briefly, 1%, 1.5%, 2%, 2.5%, and 3% per extract were prepared by weighing 10g, 15g, 20g, 25g, and 30g of each extract, and added to 250 ml Erlenmeyer flasks, with 70% alcohol (v/v) as a solvent. The mixture was then agitated in a shaker for 12 hours. The mixture was filtered using a cheesecloth, and the residue discarded, and the aliquot was then evaporated in the GeneVac (Genevac® EZ 2.3; Ipswich, UK) at 45°C for 12 hours. The crude extracts were then diluted with double sterilized distilled water accordingly to adjust to the desired concentrations. The prepared plant extracts were stored at 4°C and later used for the *in vitro* and *in vivo* experiments.

4.2.2 *In vitro* screening of plant extracts against *C. gloeosporioides*

The effect of the prepared plant extracts on the inhibition of mycelial growth of *C. gloeosporioides* was examined *in vitro*. PDA was autoclaved at 121°C for 15 minutes and cooled at room temperature. Amendment of PDA was performed according to Ahmadu *et al.* (2020), where 1 ml of the diluted concentrations of plant extract was added to 9 ml of PDA, and left to solidify on a petri dish overnight. For the control, 9 ml PDA was amended with 1ml of autoclaved distilled water. *In vitro* screening of these plant extracts against *C. gloeosporioides* was conducted according to the method by Ahmadu *et al.* (2020), after media amendment, a single mycelial plug (5x5 mm) of the pathogen was inoculated on the petri dish and then incubated at 28°C.

Plates with media amended with water were inoculated with *C. gloeosporioides* and served as a control. Each bioassay was replicated three times and incubated in the dark at 28°C and the

mycelial growth was observed at days 3, 5, and 7. Thereafter, inhibition was determined by calculating the percent of relative growth inhibition of *C. gloeosporioides*. The growth diameter of *C. gloeosporioides* exposed to the plant extracts was measured and growth inhibition percentage calculated in relation to the controls by the following formula:

$$L = \frac{C - T}{C} \times 100$$

L = inhibition of radial mycelial growth (%)

C = average radial growth of pathogen in control

T = average radial growth of pathogen in the presence of the antagonists.

Based on the results obtained from the primary and secondary screening, *P. sidoides* extract at 2.5% and 3% were selected for *in vivo* trials.

4.2.3 *In vivo* screening of *P. sidoides* extracts against *C. gloeosporioides*

Mango ‘Kent’ fruit were procured from the Durban Fresh Produce Market and transported to the Plant Pathology Laboratory Facilities in the University of KwaZulu Natal, Pietermaritzburg Campus. Fruit were then disinfected with 15% sodium hypochlorite for 1 minute then rinsed 2-3 times with autoclaved distilled water and air dried overnight. Fruit was wounded (5mm deep and 5mm wide) with a sterile cork borer at four points. The wounds were then inoculated with 30µl *P. sidoides* extract at 2.5% and 3% concentrations. The fruits were then dried for 4-5 hours then the conidial plug of *C. gloeosporioides* was also inoculated on the fruit wounds. Control fruits were only inoculated with the conidial plug of the pathogen. The fruits were then stored at 25°C with a relative humidity of 90-95% for 7 days. The disease incidence (DI) of anthracnose was determined using the following equation:

$$\text{Disease incidence} = \frac{\text{Number of decayed wounds}}{\text{Number of total wounds}} \times 100$$

4.2.4 Scanning electron microscopy analysis of the interaction between *C. gloeosporioides* and *P. sidoides*

Concentrations of *P. sidoides* that successfully inhibited the growth of *C. gloeosporioides* *in vitro* were then amended in PDA media. The mycelial disc (5 mm x 5 mm) was placed at the centre of the petri dish and incubated at 28°C. After 7 days, the inhibition of mycelial growth and sporulation of *C. gloeosporioides* was observed under the scanning electron microscopy (SEM).

For *in vivo* trials, fruits were wounded and inoculated with 2.5%, 3% of *P. sidoides* extract and inoculated with *C. gloeosporioides* then kept at 25°C for 8 days and sampled for further analysis. The morphological reaction between the plant extracts and the pathogen, the control with the pathogen only was observed using SEM Zeiss EVO LS15, Carl Zeiss NTS Ltd., Germany conducted at the Microscopy and Microanalysis Unit, University of KwaZulu-Natal, Pietermaritzburg, South Africa. Samples were cut from inoculated PDA plates and fruit then held for 2 hours in fixation of 3% buffered glutaraldehyde and washed twice in 0.05M sodium cacodylate buffer for 5 minutes.

The samples were then dehydrated with approximately 2 ml aliquots of 10%; 30%; 50%; 70% and 90% ethanol for 10 minutes per concentration. The samples were rinsed three times with 100% ethanol for 10 minutes to complete the dehydration process. Thereafter, the samples were placed in the Quorum K850 critical drying point dryer (CPD) basket with 100% ethanol. The ethanol was replaced with liquid carbon dioxide (CO₂) during CPD. The liquid CO₂ was heated and pressurized to the critical point at which the liquid turned into a gas without damaging the samples due to surface tension, leaving the samples dry and undamaged. Using black double-sided tape, the dried samples were carefully mounted onto SEM stubs. The sample stubs were transferred to the Quorum Q150R ES sputter coater. In this step, the samples were coated twice with gold and palladium to make them conductive to the electron beam. After drying, the samples were examined under the Zeiss EVO LS15 SEM.

4.2.5 Statistical analysis

The experiment was arranged in a completely randomized design. Data collected was subjected to analysis of variance (ANOVA) using the 20th edition of GenStat® software to determine differences between treatment means. The means were separated using Duncan's Multiple Range Test at $P \leq 0.05$ confidence interval.

4.3 Results

4.3.1. *In vitro* screening of plant extracts against *C. gloeosporioides*

In vitro screening of plant extracts showed that higher concentrations of *P. sidoides* extract had higher inhibition compared to lower concentrations. There was a significant difference between the treatments and the control ($p \leq 0.05$).

Table 4.1: Mean percentage inhibition of *C. gloeosporioides* by *Aloe vera* plant extracts on PDA after 7 days at 28°C.

Isolates	Average inhibition (mm)	% Inhibition	Class
1% <i>Aloe</i>	81.00bc	4.71	1
1.5% <i>Aloe</i>	77.67a	8.62	1
2% <i>Aloe</i>	79.00ab	7.06	1
2.5% <i>Aloe</i>	82.33cd	3.14	1
3% <i>Aloe</i>	79.00ab	3.14	1
Control	85.00c		
P-level	<0.001		
L.S.D	2.965		

Table 4.2: Mean percentage inhibition of *C. gloeosporioides* by *Tetradenia riparia* plant extract on PDA after 7 days at 28°C.

Isolates	Average inhibition (mm)	% Inhibition	Class
1% <i>Tetradenia</i>	42.67a	49.80	2
1.5% <i>Tetradenia</i>	45.33a	46.67	2
2% <i>Tetradenia</i>	47.00ab	44.70	2
2.5% <i>Tetradenia</i>	45.33a	46.67	2
3% <i>Tetradenia</i>	55.33b	34.91	1
Control	85.00c		
P-level	<0.001		
L.S.D	10.70		

Table 4.3: Mean percentage inhibition of *C. gloeosporioides* by *Moringa oleifera* plant extract on PDA after 7 days at 28°C.

Isolates	Average inhibition (mm)	% Inhibition	Class
1% <i>Moringa</i>	9.00a	89.41	3
1.5% <i>Moringa</i>	9.00a	89.41	3
2% <i>Moringa</i>	8.33a	90.2	3
2.5% <i>Moringa</i>	13.33ab	84.32	3
3% <i>Moringa</i>	17.33b	79.61	3
Control	85.00c		
P-level	<0.001		
L.S.D	6.617		

Table 4.4: Mean percentage inhibition of *C. gloeosporioides* by *Pelargonium sidiodes* plant extract on PDA after 7 days at 28°C.

Isolates	Average inhibition (mm)	% Inhibition	Class
1% <i>Pelargonium</i>	31.67b	62.74	2
1.5% <i>Pelargonium</i>	3.33a	96.08	3
2% <i>Pelargonium</i>	0.00a	100	3
2.5% <i>Pelargonium</i>	0.00a	100	3
3% <i>Pelargonium</i>	0.00a	100	3
Control	80.00c		
P-level	<0.001		
L.S.D	4.799		

The inhibition of mycelial growth of *C. gloeosporioides in vitro* is shown in Tables 4.1, 4.2, 4.3, and 4.4 and Figure 4.1. These results were used to eliminate the extracts that did not inhibit the mycelial growth of *C. gloeosporioides*. *P. sidiodes* extract had significant inhibition on mycelial growth compared to *M. oleifera*, *T. riparia*, and *A. vera* (Figure 4.1) Non-treated control had the most mycelial growth followed by *A. vera* which did not inhibit the growth of *C. gloeosporioides*, *Moringa*, *Tetradenia* and *Pelargonium* which inhibited the growth of the pathogen (Figure 4.1)

