Effects of Alternaria leaf spot (*Alternaria alternata* (fr.) Keissl) on the growth, yield and phytochemical profile of *Moringa oleifera* lam

by

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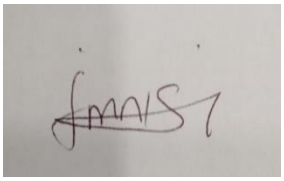
DECLARATION

I declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Agriculture (Agronomy) has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.



05/09/2024

Mnisi, F (Ms) Date

DEDICATION

To my loving family

ACKNOWLEDGEMENTS

It is not by might, nor by power, but by God’s spirit that I was able to obtain this degree. For his word is our anchor and our support through life’s deepest tribulations, God is much appreciated, thereof. God did it. I want to express my gratitude to my supervisory team for allowing me to be a student under your guidance and helping me develop into a competent individual. Thank you for bearing with me and for your steadfast commitment to seeing me finish the course rather than giving up. I have taken note of Prof. A.R. Ndhala's steadfast support and determination to achieve the goal of earning this degree. Thank you for believing in me and giving me the platform to gain more knowledge in this field. Prof. M.A. Kena, I appreciate the action you took in helping me find something to work with when nothing else was working. I'm not sure what would have happened to me if you did not come through for me. I almost lost it at that point. I highly appreciate.

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LIST OF ABBREVIATIONS

WUE- Water use efficiency

TR- Transpiration rate

SC- Stomatal conductance

MAMPs- Microbe associated molecular patterns

cm- centimetres

mm- millimetres

%- percentage

Chla- Chlorophyll a

Chlb- Chlorophyll b

TotChl- Total Chlorophyll

ROS- Reactive oxygen species

GBRCE- Green Biotechnologies Research Centre of Excellence

PDA- Potato Dextrose Agar

Ci CO_2 con- Ci intercellular CO_2 concentration

ABSTRACT

Moringa oleifera is a species of Moringa belonging to the family Moringaceae which has been used for thousands of years in less developed communities. It is a nutrient-rich plant containing a variety of minerals and phytochemicals. Moringa is affected by pests and diseases which affect their growth, yield and phytochemical composition. *Alternaria alternata* is one of the pathogens that causes Alternaria leaf spot disease in Moringa. The objective of the study was to determine whether *Alternaria* leaf spot *(caused by Alternaria alternata)* affects the growth, yield and phytochemical contents of the PKM1 cultivar of *M. oleifera.* The study was conducted at the University of Limpopo, Plant Pathology Laboratory, and the Green Biotechnologies Research Centre of Excellence. Two main treatments: *M. oleifera* seedlings inoculated with *A. alternata* and a non-inoculated control were used. The data collected for growth parameters were plant height and stem diameter. Fresh shoot mass and root length were collected as yield parameters. For the physiological attributes: photosynthetic rate, stomatal conductance, transpiration rate, water use efficiency, and Ci intercellular CO_2 concentration were collected. The L*, a*,b*,c* and h values were collected as colour parameters. Lastly, the biochemical compounds (total flavonoids, total phenols, antioxidant activity and chlorophyll). To compare differences in treatment means, paired sample t-test was performed at a 5% significance level using Sigma Stat software 2016 and Graph Prism 5. The inoculated treatment significantly ($p<0.05$) reduced the plant height and fresh shoot mass and had no significant ($p>0.05$) effect on the stem diameter and root weight.

Inoculation affected the physiological attributes as follows: photosynthetic rate, stomatal conductance and transpiration rate was decreased by 4%, 17% and 4% respectively, while the water use efficiency increased by 10% in comparison to the non-inoculated plants. There was no significant effect on the Ci intercellular CO_2 concentration. The colour parameters affected by the treatment were as follows: the mean± standard error of the treatment was higher than the control for the L*, a*, b* and c* values. There was no significant difference for the h value. The inoculation significantly ($p<0.05$) increased the total flavonoids, phenols and antioxidant activity by 69%, 4% and 4% and decreased, chla, chlb and totchl by, 48%, 43% and 44% respectively. In conclusion, the introduction of *A. alternata* into PKMI cultivar of *M. oleifera* influenced its growth and development and altered the physiological and biochemical defence mechanism.

CHAPTER 1
GENERAL INTRODUCTION

1.1. Introduction

The area of arable land for most agricultural production is expected to decline by a range of 8 – 20% in the next two decades (Chakraborty and Newton, 2011). This is mainly due to decreased agricultural investments, urbanization, deterioration of the soil, and the conversion of arable land suited for crops to non-food production by 2050. This is accelerated by the global growing population predicted to increase from 7.7 billion people to 9.7 billion people in 2050 (Mashamaite *et al*., 2021). This background poses a challenge on food security issues which contributed to the 37% increase (83-132 million) in the number of undernourished people globally in 2020 (Costa *et al*., 2022). On the other hand, matters related to climate change, which contributes to inconsistent weather conditions have now worsened the crop production sector and the food supply. There is a need for increased production that has a minimal adverse effect on the environment in order to meet the demands for food security (Costa *et al*., 2022). Growing multipurpose plants that are phenotypically and genotypically plastic enough to be grown in different growth conditions serves as an alternative to food security (Costa *et al*., 2022).