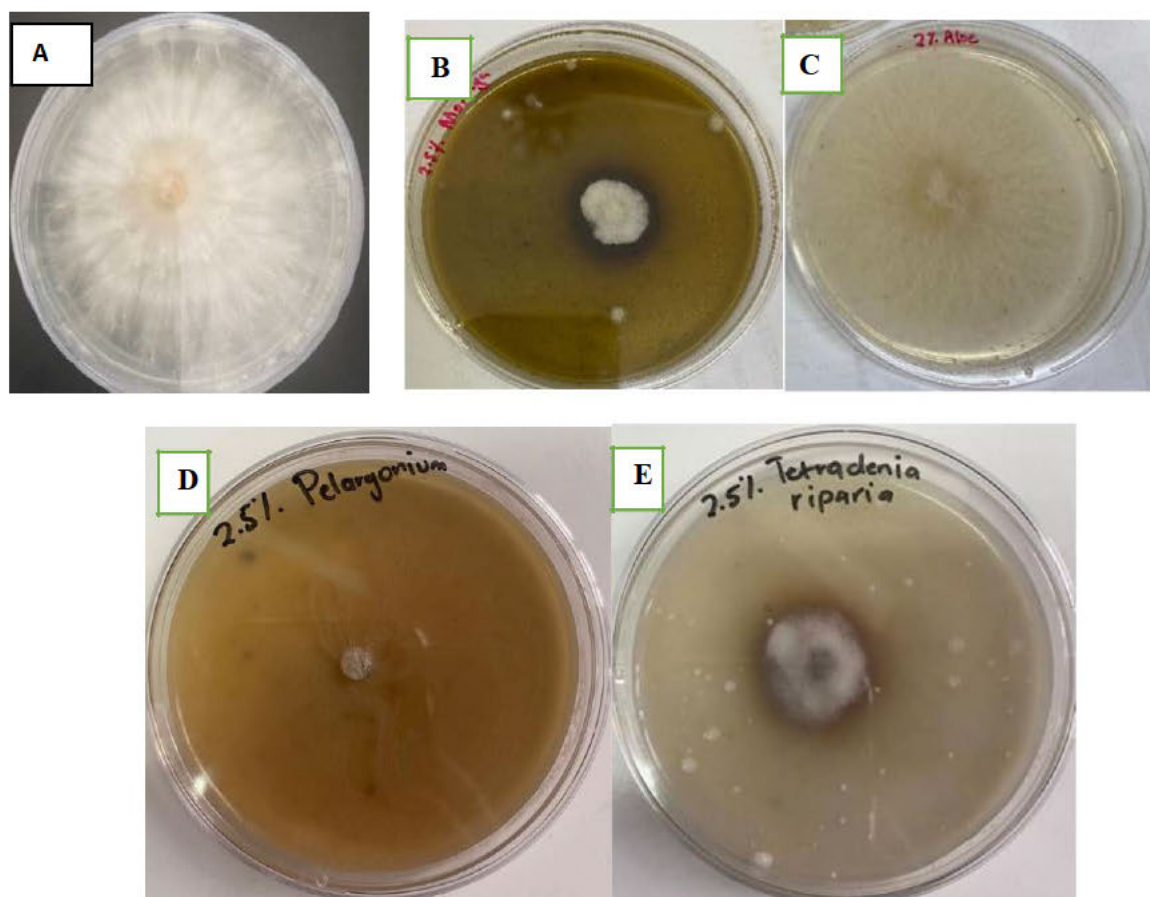


Figure 4.1: *In vitro* inhibitory activity of different plant extracts, with different concentrations, control plate showing PDA amended with water (A), *Moringa oleifera* (B), *Aloe vera* (C), *Pelargonium sidoides* (D). *Tetradenia riparia* (E) and against *C. gloeosporioides* after 7 days incubation at 28°C.

4.3.2 *In vivo* effect of plant extracts against *C. gloeosporioides*

Fruits were observed for disease incidence and mycelial growth inhibition after 7 days post-inoculation at 25°C (Figure 4.2) during *in vivo* screening. There was less disease incidence of *C. gloeosporioides* on mango fruit treated with *P. sidoides* extracts at 2.5% ($\leq 75\%$) and 3% at ($\leq 50\%$) concentrations (Figure 4.2 B and C) compared to the control fruit (Figure 4.2 A) which had 100% infection. The efficacy of the treatments was then established in Figure 4.2 (B and C).



Figure 4.2: *In vivo* anthracnose suppression with different concentrations of *Pelargonium sidoides* extracts (A) Inoculated, non-treated control; 2.5% extract concentration; (C) 3% extract concentration respectively on mango fruit after 7 days post-inoculation at 25°C.

4.3.3 Scanning Electron Microscope of the interaction between plant extracts against *C. gloeosporioides* *in vitro*

Mycelial growth and conidia of *C. gloeosporioides* was observed using the SEM (Figure 4.3) after 7 days post inoculation at 28°C. The interaction of *Pelargonium sidoides* against *C. gloeosporioides* was shown by the change in the mycelial and hyphal structures of *C. gloeosporioides*. SEM micrographs revealed that pelargonium caused the damage to the morphology of both mycelia and conidia (Figure 4.3 B and C) compared to the control (Figure 4.3 A).

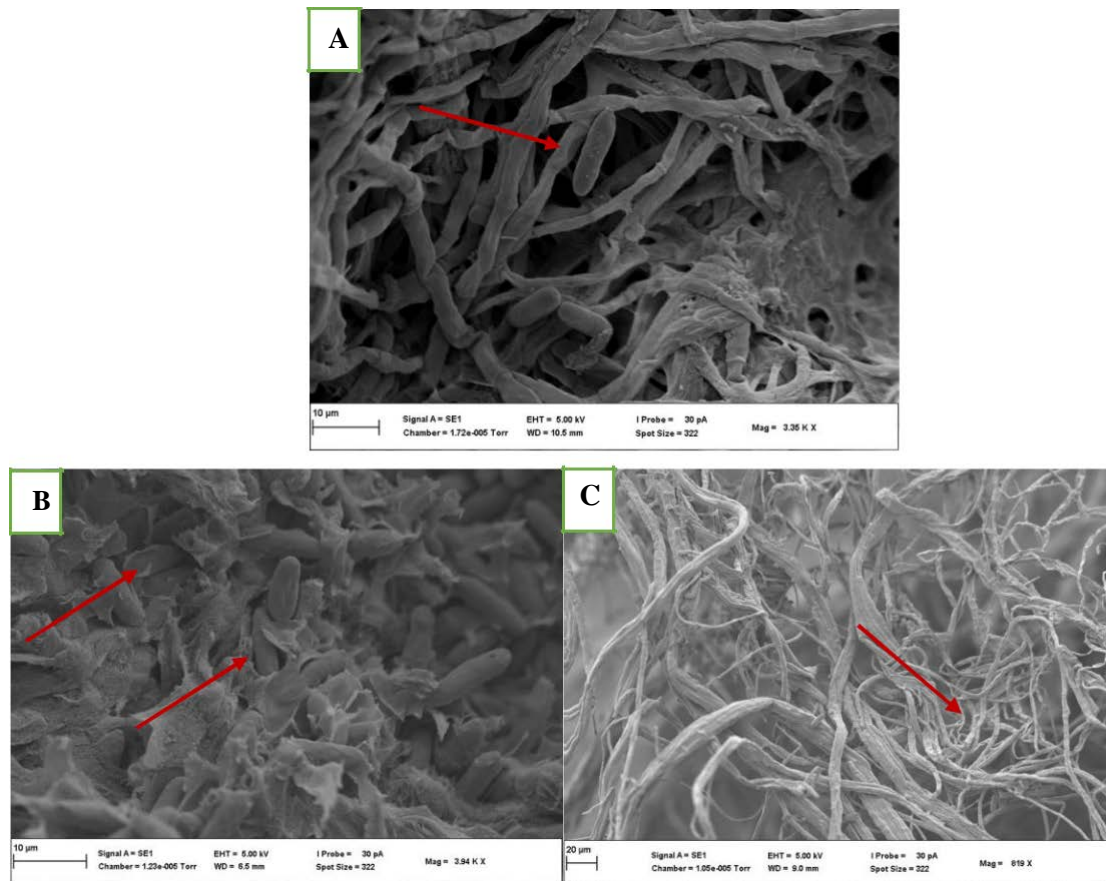


Figure 4.3: Scanning electron microscopy micrographs of interaction of *Pelargonium sidoides* extract with *C. gloeosporioides* on PDA. Control inoculated with the pathogen only (A), *P. sidoides* at 2.5% concentration (B) and *P. sidoides* at 3% concentration (C) respectively 7 days post inoculation at 28°C. Red arrows indicate the damaged mycelia and spores of *C. gloeosporioides* (B and C) and mycelia and spores of *C. gloeosporioides* on PDA (A).

The interaction of *P. sidoides* extract and *C. gloeosporioides* on mango surface is shown in Figure 4.4. The spores of the pathogen were abundant on the surface of the mango fruit inoculated with the pathogen only (Figure 4.4 A). Fruit treated with *P. sidoides* at 2.5% and 3% concentrations had no spores observed and there was shrinkage of pathogen mycelia (Figure 4.4 B and C).

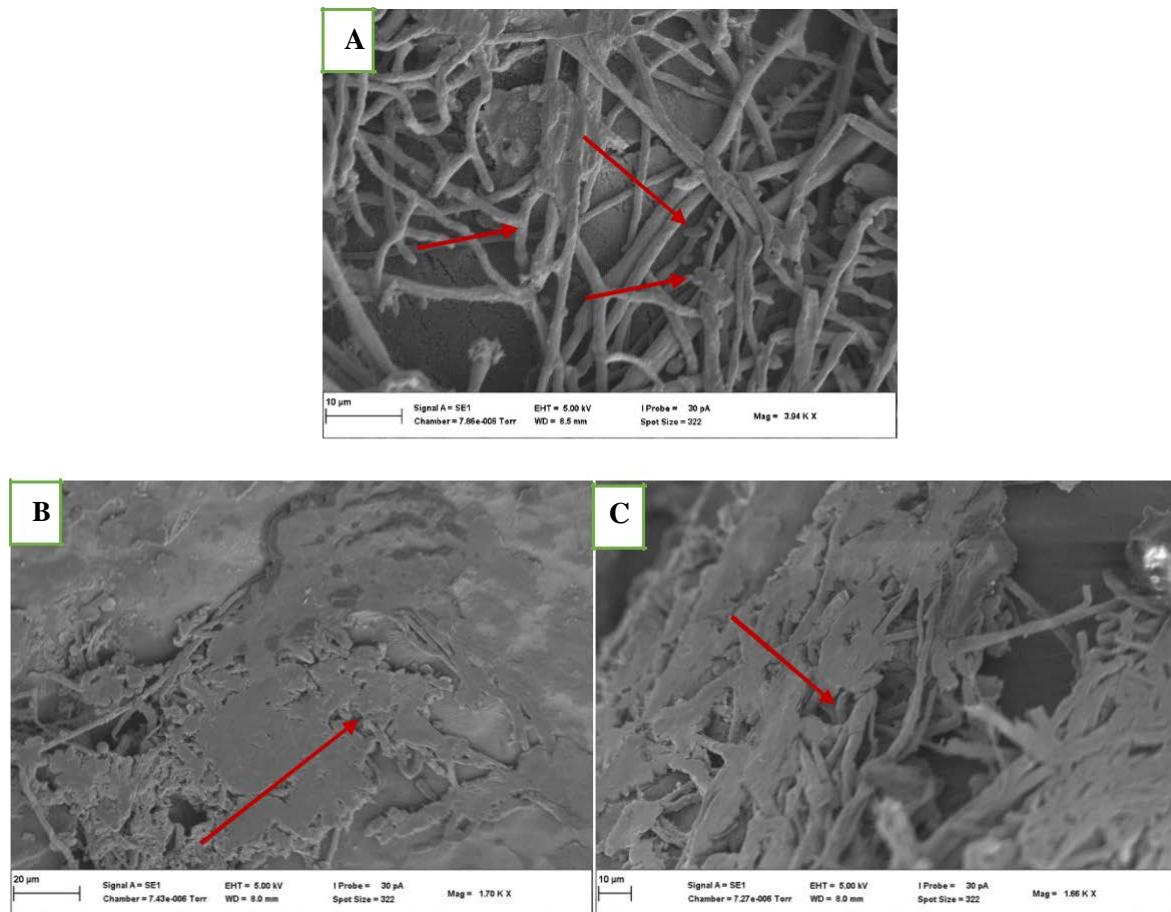


Figure 4.4: Scanning electron microscopy micrographs of the interaction of *Pelargonium sidoides* extract against *C. gloeosporioides* on mango fruit. Control inoculated with the pathogen only (A), *P. sidoides* at 2.5% concentration (B) and *P. sidoides* at 3% concentration (C) respectively 7 days post inoculation at 25°C. Red arrows indicate the damaged mycelia and spores of *C. gloeosporioides* (B and C) and mycelia and spores of *C. gloeosporioides* on PDA (A).