Moringa oleifera Lam. species is the most grown, utilized and studied member of 13 species (Leone *et al*., 2015a) of Moringaceae family. It is known for its adaptability to a wide range of growth conditions and their medico-nutritional qualities beneficial for human health. The Moringa tree, which has been used for thousands of years in less developed communities, is the most nutrient-rich variety containing minerals and phytochemicals and thus falls under the top 10 of the 500 000 plants growing in

popularity (Ndhlala and Tshabalala, 2023). As such, it is cultivated for food and medicinal purposes and thus, it is regarded ‘a gift from nature to the human population’ (Agbogidi *et al.,* 2012).

Just like any other plant, Moringa is susceptible to various pests and diseases whose presence can affect growth, yield and phytochemical composition. The most severe diseases affecting Moringa are caused by fungal pathogens (Gatan, 2020), one of which is Alternaria leaf spot, caused by *Alternaria alternata* (Fr.) Keissl. *Alternaria alternata* is a cosmopolitan necrotrophic fungus that causes destruction through surface spots on leaves of fruits and vegetables plants (Zhao *et al.,* 2012). *A. alternata* is also an endophyte that coexists with numerous plants in asymptomatic symbiosis (DeMers, 2022). The detection of the disease on the early stages is difficult, and once the spots appear, treatment is too late, and defoliation is to follow (Saint Sauveur, 2010).

Phytochemicals are fundamental metabolites that play a crucial role in plant growth, development and reproduction as well as in assisting plants in either temporarily or permanently overcoming environmental stressors (Molyneux *et al.,* 2007). Some phytochemicals such as phenolic acids and flavonoids are induced by the infection of pathogens to improve resistance (Simoes *et al.,* 2009). Ephrath *et al*. (1989) reported a decrease in the growth and development of bolls of cotton (*Gossypium hirsutum* L.) and boll shedding resulting in significant yield losses due to infection by *A. alternata*. Hosagoudar *et al.* (2014) reported that Alternaria leaf spot resulted in a 26% yield reduction on cotton. *Alternaria alternata* caused a significant increase in the appearance of spots, causing a decrease in the fruit quality, and affected the biochemical constituents of mango (*Mangifera indica* L.) fruit (Li *et al.,* 2018).

Moringa tree possess innumerable benefits for the human population and thus it is named ‘a high-quality gift from nature at a very low cost’ (Mahmood *et al*., 2010). Further research is required to determine how biological factors affect the productivity of Moringa phytochemicals. Evaluating the host-pathogen interaction between *A. alternata* and *M. oleifera* can provide better understanding on its biochemical compositions and nutritional status (DeMers, 2022).

1.2. Problem statement

The cultivation of Moringa as a perennial crop is expanding quickly, and the expected increase in cultivation might result in increased disease incidence which negatively affects the plant growth and development (Ponnuswami, 2019). According to Gatan (2020) and Ziedan *et al.* (2016), Moringa is affected by fungal diseases including those that attack leaves (defoliation) and pods causing lower yields, and detrimental effect on Moringa. *Alternaria* leaf spot is an economically important disease of Moringa as it reduces the photosynthetic capacity by causing dark brown spots which appear circular to angular on infected leaves and black or brown marks on the branches and further reduces the yield and acceptability of the crop (Ziedan *et al.,* 2016; Gatan, 2020). Plants produce a wide range of phytochemicals in response to microbial diseases and predators as a protection from the toxicity of the infecting organisms. (Rahman *et al.,* 2021). Evidently, plant diseases are regarded as one of the major causes of stunted growth and a drop in more than 50% of yield losses and total productivity (Singh *et al.,* 2021). However, as with many diseases, the impact of Alternaria leaf spot on Moringa production has not been fully exploited. These include the ability of the causal organisms to influence biochemical composition and quality. This empirically derived information is crucial for the production of high yielding Moringa trees with improved nutritional contents.

1.3. Rationale

Moringa is a multipurpose plant that can be used as an alternative solution for enhancing the livelihood of humans, marginalized communities in rural areas. The plants can be cultivated, processed into products and sold to improve income for farmers, reduce poverty and act as an efficient treatment for malnutrition (Buheji *et al.,* 2020). However, both the quantity and quality of Moringa are affected by numerous factors including pests, diseases and environmental stresses (Gatan, 2020). One of the most prevalent and economically important disease of Moringa is Alternaria leaf spot caused by *A. alternata* (Gatan, 2020). Alternaria leaf spot causes the bio-deterioration of significant phytochemicals and components important for human health (Singh *et al.,* 2017). During fungal attack, the plant metabolism is altered, resulting in the creation of new volatile chemicals or changes in the levels of existing ones (Encicas-Basutro *et al.,* 2017).

In South Africa, the effects of Alternaria leaf spot on the growth, yield, phytochemical composition and antioxidant properties of Moringa had not been investigated. This information would be imperative in understanding the underlying mechanisms involved in pathogenesis. This would lead to the selection of the necessary management techniques applicable regarding the benefits that come with yield, phytochemicals and antioxidant capacity of the tree (Arendse *et al*., 2014).

1.4. Purpose of the study

1.4.1. Aim

Establishment of analytical information on the effects of *Alternaria* leaf spot caused by *Alternaria alternata* on the growth, yield and phytochemical profile of *M. oleifera*.

1.4.2. Objective

To determine whether *Alternaria* leaf spot *caused by Alternaria alternata* affects the following parameters of a PKM1 cultivar of *M. oleifera.*

i. Growth

ii. Yield

iii. Phytochemical contents.

1.4.3. Hypothesis

Alternaria leaf spot *caused by Alternaria alternata* will not affect growth, yield and phytochemical levels of a PKM1 cultivar of *M. oleifera*.

1.5. Structure of the mini dissertation

The mini dissertation is organised such that Chapter 1 is composed of the description of the study problem, background and the significance of the study. Chapter 2 reviews work done on the problem statement and establishes work not done. Chapter 3 describes the study site, experimental design, data collection protocols used to accomplish the objective. The summary and interpretation of the experimentally generated findings are covered in Chapter 4 and the significance, suggestions for future research, and a conclusion from the findings are covered in Chapter 5. In alphabetical sequence, references are made in the document using the Harvard style.

CHAPTER 2

LITERATURE REVIEW

2. Literature review

2.1. The host: *Moringa oleifera*

2.1.1. Nomenclature, origin and distribution.

The family Moringaceae includes the medium-sized plant known as Moringa. The tree belongs to the single genus Moringa and has the botanical name *Moringa oleifera* Lamarck (Ramachandran *et al*., 1980). From India to Africa, Moringa is known by a variety of common names (Bopape-Mabapa, 2019), including drumstick tree, Moringa, horseradish tree, and benzoil tree (Azad *et al*., 2015). It is also known as Ben oil or Never Die in western African nations, Malungai or Malungay in the Philippines, (Bopape-Mabapa, 2019), Sahjan in Hindi, Surajana in Sanskrit, Sajiwan in Nepali (Patel *et al*., 2010). The Moringa plant is native to the northern Indian subcontinent's southern foothills of the Himalayas, native to India, Bangladesh, Pakistan and Afghanistan (Velázquez-Zavala *et al*., 2016) where there are hot steppe, hot subtropical summers, dry tropical summers, and subtropical-dry winters (Moyo *et al*., 2011, Mokgehle *et al*., 2022). However, it has spread to other tropical and subtropical regions of the world. The plant is distributed from Africa, Latin America and Oceania countries including Australia (Patel *et al*., 2010). The species *M. oleifera* was introduced to the Americans in the nineteenth century by sailors crewing on the Nao *de China* route between Manila and Acapulco from the Phillipines (Velázquez-Zavala *et al*., 2016).

Table 2.1. Taxonomical classification of Moringa tree (Azad *et al*., 2015).

Kingdom	*Plantae*
Sub-Kingdom	*Angiosperms*
Phylum	*Charophyta*
Class	*Equisetopsida*
Subclass	*Magnoliidae*
Order	*Brassicales*
Family	*Moringaceae*
Genus	*Moringa*
Species	*Moringa oleifera*

2.1.2. Environmental conditions for growth and development.

Moringa production is best under tropical insular climate (Moyo *et al.,* 2011). Except for waterlogged places, Moringa can withstand hard circumstances in hot, dry, and humid regions of the tropics and subtropics. The plant can survive under extreme conditions such as less fertile soils (Moyo *et al*., 2011) and can successfully generate high-quality nutritional content (Mabapa *et al*., 2017). The amount of rainfall needed by Moringa plant varies based on the characteristics of the soil, ranging from 250-2000 mm. It thrives best in dry, sandy soils and can tolerate poor soil with a pH range of 5-9 (Azad *et al*., 2015). The Moringa tree develops quickly and can withstand harsh climatic and environmental circumstances that would normally make it impossible for agricultural plants to survive and require only 400 mm of rainfall yearly (Mabapa *et al*., 2018).

2.1.3. Moringa production areas in South Africa.

Production of Moringa in South Africa is performed solely for processing of the leaves and consumption (Bopape-Mabapa *et al*., 2020). Out of the nine provinces in South Africa, Moringa is cultivated in six of them, with a great deal of its production being produced by farmers and households in the province of Limpopo. Gauteng, Mpumalanga, Kwa-Zulu Natal, Free State, and the Northwest provinces are among the six production provinces (Mashamaite *et al*., 2021). The trees were introduced to South Africa in 2006 and since has slowly gained popularity in the abovementioned provinces (Tshabalala *et al*., 2020). The primary reasons for producing Moringa in the Limpopo province are to generate revenue, ensure food security, feed livestock, purify water, treat ailments, and provide supplementation for excellent health (Bopape-Mabapa *et al*., 2020). Most people in South Africa, however, are not aware of the potential benefits of Moringa.

Figure 2.1: Production areas of Moringa in South Africa

2.1.4. Uses of Moringa

Moringa is regarded as one of the most useful plant globally. Historically, the tree was planted by various purposes by farmers and this led to an increased in its uses and application (Leone *et al*., 2015a). The entire tree may be utilized for food, medicine, and industry, with the leaves, flowers, and fresh pods being used in a vegetable form as well as for livestock feeding (Moyo *et al*., 2011).