4.4 Discussion

The use of leaf plant extracts with medicinal properties in controlling postharvest fungal diseases may provide an alternative to the use of synthetic fungicides (Bambalele *et al.*, 2021). The use of plant extracts involves the preparation of desired concentrations, and the selection of the best-performing agents *in vitro* and *in vivo*. In this study, *M. oleifera*, *A. vera*, *T. riparia*, and *P. sidoides* extracts were assessed against *C. gloeosporioides*. The inhibitory effect of some of the tested extracts was recognised by the failure of the pathogen to grow effectively and spread on the petri dish.

The growth inhibition of the fungal pathogen is due to the antimicrobial effect on the plant extracts (Samie *et al.*, 2019). The difference between the growth inhibition is likely to reflect the effect of the concentration of the plant extracts. For *Pelargonium*, as the concentration increased, the anthracnose disease incidence decreased. The efficacy of the treatments *in vitro* is prejudiced hence fruit treatment for accuracy and to explore the full potential of the treatments in controlling the pathogenic fungi (Ziena, 2019).

The 2.5% and 3% concentrations of *Pelargonium* extract had the most inhibitory effect against *C. gloeosporioides* when subjected to *in vivo* screening on healthy mango fruit. The findings showed that the 3% *pelargonium* extract significantly ($p < 0.05$) reduced the disease incidence compared to the 2.5% concentration and the control fruits. These findings are in accordance with Yeo *et al.* (2023) where the disease incidence and severity decreased by increasing the concentration of plant extracts on stem-end-rot of mango *in vivo*. The antifungal effect of plant extracts could be attributed to the presence of antioxidants such as phenols, flavonoids, alkaloids, quinones, and saponines (Villalobos *et al.*, 2016; Onaebi *et al.*, 2020).

The scanning electron microscopy images exhibited that the morphology of *Pelargonium*-treated samples changed, and the mycelia was thin and broken. The number of spores observed on the *pelargonium* treated samples was fewer as compared to the pathogen only treatment which had healthy spores and mycelia *in vitro*. Control mango fruits had fully formed spores and healthy mycelia, whereas the mycelia were shredded, and no healthy spores were observed on *Pelargonium* treated fruits. According to Ahmadu *et al.* (2020), the antifungal activity of *M. oleifera* affects the mycelial growth and sporulation of the pathogen, as it destroys the cytoplasmic organelles and nucleus, leading to the loss of viability and germination of the pathogen. Functional integrity of fungal cell components is the basic requirement for viability and germination (Ahmadu *et al.*, 2020). Notably the irreversible destructive changes caused by *moringa* deactivate the capacity of the pathogen to regenerate (Ahmadu *et al.*, 2020).

The results from this study have shown that *P. sidoides* can be used as a plant extract to control pathogenic fungi as it has displayed some antimicrobial properties and reduces the dependence on synthetic fungicides. This is supported by a study conducted by Du Toit (2020), where antimicrobial peptides such as lipopeptides on *P. sidoides* were identified. Plants producing these lipopeptides are reported to have improved health as lipopeptides induce systemic resistance and direct antagonism towards invading pathogens on humans (Du Toit, 2020). Similarly, to the study by Aboobaker *et al.* (2019), where the antimicrobial effect of endophytes isolated from *P. sidoides* was explored. These endophytes displayed a synergistic interaction with *P. sidoides* as the host plant and exhibited antimicrobial properties against fungal pathogens (Aboobaker *et al.*, 2019). *P. sidoides* has been used mostly for human viral and bacterial diseases, but the impact on fungal diseases postharvest has not been explored (Samie *et al.*, 2019).

4.5 Conclusion

Prevention of postharvest diseases with eco-friendly and relatively cost-effective methods is one of the strategies that reduce food losses and maximise the profit for the producers. This study was conducted to assess the potential of plant extracts in controlling anthracnose of mango. *P. sidoides* successfully reduced anthracnose of mango in comparison to the non-treated control. Anthracnose is a latent disease that can be present and invisible hence there was disease incidence observed on treated fruit. *P. sidoides* have antifungal and antimicrobial properties and can be recommended as an alternative with the potential to control postharvest fungal pathogens and reduce the use of fungicides.

4.5 References

- Aboobaker, Z., Viljoen, A., Chen, W., Crous, P.W., Maharaj, V.J. and Van Vuuren, S., 2019. Endophytic fungi isolated from *Pelargonium sidoides* DC: antimicrobial interaction and isolation of a bioactive compound. South African Journal of Botany, 122, pp.535-542.
- Ahmadu, T., Ahmad, K., Ismail, S.I., Rashed, O., Asib, N. and Omar, D., 2020. Antifungal efficacy of *Moringa oleifera* leaf and seed extracts against *Botrytis cinerea* causing gray mold disease of tomato (*Solanum lycopersicum* L.). Brazilian Journal of Biology, 81, pp.1007-1022.

- Arauz, L.F., 2000. Mango anthracnose: Economic impact and current options for integrated management. *Plant Disease*, 84(6), pp.600-611.
- Atiq, M., Naeem, I., Sahi, S.T., Rajput, N.A., Haider, E., Usman, M., Shahbaz, H., Fatima, K., Arif, E. and Qayyum, A., 2020. Nanoparticles: A safe way towards fungal diseases. *Archives of Phytopathology and Plant Protection*, 53(17-18), pp.781-792.
- Bambalele, N.L., Mditshwa, A., Magwaza, L.S. and Tesfay, S.Z., 2021. The effect of gaseous ozone and moringa leaf-carboxymethyl cellulose edible coating on antioxidant Activity and biochemical properties of 'Keitt' mango fruit. *Coatings*, 11(11), p.1406.
- Bambalele, N.L., Mditshwa, A., Magwaza, L.S. and Tesfay, S.Z., 2021. Recent advances on postharvest technologies of mango fruit: A review. *International Journal of Fruit Science*, 21(1), pp.565-586.
- Banya, M., Garg, S. and Meena, N.L., 2020. A review: Chilli anthracnose, its spread and management. *Journal of Pharmacognosy and Phytochemistry*, 9(4), pp.1432-1438.
- Bayoumi, A.E., 2022. Deleterious effects of banned chemical pesticides on human health in developing countries. *Pesticides-Updates on Toxicity, Efficacy and Risk Assessment*, p.169.
- Du Toit, L.A., 2020. Discovery of antifungal compounds produced by *Pelargonium sidoides*-associated microorganisms. Doctoral dissertation, Department of Plant Pathology: Stellenbosch University, Cape Town, South Africa.
- El Khetabi, A., Lahlali, R., Ezrari, S., Radouane, N., Lyouf, N., Banani, H., Askarne, L., Tahiri, A., El Ghadraoui, L., Belmalha, S. and Barka, E.A., 2022. Role of plant extracts and essential oils in fighting against postharvest fruit pathogens and extending fruit shelf life: A review. *Trends in Food Science and Technology*, 120, pp.402-417.
- El Ouadi, Y., Bendaif, H., Assaggaf, H., Abdallah, E.M., Mekkaoui, M., Mrabti, H.N., Manssouri, M., Benali, T., Bouyahya, A. and Bouyanzer, A., 2022. Efficacy of *Pelargonium graveolens* essential oils against some postharvest fungal diseases of apple. *Advancements in Life Sciences*, 9(2), pp.195-201.
- Leng, P., Zhang, Z., Pan, G. and Zhao, M., 2011. Applications and development trends in biopesticides. *African Journal of Biotechnology*, 10(86), pp.19864-19873.

- Liamngee, K., Terna, A.C. and Ussuh, M.W., 2019. Effect of *Moringa oleifera* leaf extract on the postharvest quality of tomato fruits during storage. *Journal of Postharvest Technology*, 7(3), pp.45-55
- Luanda, A. and Ripanda, A., 2022. Recent trend on *Tetradenia riparia* (Hochst.) Codd (Lamiaceae) for management of medical conditions. *Phytomedicine Plus*, p.100382.
- Masibo, M. and He, Q., 2008. Major mango polyphenols and their potential significance to human health. *Comprehensive Reviews in Food Science and Food safety*, 7(4), pp.309-319.
- Matrose, N.A., Obikeze, K., Belay, Z.A. and Caleb, O.J., 2021. Plant extracts and other natural compounds as alternatives for post-harvest management of fruit fungal pathogens: A review. *Food Bioscience*, 41, p.100840.
- Mendy, T.K., Misran, A., Mahmud, T.M.M. and Ismail, S.I., 2019. Antifungal properties of *Aloe vera* through in vitro and in vivo screening against postharvest pathogens of papaya fruit. *Scientia Horticulturae*, 257, p.108767.
- Onaebi, C., Onyeke, C., Osibe, D., Ugwuja, F., Okoro, A. and Onyegirim, P., 2020. Antimicrobial activity of *Ocimum gratissimum* L. and *Carica papaya* L. against postharvest pathogens of avocado pear (*Persea americana* Mill.). *Journal of Plant Pathology*, 102, pp.319-325.
- Samie, S., Trollope, K.M., Joubert, L.M., Makunga, N.P. and Volschenk, H., 2019. The antifungal and *Cryptococcus neoformans* virulence attenuating activity of *Pelargonium sidoides* extracts. *Journal of Ethnopharmacology*, 235, pp.122-132.
- Sudheeran, P.K., Sela, N., Carmeli-Weissberg, M., Ovadia, R., Panda, S., Feygenberg, O., Maurer, D., Oren-Shamir, M., Aharoni, A. and Alkan, N., 2021. Induced defense response in red mango fruit against *Colletotrichum gloeosporioides*. *Horticulture Research*, 8, pp.1-12.
- Tarabih, M., 2020. Maintenance quality and reduce chilling Injury of naomi mango fruits during cold quarantine. *Pakistan Journal of Biological Sciences: PJBS*, 23(2), pp.190-205.
- Tesfay, S.Z. and Magwaza, L.S., 2017. Evaluating the efficacy of moringa leaf extract, chitosan and carboxymethyl cellulose as edible coatings for enhancing quality and extending

- postharvest life of avocado (*Persea americana* Mill.) fruit. Food Packaging and Shelf Life, 11, pp.40-48.
- Tesfay, S.Z., Magwaza, L.S., Mditshwa, A. and Mbili, N., 2017, September. Carboxyl methylcellulose (CMC) incorporated with moringa leaf and seed extracts as new postharvest organic edible coating for avocado (*Persea americana* Mill.) fruit. In VII International Conference on Managing Quality in Chains (MQUIC2017) and II International Symposium on Ornamentals in 1201 (pp. 161-168).
- Tijjani, A., Bashir, K.A., Mohammed, I., Muhammad, A., Gambo, A. and Musa, H., 2016. Biopesticides for pests control: A review. Journal of Biopesticides and Agriculture, 3(1), pp.6-13.
- Villalobos, M.D.C., Serradilla, M.J., Martín, A., Ordiales, E., Ruiz-Moyano, S. and Córdoba, M.D.G., 2016. Antioxidant and antimicrobial activity of natural phenolic extract from defatted soybean flour by-product for stone fruit postharvest application. Journal of the Science of Food and Agriculture, 96(6), pp.2116-2124.
- Yeo, Y.S., Dembele, D.D., Camara, B., Ouattara, S., Rey, J.Y., Fernandez, D. and Kone, D., 2023. Effect of some biopesticides based on essential oil and plant extracts on postharvest mango Stem-end rot disease caused by *Lasiodiplodia theobromae*. Journal of Agriculture and Food Research, 14, p.100798.
- Ziena, L.W., 2019. Integration of rapid hot water treatments and biocontrol agents to control postharvest pathogens of tomato. Master of Science dissertation, Department of Plant Pathology, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Chapter 5

The effect of *Burkholderia contaminans* and *Pelargonium sidoides* infused into carboxymethyl cellulose as postharvest treatments on antioxidant activity of ‘Kent’ mango fruit.