2.1.4.1. Moringa in medicine

In medicine, Moringa is utilized as a nutritional supplement due to its exceptional nutritional and medicinal properties (Abir *et al*., 2022). Moringa provides a rich and rare combination of nutrients, amino acids, antioxidants, anti-aging and anti- inflammatory properties used for nutrition and medicine. Moringa possesses antifungal, antidiabetic, anticancer, analgesic, and antibacterial properties. The Moringa plant is said to be able to prevent 300 ailments, according to Ayurvedic traditional medicine, and its leaves can be used for the prevention and treatment of diseases (Leone *et al.,* 2015b). The medicinal benefits of Moringa have been established and has been used for millennia in many cultures throughout the world to treat a variety of illnesses, such as skin infections, asthma and respiratory ailments. (Mahmood *et al*., 2010). As a result of its capacity to increase a woman's production of milk and serve as a remedy for anaemia, the tree is regarded as a mother's best friend in the Phillipines (Anwar *et al*., 2007). *Moringa oleifera* was found to contain high levels of bioactive compounds which are essential for health (Singh *et al*., 2020). Apart from malnutrition, the above-mentioned nutrients are essential for vision, blood, strengthening of bones and skin repair (Chukwuebuka, 2015). Furthermore, the contained poly-phenolic compounds in *M. oleifera* leaves were reported to offer antioxidant and anti-inflammation properties (Siddhuraju and Becker, 2003). Moringa contains various secondary metabolites

which are useful for the treatments of communicable and non-communicable diseases in humans (Tshabalala *et al*., 2019).

2.1.4.2. Moringa in human nutrition

Moringa provides a socially beneficial way of dealing with poverty and malnutrition and is cost-effective making it even possible for poverty-stricken societies (Horn *et al*., 2022). There is a high level of elemental nutrients in *M. oleifera* compared to most conventional vegetables which makes it an important crop in combating hunger and malnutrition (Tshabalala *et al*., 2019). As a significant food component, *M. oleifera* has received a great deal of attention. In many nations, including many regions of Africa, the leaves, fruit, flowers, and immature pods are utilized as vegetables because they are extremely rich in nutrients (Anwar *et al*., 2007). Carbohydrates and proteins in *M. oleifera* leaves were reported to have potential on enhancing human diet (Bao *et al*., 2020). In addition, the Moringa tree produces leaves, fruits, flowers, and immature pods that are used as a source of nutrients such as β-carotene, protein, vitamin C, calcium, and potassium (Tetteh *et al*., 2019). Reports indicate that the leaves of *M. oleifera* act as a good source of natural antioxidants and carotenoids which can be used in countries affected by malnutrition (Singh *et al*., 2020). Research reports shows that *M. oleifera* contains seven times more vitamin C than oranges (*Citrus sinensis* L.), fifteen times potassium compared to bananas (*Musa balbisiana* L.), seventeen times calcium compared to milk, nine times proteins compared to yoghurt, ten times vitamin A compared to carrots (*Daucus carota* L.) and twenty-five times iron compared to spinach (*Spinacia oleracea* L. (Shareef *et al*., 2019).

2.1.4.3. Moringa as a yield enhancer

Moringa leaf extracts are considered one of the modern bio-stimulants and growth promoters because they include an abundance of antioxidants, important nutrients,

phytohormones like zeatin reported to increase yield by 10-45% (Maishanu *et al*., 2017), auxins, and gibberelins (Alkuwayti *et al*., 2020). As a biofertilizer, the extracts have been utilized to mitigate the harmful effects of several abiotic stressors such as salinity and drought (Alkuwayti *et al*., 2020). According to Mahmood *et al.* (2010), laboratory tests showed that Moringa spray had a positive effect on plants due to improved overall growth and firmness, increased resistance to pests and diseases, extended shelf lives, and heavier roots, stems, and leaves. This resulted in more and larger fruits as well as an overall increase in yield. Maishanu *et al.* (2017) found that plants treated with Moringa extracts had the highest means for stems, the number of branches and leaves, the length of the branches and leaves, and the stem thickness of cowpea (*Vigna unguiculata*) in comparison to plants treated with urea fertilizer and the control. Moringa extracts have been shown to increase the ability of the plant to endure abiotic stresses and performance when applied to the seeds or foliage of the plant (Rady and Mohammed, 2015). The extracts function by maintaining an optimal tissue water status, enhancing the stability of the membranes, increasing the production of secondary metabolites, reducing the uptake of undesirable Na^+ and/or Cl, improving shoot or leaf K^+, increasing antioxidant levels, and strengthening the inherent defences of the plant (Rady and Mohammed, 2015). Foliar application of Moringa leaf extracts enhances fruit quality and yield while delaying fruit senescence and promoting more vigorous growth, deeper rooting, and greater seed germination (Iqbal *et al*., 2020). Moringa extracts are also reported to suppress soil-borne diseases in vegetable production (Hlokwe *et al*., 2020).