Abstract

Mango fruits have a high nutritional value and are beneficial to human health. However, fruit losses frequently occur after harvest because of their high perishability. The antioxidant capacity of the fruit is also compromised during postharvest handling and storage. Therefore, this study investigated the effect of *Pelargonium sidoides*–carboxymethyl cellulose (PE) edible coating, biocontrol agent *Burkholderia contaminans* infused with carboxymethyl cellulose (BCA) and carboxymethyl cellulose (CMC) on the antioxidant activities of ‘Kent’ mango fruit. For this purpose, mango fruit was procured, disinfected, and treated with CMC, BCA, PE, and the combination of treatments, untreated fruits were used as a control treatment. Fruits were stored at 10 °C for twenty-one days, then ripened at ambient temperature for seven days. Mango fruit was assessed for mass loss percentage, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), total phenolic content, and ascorbic acid (AA). The treatments had a significant effect ($p < 0.05$) on mass loss %. Untreated fruit had the highest mass loss (18.53%) compared to all treatments (CMC, BCA, BCA+PE and PE) which had 15.3%, 14.64%, 14.18% and 14.04%, respectively. Mango fruit coated with PE had DPPH scavenging activity at 51.11 $\mu\text{M TE/g DM}$, whereas CMC treated fruit had 29.66 $\mu\text{M TE/g DM}$, even lower than untreated fruit which had 36.52 $\mu\text{M TE/g DM}$. Phenolic content was recorded at 116.4 $\mu\text{g GEA/g DM}$ on PE treated fruit compared to 95.1 $\mu\text{g GEA/g DM}$ for the control fruit. The concentration of AA was also at 51.95 mg/g on PE treated mango compared to 24.61 mg/g on the control fruit. These findings demonstrate that *P. sidoides*–carboxymethyl cellulose (PE) reduced ripening and enhanced the antioxidants of mango fruit during storage. *P. sidoides* infused with CMC can be used to prolong the shelf life of mango while retaining nutrients, not compromising the fruit quality.

Keywords: Mango fruit; carboxymethyl cellulose; antioxidants; biocontrol agent; edible coating

5.1 Introduction

Mango is the third most important tropical and subtropical fruit, due to its health-related properties on human (Bambalele *et al.*, 2019). It is rich in compounds such as carotenoids, amino acids, ascorbic acid, antioxidants and phenolics (Swaroop *et al.*, 2018; Lenucci *et al.*, 2022). The consumption of fruit that is rich in natural antioxidants is linked to reduced risks of diseases such as Alzheimer's, diabetes, cardiovascular disease, and cancer (Burton-Freeman *et al.*, 2017; Yap, *et al.*, 2021; Bambalele *et al.*, 2021a). The wide distribution and demand have created a gap in the sustainable production of fresh mango (Ribeiro and Schieber, 2010).

Mango is sold on mass basis, thus, mass loss results in huge income loss for producers (Ntsoane *et al.*, 2019). Also, tends to have limited shelf-life, due to its climacteric nature (Odetayo *et al.*, 2022). This is due to ethylene production, causing drastic changes in enzyme activity leading to colour change and overripening (Bambalele *et al.*, 2019). Transportation of mango fruit for a long period of time is disadvantaged by the high perishability (Arauz 2000). Prolonging shelf life is essential to maximise supply while preserving fruit quality (Bibi and Baloch, 2014). Ambient temperature catalysis the ripening process and reduces shelf life (Hmam, *et al.*, 2021). Hence, controlled temperature is required to minimize respiration and fruit softening, and this is essential during peak season (Arampath and Dekker, 2019).

Disinfection, packaging, control of storage temperature, and the use of chemicals such as 1-Methylcyclopropene (1-MCP) have been used effectively in prolonging the shelf life of mango and retaining the quality of fruits (Tefera *et al.*, 2007; Mditshwa *et al.*, 2020). However, low temperatures under 10°C expose the fruit to chilling injury (Kebbeh *et al.*, 2023). The research conducted by Xu *et al.* (2023) revealed that melatonin treatment enhanced cold storage tolerance and reduced chilling injury in mango fruit by regulating lipid metabolic enzymes during refrigeration. As the demand for pesticide-free fruits increases, treatment of fruits in cold storage with edible coatings such as pectin, *M. oleifera* and essential oils are some of the explored environmentally friendly products (Moalemiyan *et al.*, 2012; Bambalele *et al.*, 2019; Perumal *et al.*, 2021).

Edible coatings are regarded as a dependable approach to reduce moisture and firmness loss, penetration of oxygen, water uptake by fruits and vegetables, and also improve the product appearance (Armghan Khalid *et al.*, 2022; Chavan *et al.*, 2023). Moreover, the coatings prolong the shelf life by creating a semi-permeable barrier to gases and moisture (Jafarzadeh *et al.*, 2021).

Most edible coatings have been explored, however the effect of *P.sidoides* as a plant extract has not yet been investigated. Hence, the objective of this study was to investigate the effect of *P. sidoides*–carboxymethyl cellulose edible coating, *Burkholderia contaminans* biocontrol agent and their combined effect on the antioxidant activities of ‘Kent’ mango fruit.

5.2 Materials and methods

5.2.1 Edible coatings

Carboxymethyl cellulose (Alfa Aesar, Lancashire, UK) was prepared using 500ml of autoclaved distilled water added to 5g of carboxymethyl cellulose powder. *P. sidoides* was extracted as described by Tesfay *et al.* (2017). 3% extract was prepared by weighing 30 g of extract, added to a 250 ml Erlenmeyer flask, and 70% (v/v) alcohol solvent was added. The mixture was then agitated in a shaker for 12 hours. The mixture was filtered with a cheesecloth, and the residues were discarded, and the aliquot was then evaporated in the GeneVac (Genevac® EZ 2.3; Ipswich, UK) at 45°C for 12 hours. The crude extract was suspended with 500 ml of distilled water and integrated with 5g of carboxyl methylcellulose (CMC). The prepared plant extracts were stored at 4°C.

5.2.2 Isolation of *Burkholderia contaminans* and preparation of coating

The leaf samples from different plant species were cut into pieces and rinsed with autoclaved tap water to wash off the soil and other microorganisms that might be on the leaf surface. Approximately 80g of each sample was weighed and placed in a 250 ml Erlenmeyer flask containing 100 ml of double sterilized distilled water. The Erlenmeyer bottles were then placed in a water bath at 130 rotations per minute for 1 hour at 80°C, to create a stock solution. A serial dilution was prepared from the stock solution and 1 ml of each dilution was added to 9 ml of double-sterilized distilled water. A volume of 200 µl of each dilution was inoculated in PDA plates and incubated at 28°C for 3 days. The PDA plates were washed with autoclaved distilled water, and the concentration of *B. contaminans* was adjusted to $1 \times 10^8 \text{ ml}^{-1}$ using a hemocytometer. A volume of 500 ml of *B. contaminans* ($1 \times 10^8 \text{ ml}^{-1}$) infused into 5g of CMC powder was prepared to make CMC+BCA 1% coating.

5.2.3 Fruit material

Mango fruit (cv. Kent) was procured from the Durban Fresh Produce Market and transported to the storage and research laboratory at the University of KwaZulu Natal in Pietermaritzburg. The fruit was then disinfected with 15% sodium hypochlorite for 1 minute then rinsed 2-3 times with autoclaved distilled water and air dried. A total of 225 fruit used were graded for uniformity (in size and colour) and assigned to different postharvest treatments. The experiment was replicated three times using three fruits per replicate. The treatments explored in the study were control, which was untreated fruit, fruits coated with 1% CMC only, *B. contaminans* infused with CMC, *P. sidoides* infused with CMC and lastly the combination of treatments infused with CMC:

Control: Untreated fruit,

T1: CMC 1% (EC),

T2: CMC 1% + PE,

T3: CMC 1% + BCA and

T4: CMC 1% + PE+BCA.

5.2.4 Sample preparation

The flesh and peel of mango fruit were freeze-dried using a Vir Tis Bench Top Pro freeze drier (SPScientific, Warminster, PA, USA). After that, samples were crushed into powder and stored at -80°C until used.

5.2.5 Mass loss

Mass loss was measured with a measuring scale and recorded on every sampling day during storage. Mass loss percentage was computed using the following equation, as described by Akhtar *et al.* (2016):

$$Weightloss(\%) = \frac{A-B}{A} \times 100$$

Where A indicates the fruit mass upon arrival and B indicates the fruit weight after storage intervals.

5.2.6 1,1-Diphenyl-2-picrylhydrazyl (DPPH)

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was determined as described by Allothman *et al.* (2010), with some modifications. Briefly, 150 mg sample was added to 3 ml methanol 80% (v/v) and incubated for 1.5 h at 35°C. After that, samples were cooled down and filtered with a 0.45 µm syringe filter (Merck, Warmstadt, Germany). Approximately 3.9 ml of methanolic DPPH (2.5 mg/100 ml) was added to the extract (100 µL) and incubated for 60 min at room temperature. The absorbance was measured with a spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA) at 515 nm, using methanol as blank. The DPPH radical scavenging activity was calculated and plotted against Trolox standards and expressed as µM TE/g DM.

5.2.7 Total phenolic content

The total phenolics were determined as described by Lamien-Meda *et al.* (2008), with minor modifications. Mango peel powder (1 g) was added to 10 ml acetone (80% v/v) at room temperature for 30 min with constant agitation. After that, samples were centrifuged (Avanti J-265XP, Beckman Coulter, Indianapolis, IN, USA) for ten minutes at 10 000 rpm (5°C). Total phenolic compounds were determined using the Folin– Ciocalteu method. In triplicates, sample extract (0.1 ml) was added to 2.5 ml Folin– Ciocalteu reagent (2 N). After five minutes, 2 ml sodium carbonate (75 g/L) was added, and samples were incubated at 65°C for 2 h. Thereafter, samples were cooled down, and absorbance was measured with a spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA) at 750 nm using acetone as blank. Total phenolic compounds were expressed as µg (GAE)/g dry matter.