2.2. The pathogen: *Alternaria alternata ((Fr.) Keissl)*.

2.2.1. The genus Alternaria

The genus *Alternaria*, is a large group of filamentous fungi (He *et al*., 2023) which can act as saprophytes, endophytes, plant pathogens, and weak facultative parasites. It is

a member of the Hyphomycetes group of Fungi imperfecti due to most isolates lacking sexual stage (Marais, 2013). The saprophytic fungus is involved in the decomposition and mineralization of plant residues and other products (Dube, 2014, Dalinova *et al*., 2020). The genus produces metabolites including phytotoxins and mycotoxins, which can be harmful to both plants and animals, according to Lou *et al*. (2013). The fungi are ubiquitous and can be found in large quantities in organic matter in the soil (Nadziakiewicz *et al*., 2018). Although they are capable of mutation and change in certain circumstances to cause an infection to new plants, several species from the genus often infect a variety of well-defined hosts (Baranowski *et al.,* 2015, Salih *et al*., 2018). More than 350 species of the genus exist in the world (Dalinova *et al*., 2020), and they have an impact on a variety of biological systems by creating severe leaf and stem spots that lead to premature defoliation, stem breakage, and seedling blight (Kgatle, 2013). The genus has several recognized species with overlapping morphological traits, making it challenging to distinguish between actual species (Haque and Parvin, 2021). The growing factors such as light, substrate, humiditythat the species is exposed to have an impact on the morphology of the conidia, making the use of morphological traits for species categorization unreliable (Kgatle *et al*., 2018). The genus has a distinctive characteristic that is the development of conidia with transverse and longitudinal septa, massive, multicellular, and dark in colour (Dalinova *et al*., 2020).

2.2.2. Alternaria leaf spot caused by *Alternaria alternata*

Alternaria alternata (Fr). Keissler is one of the most cosmopolitan fungal pathogen (Ito *et al*., 2004). Over 380 species of plants are hosts to *A. alternata* resulting in a wide range of symptoms including rots, leaf spots and blights on different parts (Tozlu *et al*., 2018). *A. alternata* has the ability to live asymptomatically with numerous plants as well as be pathogenic on a significant number of crops (DeMers, 2022). *A. alternata*

contains various strains which causes necrotic disease on different host plants by the production of host-specific toxins (Ito *et al*., 2004). As a saprophyte, it can infect senescing plants, but it also behaves as a latent fungus that can develop during the cold storage of fruits or remain quiescent for weeks until the fruit ripens (Troncoso-Rojas *et al.,* 2014).

2.2.3. Morphology and cultural characteristics.

Alternaria alternata is part of the black-pigmented mould family. It is fast growing and produces colonies with dark colours ranging from grey to olive to olive brown (Kustrzeba-Wójcicka *et al*., 2014). The colonies of *A. alternata* exhibit a distinctive (2–5 mm) white edge when grown on Potato Dextrose Agar (PDA), and they typically range in colour from lettuce-green to olive green. After 7–10 days, the isolates form colonies that are more than 70mm in diameter (Troncoso-Rojas *et al.,* 2014). In the mycelium described by Nagrale *et al.* (2013), the hyphae were originally thin and narrow with a diameter of 2.84 µm, but as they became older, they became slightly thicker (4.42 µm in diameter), multicelled, septate, and irregularly branched. According to Kustrzeba-Wójcicka *et al*. (2014), the conidia are simple, bigger, and 45–50 36 µm in size. They can form in chains or singly and are said to be larger and uniform in their natural habitats. The conidia are shorter when they are developed in cooler, drier environments. Basim *et al*. (2017) reported a basally placed 2-6 transverse septa and one or two longitudinal septa present on dark brown conidia that ranged in size from 7 to 45.9 µm and some of which had side branches. Conidia and conidiophore were typically ovoid-shaped, light to pale brown, and developed short conical beaks at the tip. The spores of Alternaria taper gradually to an elongate beak and are broadest near the base (Mamgain *et al*., 2013).

Figure 2.2. Morphological characteristics of *A. alternata* (Kumar *et al*., 2018).

2.2.4. Economic importance

Alternaria alternata infects mostly leaves and fruits in many hosts resulting in reduced yield and crop quality. Lesions on susceptible hosts can range in size from small necrotic patches to massive sunken pock-marks, which makes the produce undesirable to the markets and causes producer losses (Carvalho *et al.,* 2011). In crops such as potato (Solanum tuberosum L.), the infection by *A. alternata* can reduce crop yield by more than 40%, making it one of the most destructive fungal pathogens worldwide (Mathelemuse *et al*., 2022). The most effective and commonly used control measure against Alternaria leaf spot is the use of synthetic fungicides. However, this method of control continue to grow increasingly challenging and expensive as it requires many applications to maintain crop quality (Reis *et al*., 2007). *Alternaria alternata* is one of the pathogens that have evolved a resistance to agrochemicals, which makes it difficult to cultivate susceptible crops like tomatoes (*Solanum lycopersicum* L.), leading to significant losses and decreased nutritional value of the

crop. The pathogen can cause up to 79% yield reduction in tomatoes and seedling losses of about 20-40% in the field (Martinko *et al*., 2022). Due to its potential to cause significant postharvest losses due to the contamination of food and feed, *A. alternata* is an economically significant pathogen (Wenderoth *et al.,* 2017).

2.2.5. Disease epidemiology and dissemination.

Alternaria alternata epidemics can occur in climates with heavy rainfall, high humidity levels, and moderately high temperatures with a range of 24 to 29 °C (Pane and Zaccardeli, 2015). Long dew periods and temperatures above 18 °C are favourable for spore germination and penetration, whereas temperatures between 10 and 35 °C are needed for infection. Between growth seasons, mycelia can survive in soil, infested plant debris, susceptible crops, and weeds (Dube, 2014). Although *A. alternata* will survive in temperatures from 2 to 32 °C, its temperature optimum is around 20 °C. Pose *et al*. (2010) studied the influence of temperature on the germination and growth of *A. alternata* on a synthetic medium. The shortest germination time was observed at 21 to 35 °C, while the fastest growth rate was registered at 21 °C. The pathogen is mostly transported by infected seeds with spores on the seed coat or mycelium under the seed coat and is spread by wind, water, tools, and animals (Mamgain *et al*., 2013). For Alternaria spores to disperse, dry weather intervals followed by higher wind velocity and lower relative humidity are necessary. As a result, peak levels occur during sunny afternoon intervals (Bush and Prochnau, 2004). A constant moisture for 24 hours is a guarantee of infection, and a relative humidity of 91.5% or greater will cause the generation of mature spores in 24 hours according to Mamgain *et al.* (2013).