5.2.8 Ascorbic acid

The ascorbic acid was measured as described by More and Rao (2019), with slight modification. Sample powder (1 g) was added to 9 ml of metaphosphoric acid (1%) and sonicated in ice for 3 min. Thereafter, samples were centrifuged (Avanti J-265 XP, Beckman Coulter, Indianapolis, IN, USA) at 10,000 rpm (4°C) for five minutes. The extract (1 ml) was added to 9 ml of 2,6-dichlorophenolindophenol dye (0.025%) and incubated in the dark at room temperature for ten minutes. The absorbance was measured with a spectrophotometer

(Shimadzu Scientific Instruments Inc., Columbia, MD, USA) at 515 nm using 1% metaphosphoric acid as a blank. Ascorbic acid was expressed as mg/g on dry mass basis.

5.2.9 Statistical analysis

The Data were subjected to the analysis of variance (ANOVA) using GenStat statistical software (GenStat ®, 18 edition, VSN International, Hemel Hempstead, UK). Fischer's least significant difference (LSD) was used to separate means at 5% level of significance.

5.3 Results and discussion

5.3.1 Mass loss

Fruit mass loss (%) continued to increase during the storage period (Figure 5.1). The plant extract *P. sidoides* significantly ($p < 0.05$) reduced mass loss % of the fruit. The untreated fruit had the highest mass loss (18.53%) compared to all treatments (CMC, BCA, BCA+PE and PE) which had 15.3%, 14.64%, 14.18% and 14.04%, respectively. Current results are similar to those reported by Phuangto *et al.* (2019) where the application of CMC and chitosan significantly reduced mass loss on 'nam dok mai' mango fruit that was stored at 25°C for a period of 14 days. Physiological processes postharvest impact the overall shelf life of climacteric fruits because water loss initiates wilting, loss in fruit texture, and accelerates senescence (Lufu *et al.*, 2020). A study conducted by Burdon and Clark. (2001) on the effect of water loss on the 'Hayward' kiwifruit resulted in decreased fresh weight. This confirms that edible coatings retain water in fruit postharvest leading to a reduced mass loss.

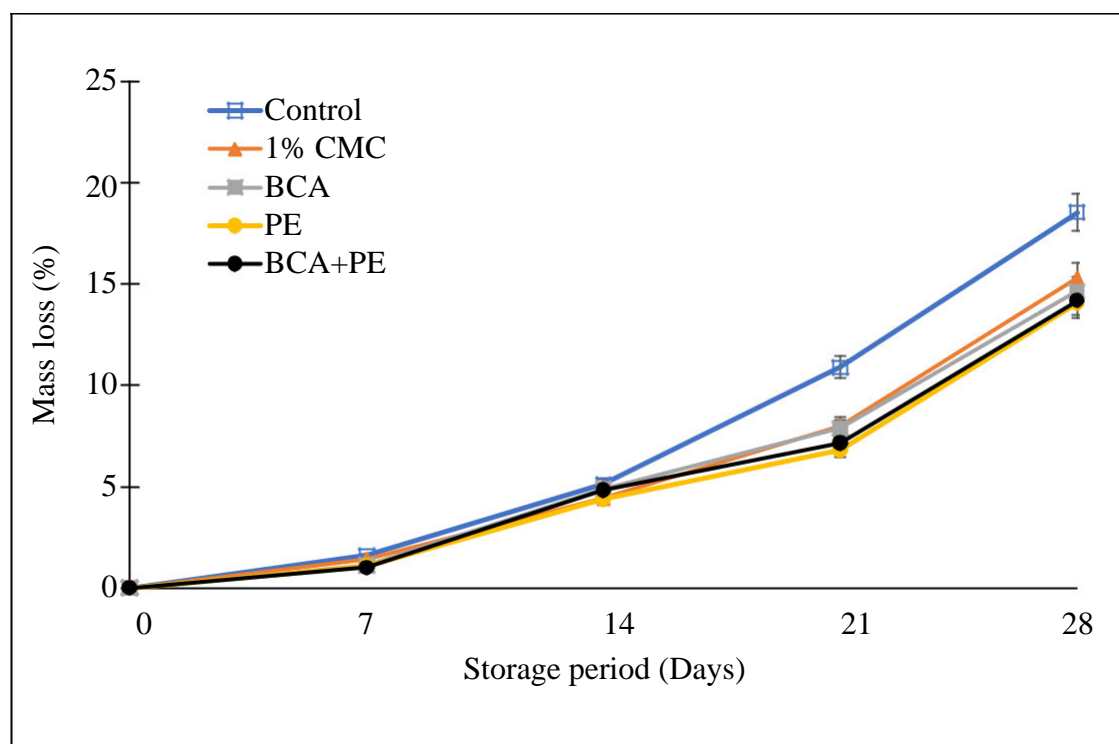


Figure 5.1: The effect of biocontrol agent, plant extract, CMC, and their combination on mass loss % of ‘Kent’ mangoes stored at 10°C for twenty-one days and seven days at room temperature. Data represents (\pm SE, n=9).

5.3.2 Antioxidant activity

The DPPH scavenging activity decreased significantly in all the treatments. All treatments were significantly different ($p < 0.05$) throughout the storage duration. The mango fruit coated with PE had the highest scavenging activity at the end of the storage, however, CMC treated fruit had the least amount antioxidant activity even lower than the control treatment (Figure 5.2). Contradictory to the results by Bambalele *et al.* (2019) on edible coatings on ‘Keitt’ mango, where the DPPH scavenging activity increased on the first fourteen days of storage. Win and Setha (2022) also demonstrated that salicylic acid reduced the DPPH scavenging activity after fourteen days of storage.

There are no studies currently reporting on the effect of biocontrol agents (*B. contaminans*) and *P. sidoides*-CMC coatings on DPPH scavenging activity in mango fruit. This study is the first to seek an understanding of these treatments on the antioxidant activity of mango fruit. The current results are comparable to those reported by Siddiqui *et al.* (2022) on banana ‘Grand Naine’ cultivar where treatment with hydrogen sulphide decreased the DPPH scavenging

activity during cold storage. This may be because the treatments used did not trigger the antioxidant stress to increase the level of DPPH scavenging activity.

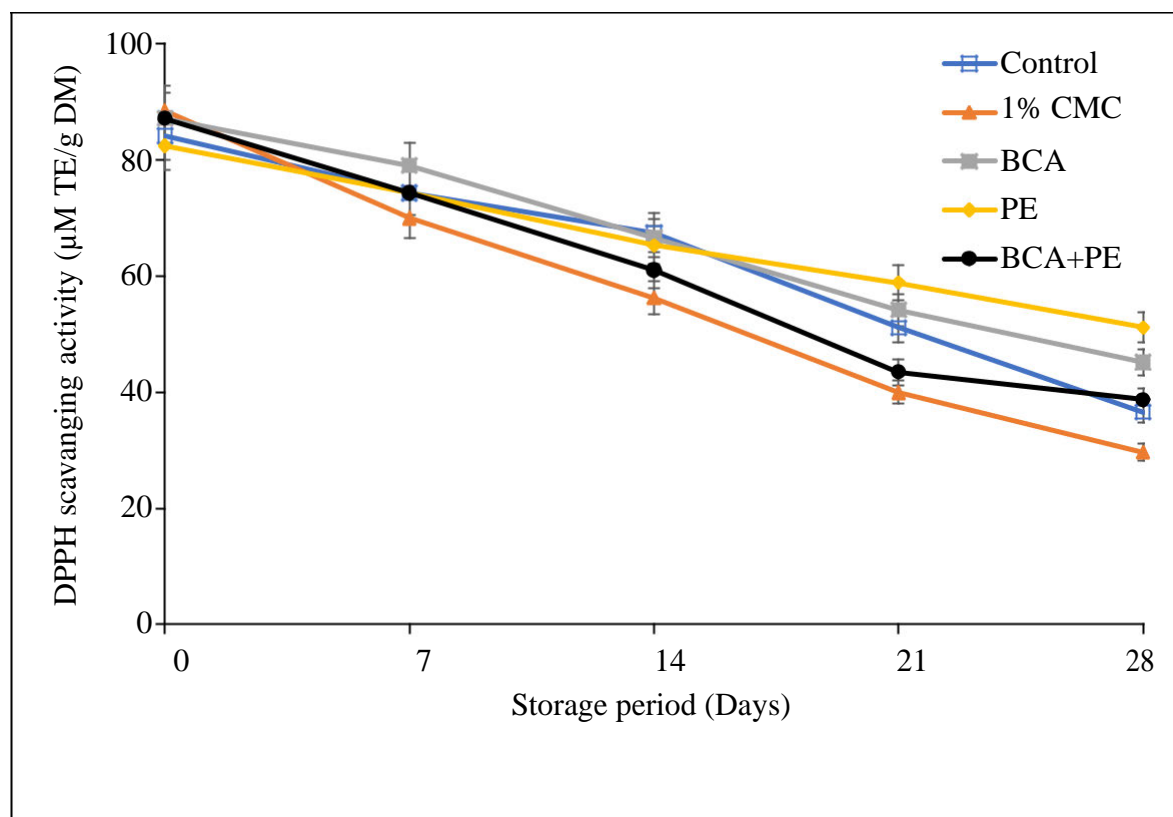


Figure 5.2: The effect of biocontrol agent, plant extract, CMC, and combination on DPPH scavenging activity of mango fruit during storage at 10°C for twenty-one days and seven days at room temperature (\pm SE, n=9).

5.3.3 Total phenolic content

The total phenolic compounds on the mango fruit decreased significantly ($p < 0.05$) during storage. The phenolic content on the fruit treated with PE was higher, with 116.4 $\mu\text{g GEA/g DM}$ than fruit treated with BCA+PE, CMC, BCA and untreated fruit at the end of storage with 112.3 $\mu\text{g GEA/g DM}$, 105.5 $\mu\text{g GEA/g DM}$, 101.8 $\mu\text{g GEA/g DM}$ and 95.1 $\mu\text{g GEA/g DM}$ respectively. Phenolic compounds are secondary metabolites in fruits. A recent study was conducted by Salehi *et al.* (2023) on the effect of edible coatings (xanthan, guar, and wild sage seed gums) on the phenolic content of sweet cherries. Their findings revealed that sweet cherries treated with edible coatings had a higher phenolic content compared to untreated fruit. This supports the findings of the current studies, where untreated mango fruit had reduced phenolic content of 95.1 $\mu\text{g GEA/g DM}$ compared to treated fruit with 116.4 $\mu\text{g GEA/g DM}$.

Despite the decrease in total phenolic content on all treatments, the PE treated fruit maintained a higher concentration of phenols at the end of storage. Phenolic compounds are antioxidants that act as a protective mechanism in fruits, also has a defence mechanism against diseases caused by pathogens (El-Gioushy *et al.*, 2022). Retaining phenolic compounds is reported to significantly reduce reactive oxygen species and prevent lipid peroxidation in plant tissue (Gill and Tuteja, 2010).