2.2.6. Life cycle and host range.

A pathogen must have favourable environmental conditions in order to infect a host plant (Marais, 2013). *Alternaria alternata* survives on soil or infected plant debris

saprophytically between growing seasons as conidia and mycelia (Kandolo *et al*., 2016). Multiple *A. alternata* spore formation occurs during a single growing season, resulting in secondary inoculum in the disease cycle (Marais, 2013). Upon *A. alternata* conidia dispersion, the conidia that land on a host plant with favourable conditions may initiate an infection by germinating and producing germ tubes in a few hours (Rotem, 1994). The cuticles, stomata, wounds, and cells serve as the point of entry for the germ tube (Rotem, 1994). The germ tube secretes extracellular materials to adhere to the surface of the plant and apply pressure to aid in penetration (Pitsi, 2013). Stomatal penetration typically occurs by chance, and hyphae often grow on top of the stomata without any penetration (Kgatle, 2013). Over time, the germ tubes formed at random locations will proliferate randomly across the surface of the host (Dehpour *et al*., 2009). In some cases, the extensive growth of the germ tubes results in the formation of a hyphal network on the tissue of the host. Some of the hyphal network forms in intercellular space of mesophyll and parenchyma tissue surroundings and produces toxins like Host Sensitive Toxins (HST) and Non-Host Sensitive Toxins (NHST). The toxins produced causes cell damage and cell wall diuration (Dehpour *et al*., 2009). Conidia may emerge from wounds and stomata on the surface of the host, increasing the risk of secondary infection (Van den Berg *et al*., 2003).

2.2.7. Pathogenicity

The pathogenic variants of *A. alternata* produce a broad range of chemically varied host-specific toxins, from low-molecular weight molecules to cyclic peptides, which can infect host plants and cause a disease (Chung, 2012). *Alternaria alternata* has evolved dramatically flexible signalling pathways that are distinct from other organisms in order to respond to many signals from the environment and survive in host plants (Chung, 2012). According to research by Droby *et al*. (1984), *A. alternata* was shown to have directly infected potato leaves by penetrating the epidermal cells and stomata and

spreading from there through the inter- and intracellular space. Necrotic cells were also identified to surround the site of penetration.

2.3. Host-pathogen interaction.

2.3.1. The importance of plant health to food security.

Plant pests and diseases have caused agricultural losses in societies throughout history, which have resulted in hardships and an inadequate supply of food (MacLeod *et al.*, 2010). The effects of plant pests and pathogens today range from low, causing only minor harm, through medium, causing only mild symptoms, and high, causing extensive crop destruction (MacLeod *et al.*, 2010). Plant health is a crucial aspect of plant production because plant diseases impair plant performance by lowering yield and reducing crop quality after harvest. The term "plant health" refers to any undesirable biological, chemical, or physical component that could compromise the physiology and performance of the crop (Savary *et al*., 2017). The adverse effects of plant diseases have a significant impact on the food and food security systems (Savary, 2020). According to literature, plant pests commonly cause annual crop output losses of about 30 - 40%. Future strategies to increase crop productivity and food security must address losses by enhancing plant health (Flood, 2010). As a major factor in ensuring food security and safety, a source of livelihood for those engaged in plant-based agriculture, a source of pharmaceuticals, and a component of healthy environments, maintaining plant health is essential for maintaining human and animal health as well as significant elements of complex interactions among the environment (Rizz *et al*., 2021).

2.3.2. *Alternaria alternata* in plant production.

Alternaria alternata is an infectious and polyphagous fungi (Salih *et al.,* 2018), which causes leaf spots in various crop plants such as tomato. *Alternaria alternata* is the

main causal agent of one of the foliar diseases, which is regarded as highly destructive under field and post-harvest stages. Due to its impacts on the leaves, *Alternaria alternata* restricts production by significantly reducing the net surface area needed for photosynthesis (Pane and Zaccardeli, 2015). In extreme circumstances, plants may completely defoliate, which would reduce their ability to produce fruit or other crops (Pane and Zaccardeli, 2015). According to Lastochkina *et al.* (2018), Alternaria fungi were the root cause of sugar beet leaf disease, which had economic importance because it resulted in decreased root yield and quality. He further indicated that the fungi disrupt the physiological functions causing morphological indicators to change, individual plant parts to die, and a decrease in yield. One of the most significant plant diseases, *Alternaria alternata*, results in considerable losses and decreased nutritional value in agriculturally important crops. The pathogen caused losses ranging from 35 to 80% on plantations in the USA, Australia, Israel, the UK, Brazil, and India (Martinko *et al*., 2022). It is a disease which commonly affects crops around the world, with yield reduction becoming a limiting factor with increasing severity and being accountable for losses of between 47.5 and 79% in fruit yield (Sharma *et al*., 2021).