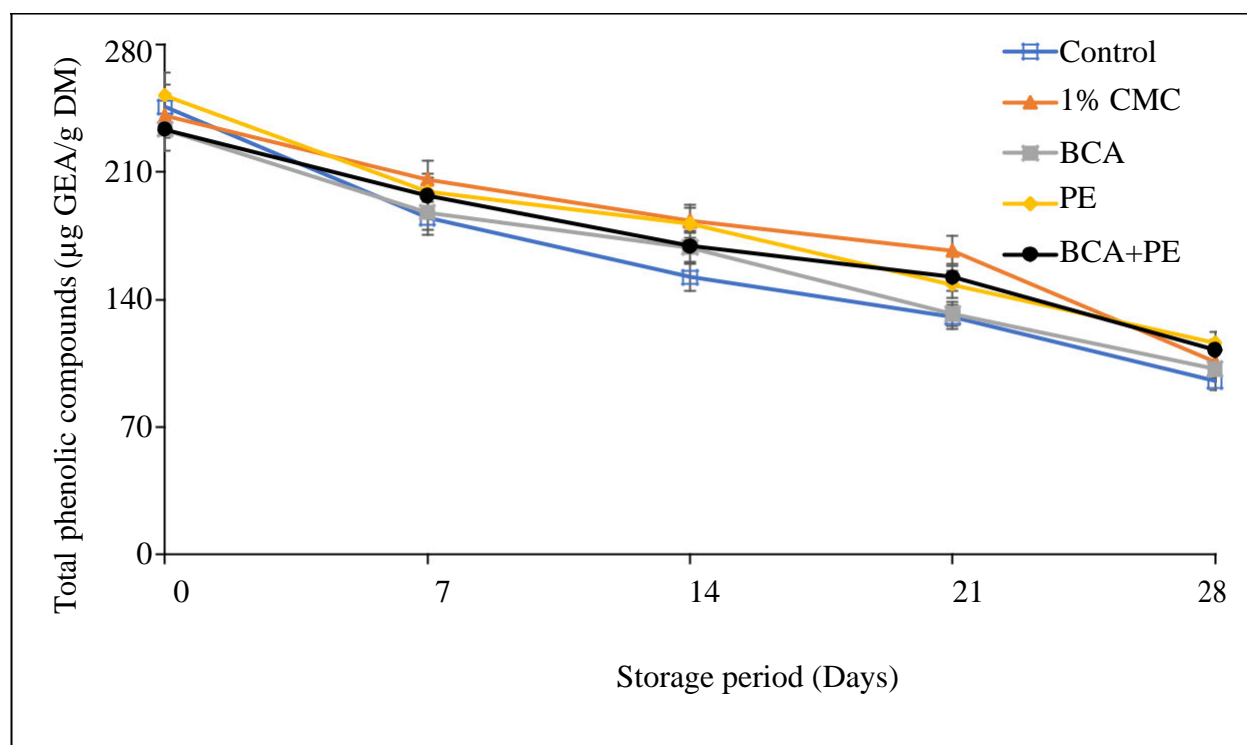


Figure 5.3: The changes in phenolic content of 'Kent' mango fruit peel after treatment with biocontrol agent, plant extract, CMC, and integrated treatment, during storage at 10°C for twenty-one days and shelf-life at ambient temperature for seven days (\pm SE, $n=9$).

5.3.4 Ascorbic acid

The concentration of ascorbic acid (AA) between the treatments was significant ($p<0.05$) over storage time (Figure 5.4). The fruit treated with PE has a decrease in AA from day 7, however maintained the highest AA at the end of storage compared to BCA+PE, CMC, BCA, and untreated fruit with 51.95mg/g, 41.95mg/g, 39.28mg/g, 33.95mg/g and 24.61mg/g respectively. As mango ripens, the level of AA decreases during storage due to the oxidation process (Ali *et al.*, 2022). Mango fruit treated with PE had a reduced oxidation hence maintaining a higher level of AA in comparison to the control after storage. These results are

comparable to Carrillo-Lopez *et al.* (2000), where ascorbic acid decreased in mango ‘Haden’ fruit which was stored at 13°C for 32 days, but this was slower in fruit coated with “Semperfresh” edible film. Contrary to the current study, a review by Mditshwa *et al.* (2017) on the effect of edible coatings on citrus reported that there was an insignificant effect of edible coatings on AA in citrus fruits. However, the research by Bambalele *et al.* (2021) where mango ‘Keitt’ was treated with EC preserved the AA content of mango fruit during storage. Treating fruits with coatings such as chitosan increases cytochrome oxidase activity by decreasing the internal oxygen content, which leads to a decreased decomposition rate of AA (Zahedi *et al.*, 2019). The combination of PE and BCA relatively maintained high concentration of AA after storage, this suggests that the combined effect of the combination may be explored further in preserving the concentration of AA.

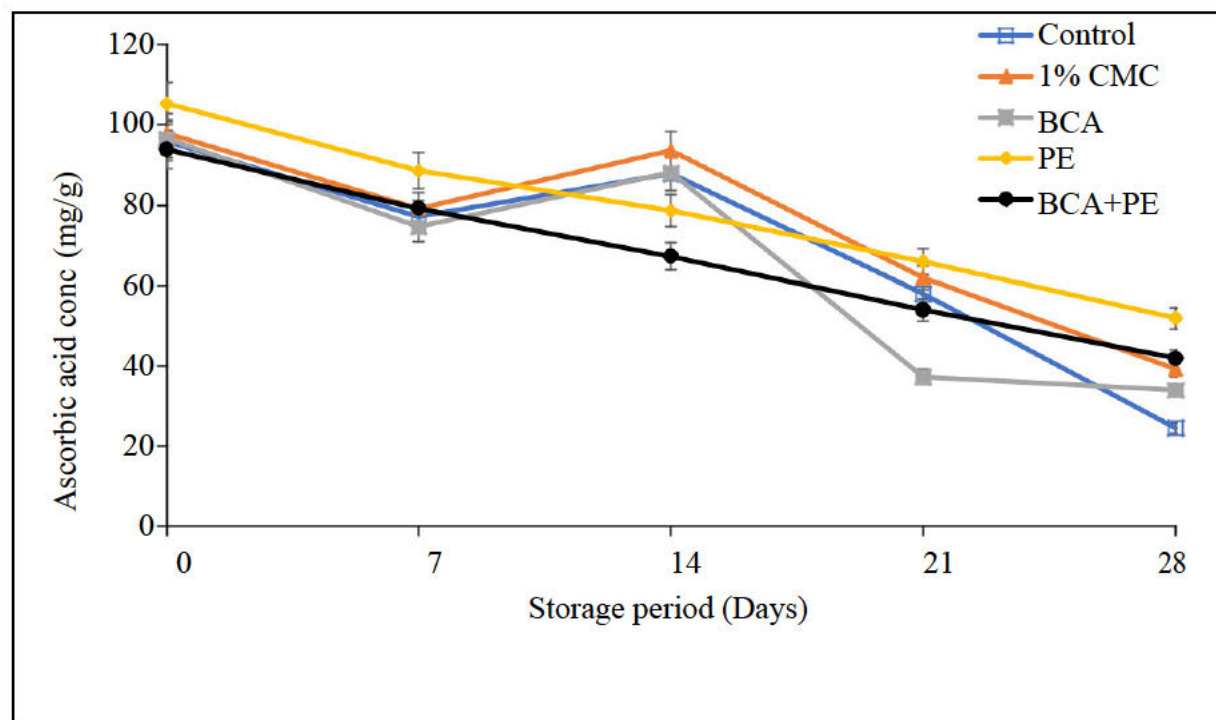


Figure 5.4: The changes in ascorbic acid concentration of ‘Kent’ mango fruit after treatment with biocontrol agent, plant extract, CMC, and integrated treatment, during storage at 10°C for twenty-one days and shelf-life at ambient temperature for seven days (\pm SE, n=9)

5.4 Conclusion

Plant extracts and biocontrol agents have been explored postharvest mostly for treating fungal diseases. This study presents the findings of *P. sidoides*-CMC, *B. contaminans*-CMC coatings, and the combined effect on postharvest treatment of mango fruit. The results indicated that the *P. sidoides*-CMC effectively reduced mass loss%, maintaining high DPPH scavenging activity. Mango fruit treated with PE had the highest level of phenolic content and also maintained the AA content. The current results lay a foundation for researchers on using the *P. sidoides* as plant extract in postharvest treatments to preserve quality, antioxidants and extend shelf life of fresh horticultural produce.

5.5 References

- Ali, S., Anjum, M.A., Khan, A.S., Nawaz, A., Ejaz, S., Khaliq, G., Iqbal, S., Ullah, S., Rehman, R.N.U., Ali, M.M. and Saleem, M.S., 2022. Carboxymethyl cellulose coating delays ripening of harvested mango fruits by regulating softening enzymes activities. *Food Chemistry*, 380, p.131804.
- Arauz, L.F., 2000. Mango anthracnose: Economic impact and current options for integrated management. *Plant Disease*, 84(6), pp.600-611.
- Armghan Khalid, M., Niaz, B., Saeed, F., Afzaal, M., Islam, F., Hussain, M., Mahwish, Muhammad Salman Khalid, H., Siddeeg, A. and Al-Farga, A., 2022. Edible coatings for enhancing safety and quality attributes of fresh produce: A comprehensive review. *International Journal of Food Properties*, 25(1), pp.1817-1847.
- Bambalele, N., Mditshwa, A., Tesfay, S.Z. and Magwaza, L.S., 2019, November. Moringa leaf extract infused into carboxymethyl cellulose edible coating combined with gaseous ozone as postharvest treatments maintain quality and extend shelf life of mango fruit. In *II International Symposium on Moringa* 1306 (pp. 225-232).
- Bibi, F. and Baloch, M.K., 2014. Postharvest Quality and Shelf Life of Mango (*Mangifera indica* L.) Fruit as Affected by Various Coatings. *Journal of Food Processing and Preservation*, 38(1), pp.499-507.
- Burdon, J. and Clark, C., 2001. Effect of postharvest water loss on 'Hayward' kiwifruit water status. *Postharvest Biology and Technology*, 22(3), pp.215-225.