2.4. Phytochemicals

The term phytochemicals refer to a class of secondary metabolites that assist plants in controlling growth and reproduction as well as overcoming periodic or ongoing environmental stressors (Molyneux *et al*., 2007). They attract beneficial insects and birds that are essential for pollination processes and protect plants from harmful agents and extreme conditions. They provide the plant colour and a variety of flavours (Martinez *et al.,* 2017). Fruits and vegetables are gaining popularity because they are abundant sources of phytochemicals and because these substances have positive health effects (Mpai *et al.,* 2018). Moringa plant contain various types and quantities of phytochemicals at different tissues of the plant. Table 2.2 below shows how different

phytochemicals are distributed in different parts of Moringa.

Table 2.2. The distribution of various phytochemicals in different parts of Moringa

Plant parts	Phytochemicals	Reference
Leaves	Rich in β-carotene Carotenoids Phenolic acids Flavonoids	Amaglo *et al*. (2010) Bhattacharya *et al*. (2018)
All tissues	Flavonoids (glucosides, rutinosides, malonylglucosides, and traces of acetylglucosides of kaempferol, quercetin, and isorhamnetin	Amaglo *et al*. (2010)
Leaves	Phenolic compounds (quercetin and kaempferol, and salicylic and ferulic acids)	Leone *et al*. (2015b)
Leaves and fruits	Carotenoid (All-E lutein, all-*E* luteoxanthin, 13-Z lutein, all-*E* zeaxanthin, and 15-Z β-carotene)	Saini *et al*. (2014)
Leaves	β -carotene Lutein α-tocopherol	Sriwichai *et al.* (2017)
Leaves	alkaloids (2-Pyrazoline and Pyrrolidine)	Kadhim *et al*. (2014)
Leaves	Indole alkaloid (N, α-L-rhamnopyranosyl vincosamide)	Panda *et al.* (2013).

2.5. Conclusion

Due to the growing significance of medicinal plants, a number of factors need to be investigated into in order to enhance the growth, yield, and phytochemical composition for human benefit. Moringa is an important food commodity that is highly nutritious and grows in many nations. It is an important food product that has received a lot of attention and is a natural source of nutrition in

the tropics. It has the capacity to sustain human nutrition demands on a daily basis. While the effects of *A. alternata* on the growth, yield, and phytochemical composition of many plants are well-established, the effects on Moringa plants are unknown. The impact of the disease on Moringa, a plant that is becoming more and more popular, will aid in the implementation of production techniques that will maximize output. *Alternaria alternata* was used as an inoculant on the PKM1 cultivar of Moringa in order to observe the effects of infection on growth, yield, and phytochemical content.

CHAPTER 3: MATERIALS AND METHODS

3. Materials and Methods

3.1. Description of study site

The study was conducted at the University of Limpopo, Plant Pathology laboratory, Agro-Processing laboratory and the Green Biotechnologies Research Centre of Excellence (GBCRE) greenhouse, in Limpopo Province, South Africa (23°53′10″S, 29°44′15″E). The day/night temperatures and relative humidity in the greenhouse averaged 28/18 °C and 40-45% respectively, with maximum temperatures controlled using thermostatically activated fans and a wet wall on opposite ends (Mashela *et al*., 2022).

3.2. Treatments and research design

The study was made up of two main treatments: *M. oleifera* seedlings inoculated with *A. alternata* and a non-inoculated control. Both treatments were laid-out in a Randomised Complete Block Design (RCBD) to block windblown streams that were generated by the heat-extracting fans. Each treatment was replicated eight times (2 treatments x 8 replications). Overall plant population summed up to sixteen.

3.3. Procedures and cultural practices

i. *Alternaria alternata* isolation from infected leaves and preparation of pathogen inoculum.

The test pathogen was isolated from Moringa plants showing symptoms of Alternaria leaf spot at the GBRCE plots. The infected leaves were cut into approximately 1 cm x 1 cm pieces, surface sterilised with 1% sodium hypochlorite solution for 2 minutes and rinsed three times with sterile distilled water. After rinsing, plant pieces were dried on filter paper and placed in 90 cm petri dishes (four pieces per petri dish) containing

Potato Dextrose Agar (PDA) medium. Petri plates were then incubated at 25 ± 2 °C for seven days. After seven days, a small piece of mycelia growing from infected leaves was transferred to fresh PDA plates using a flame sterilised inoculation needle. Inoculated plates were incubated as above for seven days to obtain a pure culture of *A. alternata.* The isolated fungi was identified according to the morphological characteristics (conidia shape and colour, brown lesions) (Gatan, 2020) using a Zeiss light microscope with a magnification of 40X/0.65. *Alternaria alternata* isolated from the infected Moringa plants was grown on Potato Dextrose Agar (PDA) (Merck, Johannesburg, South Africa) and incubated at 25 ± 2°C for 7 days as shown in Figure 3.1. Koch's postulates were carried out for the test pathogen to confirm their pathogenicity. The test pathogen was maintained on PDA slants and cultures were kept at 4±1°C until further use. An *A. alternata* spore suspension was prepared following the methods described by Mphahlele *et al*. (2020). Ten millilitres of sterile distilled water was added to 7 days old culture growing on PDA plates. The surface of the agar was scraped using a sterilized spatula to remove the spores and the hyphae. The conidial suspension resulting from the isolates were added into sterilised bottles containing 100 mL distilled water. Using a haemocytometer, pathogen concentration was adjusted to 10^5 conidia per mL of sterile distilled water.