- Burton-Freeman, B.M., Sandhu, A.K. and Edirisinghe, I., 2017. Mangos and their bioactive components: Adding variety to the fruit plate for health. *Food and Function*, 8(9), pp.3010-3032.
- Carrillo-Lopez, A., Ramirez-Bustamante, F., Valdez-Torres, J.B., Rojas-Villegas, R. and Yahia, E.M., 2000. Ripening and quality changes in mango fruit as affected by coating with an edible film. *Journal of Food Quality*, 23(5), pp.479-486.
- Chavan, P., Lata, K., Kaur, T., Jambrak, A.R., Sharma, S., Roy, S., Sinhmar, A., Thory, R., Singh, G.P., Aayush, K. and Rout, A., 2023. Recent advances in the preservation of postharvest fruits using edible films and coatings: A comprehensive review. *Food Chemistry*, p.135916.
- El-Gioushy, S.F., Abdelkader, M.F., Mahmoud, M.H., Abou El Ghit, H.M., Fikry, M., Bahloul, A.M., Morsy, A.R., Abdelaziz, A.M., Alhaithloul, H.A., Hikal, D.M. and Abdein, M.A., 2022. The effects of a gum arabic-based edible coating on guava fruit characteristics during storage. *Coatings*, 12(1), p.90.
- Gill, S.S. and Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, 48(12), pp.909-930.
- Jafarzadeh, S., Nafchi, A.M., Salehabadi, A., Oladzad-Abbasabadi, N. and Jafari, S.M., 2021. Application of bio-nanocomposite films and edible coatings for extending the shelf life of fresh fruits and vegetables. *Advances in Colloid and Interface Science*, 291, p.102405.
- Kebbeh, M., Dong, J.X., Chen, H.U.A.N., Yan, L.I.U. and ZHENG, X.L., 2023. Melatonin treatment alleviates chilling injury in mango fruit 'Keitt' by modulating proline metabolism under chilling stress. *Journal of Integrative Agriculture*, 22(3), pp.935-944.
- Lenucci, M.S., Tornese, R., Mita, G. and Durante, M., 2022. Bioactive compounds and antioxidant activities in different fractions of mango fruits (*Mangifera indica* L., cultivar Tommy Atkins and Keitt). *Antioxidants*, 11(3), p.484.
- Lufu, R., Ambaw, A. and Opara, U.L., 2020. Water loss of fresh fruit: Influencing pre-harvest, harvest, and postharvest factors. *Scientia Horticulturae*, 272, p.109519.

- Mditshwa, A., Magwaza, L.S., Tesfay, S.Z. and Mbili, N., 2017. Postharvest quality and composition of organically and conventionally produced fruits: A review. *Scientia Horticulturae*, 216, pp.148-159.
- Mditshwa, A., Magwaza, L.S., Tesfay, S.Z. and Opara, U.L., 2017. Postharvest factors affecting vitamin C content of citrus fruits: A review. *Scientia Horticulturae*, 218, pp.95-104.
- Moalemiyan, M., Ramaswamy, H.S. and Maftoonazad, N., 2012. Pectin-based edible coating for shelf-life extension of ataulfo mango. *Journal of Food Process Engineering*, 35(4), pp.572-600.
- Ntsoane, M.L., Zude-Sasse, M., Mahajan, P. and Sivakumar, D., 2019. Quality assesment and postharvest technology of mango: A review of its current status and future perspectives. *Scientia Horticulturae*, 249, pp.77-85.
- Odetayo, T., Tesfay, S. and Ngobese, N.Z., 2022. Nanotechnology-enhanced edible coating application on climacteric fruits. *Food Science and Nutrition*, 10(7), pp.2149-2167.
- Perumal, A.B., Nambiar, R.B., Sellamuthu, P.S. and Emmanuel, R.S., 2021. Use of modified atmosphere packaging combined with essential oils for prolonging post-harvest shelf life of mango (cv. Banganapalli and cv. Totapuri). *Lebensmittel-Wissenschaft & Technologie Food Science and Technology* 148, p.111662.
- Phuangto, S., Chandee, O., Subsomboon, T. and Wattanakaroon, W., 2019. Post-harvest shelf-life extension of mango using chitosan and carboxymethyl cellulose-based coatings. *Key Engineering Materials*, 824, pp.81-86.
- Ribeiro, S.M.R. and Schieber, A., 2010. Bioactive compounds in mango (*Mangifera indica* L.). In *Bioactive foods in promoting health*, Academic Press, pp. 507-523.
- Salehi, F., Ghazvineh, S. and Inanloodoghous, M., 2023. Effects of edible coatings and ultrasonic pretreatment on the phenolic content, antioxidant potential, drying rate, and rehydration ratio of sweet cherry. *Ultrasonics Sonochemistry*, 99, p.106565.
- Siddiqui, M.W., Homa, F., Lata, D., Mir, H., Aftab, T. and Mishra, P., 2022. Hydrogen sulphide infiltration downregulates oxidative metabolism and extends postharvest life of banana. *Plant Biology*, 24(4), pp.697-703.

- Swaroop, A., Bagchi, M., Moriyama, H. and Bagchi, D., 2018. Health benefits of mango (*Mangifera indica* L.) and mangiferin. *Japan Journal of Medicine*, 1(2), pp.149-154.
- Tefera, A., Seyoum, T. and Woldetsadik, K., 2007. Effect of disinfection, packaging, and storage environment on the shelf life of mango. *Biosystems Engineering*, 96(2), pp.201-212.
- Win, S.T. and Setha, S., 2022. Enhancement of anti-inflammatory and antioxidant activities of mango fruit by pre-and postharvest application of salicylic acid. *Horticulturae*, 8(6), p.555.
- Xu, P., Huber, D.J., Gong, D., Yun, Z., Pan, Y., Jiang, Y. and Zhang, Z., 2023. Amelioration of chilling injury in ‘Guifei’ mango fruit by melatonin is associated with regulation of lipid metabolic enzymes and remodeling of lipidome. *Postharvest Biology and Technology*, 198, p.112233.
- Yap, K.M., Sekar, M., Seow, L.J., Gan, S.H., Bonam, S.R., Mat Rani, N.N.I., Lum, P.T., Subramanian, V., Wu, Y.S., Fuloria, N.K. and Fuloria, S., 2021. *Mangifera indica* (Mango): A promising medicinal plant for breast cancer therapy and understanding its potential mechanisms of action. *Breast Cancer: Targets and Therapy*, pp.471-503.
- Zahedi, S.M., Hosseini, M.S., Karimi, M. and Ebrahimzadeh, A., 2019. Effects of postharvest polyamine application and edible coating on maintaining quality of mango (*Mangifera indica* L.) cv. Langra during cold storage. *Food Science and Nutrition*, 7(2), pp.433-441.

Chapter 6

Thesis overview

6.1 Introduction

Mango is known for its strong aroma, delicious taste, and high nutritional value (Tharanathan *et al.*, 2006). This fruit is grown worldwide with India, Pakistan, China, and Thailand as the leading producers (Thakor, 2019). Mango production in South Africa is growing, however is recorded to contribute 0.2% of worldwide production (Fivaz, 2006). Production of fruits and vegetables is greatly impacted by postharvest diseases resulting in significant losses. Diseases caused by opportunistic pathogens such as anthracnose have been reported to be destructive if not prevented or controlled early (Lima *et al.*, 2013; Rahul *et al.*, 2015). Anthracnose has a very wide host range including almond, avocado, apple, guava, strawberry, papaya, and banana (Sharma and Kulshrestha, 2015; Micah and Inkoom, 2016). These pathogenic fungi colonise the surface of immature fruit as a latent infection and may later develop under favourable conditions (Konsue *et al.*, 2020).

Fruit softening is one of the factors affecting the production of quality fruits (Payasi and Sanwal 2010). Climacteric nature of fruits, environmental conditions, degree of ripeness during harvest, and storage conditions also affect the production of mango fruit (Singh *et al.*, 2013). There are several control measures in place for reducing loss while maintaining the quality of fruits (Qadri *et al.*, 2020). Synthetic fungicides have been used effectively in the past, however, the demand for non-hazardous methods is being explored relentlessly (Arauz, 2000). Controlling temperature between the harvest period and consumption is an important factor in maintaining fruit quality (Xu *et al.*, 2023). The introduction of biocontrol agents and plant extracts as an alternative control against fungal diseases and treatment during storage is also important as it has a positive impact on fruit quality.

6.2 Research objectives and major findings

This study aimed to investigate the effect of biocontrol agents and plant extracts to control *C. gloeosporioides* of mango *in vitro* and *in vivo*. The specific objectives were as follows: (1) Evaluate the *in vitro* and *in vivo* screening of antagonistic microorganisms against *C. gloeosporioides*. (2) Evaluate the *in vitro in vivo* screening of plant extracts against *C. gloeosporioides* of mango. (3) Determine the mode of action of biocontrol agents and plant

extracts against *C. gloeosporioides* on mango fruit. (4) Evaluate the combined effect of plant extracts and biocontrol agents in prolonging the shelf life of mango in cold storage. The major research findings are highlighted below.

Chapter 3: *In vitro* and *in vivo* screening of antagonistic microorganisms against *C. gloeosporioides* of mango

- YBa (*Meyerozyma guilliermondii*), BSs and BSL (*Burkholderia contaminans*), isolates successfully suppressed the pathogen and depicted high efficacy against *C. gloeosporioides* both *in vitro* and *in vivo*.
- Both strains of *B. contaminans* had the highest antifungal effect against the pathogen.
- The SEM images clearly indicated the presence of bacteria and yeast on mango peel where few spores were produced.
- The bacteria compete for nutrients and space resulting in reduced growth of the pathogen.

Chapter 4: The antifungal effect of different plant extracts against *Colletotrichum gloeosporioides* on ‘Kent’ mangoes

- *Pelargonium sidoides* at 2,5% and 3% showed the antifungal effect against the mycelial growth of *C. gloeosporioides* *in vitro* and *in vivo*.
- The SEM images showed that the *P. sidoides* affected the morphology of the pathogen hyphae *in vitro*.
- The SEM images showed that *P. sidoides* extract deposits were found on mango peel which reduced the spread of the pathogen on the fruit resulting in a reduced disease incidence of anthracnose *in vivo*.

Chapter 5: The effect of *Burkholderia contaminans* and *Pelargonium sidoides* infused into carboxymethyl cellulose as postharvest treatments on antioxidant activity of ‘Kent’ mango fruit.

- *P. sidoides*-carboxymethyl cellulose (PE) treated mango fruit had a reduced mass loss after being exposed to 10°C for twenty-one days and seven days ambient temperature with 14.04% in comparison to 18.53% on untreated fruit.
- The effect of *P. sidoides*-carboxymethyl cellulose (PE) on antioxidants was studied, PE maintained 1.1-Diphenyl-2-picrylhydrazyl (DPPH) during storage with 51.11 µM TE/g DM.
- The phenolic content on mango peel was preserved on fruit treated with PE compared to other treatments.
- The ascorbic acid AA concentration decreased for all the treatments during storage however mango fruit treated with PE preserved more AA which was 51.95 mg/g.

6.3 Conclusion and recommendations

In order to minimise postharvest anthracnose on mango, economically viable methods are required. Antimicrobial properties found in most plants may be used for crop protection. These pose as natural predators of pathogenic fungi. Biocontrol agents and plant extracts used in this study have shown an inhibitory and antifungal effect on anthracnose of mango. These also have been explored on postharvest quality of mango and may be considered as a potential method of maintaining quality while preserving nutrients. This study leaves a room for further research on different concentrations of plant extracts and integration with biocontrol agents against fungal pathogens of mango including *C. gloeosporioides*. Future research may also investigate scavenging abilities in H₂O₂ content, enzyme activity on treated fruits and *C. gloeosporioides* protein abundance using 1D SDS-PAGE coupled with LC-MS/MS. This study shows a significant outcome of the effectiveness and potential use of biocontrol agents and *P. sidoides* as an alternative to synthetic fungicides while prolonging the shelf life of mango postharvest.

6.4 References

- Arauz, L.F., 2000. Mango anthracnose: Economic impact and current options for integrated management. *Plant Disease*, 84(6), pp.600-611.
- Fivaz, J., 2006, February. Mango production in South Africa as compared to the rest of the world. In *VIII International Mango Symposium* 820 (pp. 29-46).
- Konsue, W., Dethoup, T. and Limtong, S., 2020. Biological control of fruit rot and anthracnose of postharvest mango by antagonistic yeasts from economic crops leaves. *Microorganisms*, 8(3), p.317.
- Lima, N.B., de A. Batista, M.V., De Moraes, M.A., Barbosa, M.A., Michereff, S.J., Hyde, K.D. and Câmara, M.P., 2013. Five *Colletotrichum* species are responsible for mango anthracnose in northeastern Brazil. *Fungal Diversity*, 61, pp.75-88.
- Micah, J.A. and Inkoom, E.W., 2016. Overcoming constraints in mango production in Ghana. *International Journal of Agricultural Science and Research (IJASR)*, 6, pp.147-154.
- Payasi, A. and Sanwal, G.G., 2010. Ripening of climacteric fruits and their control. *Journal of Food Biochemistry*, 34(4), pp.679-710.
- Qadri, R., Azam, M., Khan, I., Yang, Y., Ejaz, S., Akram, M.T. and Khan, M.A., 2020. Conventional and modern technologies for the management of post-harvest diseases. *Plant Disease Management Strategies for Sustainable Agriculture through Traditional and Modern Approaches*, pp.137-172.
- Rahul, S.N., Khilari, K., Sagar, S., Chaudhary, S., Kumar, S., Vihan, N. and Tomar, A., 2015. Challenges in postharvest management of fungal diseases in fruits and vegetables-a review. *South Asian Journal. Food Technology Environmental*, 1(2), pp.126-130.
- Sharma, M. and Kulshrestha, S., 2015. *Colletotrichum gloeosporioides*: an anthracnose causing pathogen of fruits and vegetables. *Biosciences Biotechnology Research Asia*, 12(2), pp.1233-1246.
- Singh, Z., Singh, R.K., Sane, V.A. and Nath, P., 2013. Mango-postharvest biology and biotechnology. *Critical Reviews in Plant Sciences*, 32(4), pp.217-236.
- Thakor, N.J., 2019. Indian mango–production and export scenario. *Peach*, 18(107), pp.0-12.

- Tharanathan, R.N., Yashoda, H.M. and Prabha, T.N., 2006. Mango (*Mangifera indica* L.), “The king of fruits”—An overview. *Food Reviews International*, 22(2), pp.95-123.
- Xu, P., Huber, D.J., Gong, D., Yun, Z., Pan, Y., Jiang, Y. and Zhang, Z., 2023. Amelioration of chilling injury in ‘Guifei’ mango fruit by melatonin is associated with regulation of lipid metabolic enzymes and remodeling of lipidome. *Postharvest Biology and Technology*, 198, p.112233.

Appendices

Appendix 1: Primary screening of yeast isolates against the fungal pathogen *C.*

gloeosporioides in vitro

Source of isolation (Leaves)	Treatment	Mean±SE	% Inhibition	Class
	Control	66.78±6.51	0,00	0
<i>Musa acuminata</i>	Ba	18.00±1.35	74,59	3
<i>Datura stramonium</i>	TA	19.00±2.12	71,25	3
<i>Datura stramonium</i>	500	22.22±2.32	67,50	2
<i>Eucalyptus mannifera</i>	Im	23.11±2.54	66,25	2
Cape ivy	445	23.33±2.46	66,25	2
Common lantana	CL	24.33±2.63	65,41	2
Cape ivy	CM	24.67±2.63	65,00	2
<i>Cathe edulis</i>	Kh	23.89±2.63	65,00	2
<i>Eucalyptus mannifera</i>	Gm	24.44±2.73	64,16	2
Papaya	Pa	25.05±2.65	63,13	2
Spiked speedwell	Ss	25.11±2.70	62,91	2
Papaya	173	25.33±2.69	62,50	2
<i>Stephanie longa</i>	SL	25.33±3.69	62,50	2
Incense	410	25.56±2.60	62,50	2
Incense	414	25.56±2.60	62,50	2
Incense	G	26.00±3.79	61,66	2
Incense	467	26.22±2.79	61,25	2
English lavender	EL	26.33±3.79	61,25	2
<i>Aloe vera</i>	Po	27.00±2.80	60,00	2
Sage	Sa	27.28±2.89	59,59	2
Sage	OF	27.33±3.89	59,16	2
<i>Solanum tuberosum</i>	Pt	27.89±2.81	58,75	2
<i>Solanum tuberosum</i>	415	28.33±2.69	58,75	2
<i>Cuban oregano</i>	CO	28.67±3.79	58,34	2
<i>Cuban oregano</i>	Cs	29.11±3.92	57,91	2
<i>Tetradenia riparia</i>	W	30.33±3.02	57,50	2
<i>Tetradenia riparia</i>	443	29.78±3.80	57,09	2
<i>Tetradenia riparia</i>	IBF	30.67±3.90	56,25	2

<i>Tetradenia riparia</i>	425	30.33±3.80	56,25	2
Olive	444	30.56±3.79	55,84	2
<i>Podocarpus elongatus</i>	15	30.89±3.79	55,41	2
Fern	Fe	31.11±3.89	55,00	2
Hard aloe	HA	31.33±3.89	55,00	2
Olive	507	31.55±3.51	55,00	2
Cape ivy	CI	31.67±3.50	55,00	2
<i>Cuban oregano</i>	CoM	32.00±3.50	54,59	2
<i>Dovyalis caffra</i>	188	32.22±3.51	54,16	2
<i>Dovyalis caffra</i>	419	33.22±3.08	51,66	2
<i>Persea americana</i>	Avo	33.44±3.99	51,66	2
<i>Citrus limon</i>	Le	33.66±3.18	50,84	2
<i>Citrus limon</i>	169	34.00±3.18	50,41	2
<i>Citrus limon</i>	O	34.33±4.47	49,16	2
Cherry laurel	ChL	35.33±3.67	47,50	2
<i>Tetradenia riparia</i>	IBL	35.00±4.57	47,50	2
<i>Mangifera indica</i> L	Ma	35.78±3.56	47,50	2
<i>Mangifera indica</i> L	513	36.11±3.66	46,66	2
<i>Prunus persica</i>	Pe	36.78±3.05	45,00	2
<i>Prunus persica</i>	498	37.33±3.25	43,75	2
<i>Prunus persica</i>	MT	37.67±4.98	42,50	2

Appendix 2: Primary screening of *Bacillus* isolates against the fungal pathogen *C. gloeosporioides* *in vitro* after 7 days at 25°C.

Source of isolation (Leaves)	Treatment	Mean±SE	% Inhibition	Class
	Control	63.33±6.64	0.00	0
<i>Solanum tuberosum</i>	Pt	17.44±0.59	77,37	3
<i>Datura stramonium</i>	TA	18.33±1.07	75,31	3
<i>Tetradenia riparia</i>	IBF	19.44±0.59	74,90	3
<i>Solanum tuberosum</i>	415	20.67±0.70	73,25	3
Spiked speedwell	Ss	21.00±0.88	72,43	3
<i>Dovyalis caffra</i>	188	21.45±0.78	72,01	3
Fern	Fe	21.56±0.80	72,01	3
Sage	Sa	22.00±0.88	71,20	3
<i>Tetradenia riparia</i>	IBL	22.22±0.87	70,78	3
<i>Citrus limon</i>	169	22.67±0.77	70,37	3
Incense	414	22.89±0.87	69,96	2
<i>Mangifera indica</i> L	Ma	23.00±0.77	69,96	2
Cape ivy	445	23.45±0.91	69,54	2
Papaya	173	23.89±0.99	68,73	2
<i>Musa aciminata</i>	Ba	24.56±0.87	67,90	2
<i>Tetradenia riparia</i>	W	24.78±0.78	67,90	2
<i>Persea americana</i>	Avo	25.00±0.77	67,49	2
<i>Dovyalis caffra</i>	419	25.55±0.97	66,26	2
Sage	OF	25.78±0.87	66,26	2
<i>Stephanie longa</i>	SL	26.22±0.87	65,84	2
Cherry laurel	ChL	26.78±1.25	64,20	2
Papaya	Pa	26.78±1.25	64,20	2
<i>Mangifera indica</i> L	513	27.22±1.25	63,79	2
Olive	444	27.56±1.37	63,37	2
<i>Prunus persica</i>	498	28.00±1.20	63,37	2
<i>Cuban oregano</i>	Cs	31.33±2.22	56,38	2
Hard aloe	HA	33.33±3.10	51,85	2
<i>Cuban oregano</i>	CoM	33.67±3.29	51,02	2
<i>Tetradenia riparia</i>	425	34.11±3.37	50,62	2

Incense	G	34.44±3.38	50,62	2
<i>Podocarpus elongatus</i>	15	34.83±3.33	50,21	2
Olive	507	35.22±3.20	50,21	2
<i>Prunus persica</i>	Pe	35.55±2.90	50,21	2
Incense	410	35.78±2.89	49,79	2
<i>Eucalyptus mannifera</i>	Gm	35.89±2.90	49,79	2
Incense	467	36.45±2.70	49,38	2
<i>Cuban oregano</i>	CoM	36.67±2.69	48,98	2
<i>Cathe edulis</i>	Kh	36.89±2.60	48,98	2
<i>Aloe vera</i>	Po	37.34±2.60	48,56	2
<i>Datura stramonium</i>	500	37.67±2.71	48,15	2
Cape ivy	CI	37.89±2.82	47,74	2
<i>Prunus persica</i>	MT	38.00±2.83	47,74	2
<i>Tetradenia riparia</i>	443	38.56±2.75	47,32	2
<i>Cuban oregano</i>	CO	38.73±2.69	47,09	2
English lavender	EL	40.56±3.35	44,44	2
<i>Citrus limon</i>	O	41.22±3.35	43,62	2
Common lantana	CL	42.56±4.35	38,68	1
<i>Eucalyptus mannifera</i>	Im	43.33±4.55	37,44	1
<i>Citrus limon</i>	Le	44.78±5.39	33,33	1

Standard curves

Ascorbic acid : $y = (-0.005)x + 0.5864$

$$\text{DPPH : } \text{Inhibition(\%)} = \frac{A_c - A_t}{A_c} \times 100$$

Where A_c = absorbance of control; A_t = absorbance of the extract
 Phenolic content: $y = 0.0041x + 0.0292$