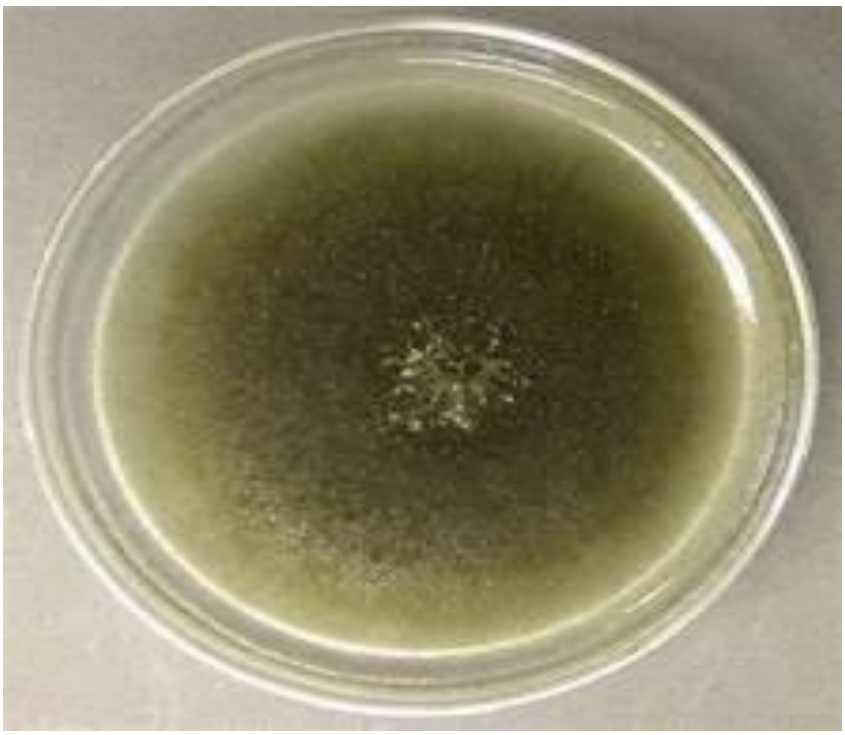

Figure 3.1. *A. alternata* growing in a petri-dish 7 days after isolation.

ii. Establishment of Moringa seedlings and inoculation

Moringa seeds of the PKM1 cultivar obtained from the DSI were directly planted into 25 cm diameter plastic pots having a capacity to hold 2700 cm^3 of a growth medium. The used growth medium consisted of Hygromix growth medium, steam-pasteurized loam and sandy soil at a ratio of 3:1:1 v/v. On the benches in the greenhouse, the pots were arranged with a

0.50 m inter-row and 0.25 m intra-row spacing. Four weeks after emergence, seedlings were inoculated evenly by spraying all the leaves with *A. alternata* conidial suspension (10^5 mL^{-1}) prepared as described under 3.3.(i) following a description by Mphahlele *et al*. (2020). For the control, the leaves were sprayed with distilled water. After inoculation, each plant was covered with a clear polyethylene plastic bag to enhance humidity and accelerate the infection process.

Figure 3.2. Moringa inoculated with *A. alternata*.

3.4. Data collection

3.4.1. Disease incidence and severity

Alternaria leaf spot was determined bi-weekly using the severity scale of 0-5 where: 0 = no lesion, 1 = small lesion; 2 = medium size; 3 = large; 4 = whole leaf affected; 5 = dead leaf (Mphahlele *et al.,* 2020). Final disease severity was calculated using a formula:

Disease severity (%) = [(Number of leaves scored for each rating x the rating value)/ Total plants scored] x 100.

The disease incidence was determined using a formula:

DI (%) = (total plants infected/ total plants evaluated) x 100

3.4.2. Determination of growth parameters (Collected during the experiments)

Data on growth parameters including plant height and stem diameter were measured once every week until the termination date (35 days after inoculation) (Mathelemuse *et al.,* 2022). Plant height was measured from the soil level in the pot to the elongation tip

using a measuring tape. The stem diameter was measured five centimetres above the soil using a digital Vernier caliper.

3.4.3. Determination of yield parameters (Collected at termination day of the trial)

Fresh shoot (stem and leaves) mass was determined using a weighing balance and reported as fresh mass (g) per plant. The root length was measured using a measuring tape and reported as root length (cm).

3.4.4. Determination of colour attributes

The Minolta CR-400 chromatometer (Minolta, Osaka, Japan) was used to measure the color of the leaves of the moringa plant. To ascertain the color changes, measurements were made three times on each replicate for each treatment (Managa *et al*., 2019).

3.4.5. Determination of plant physiological attributes

i. The chlorophyll content was determined using the Konica Minolta chlorophyll meter spad-502plus.

ii. Photosynthetic rate, stomatal conductance, Ci Intercellular CO_2 concentration, transpiration rate and water use efficiency were measured using LI-6000 Portable Photosynthesis System out on fully expanded young leaves.

3.4.6. Biochemical analysis in dried Moringa plant leaves

i. Preparation of the extract: the healthy and diseased dried leaves were harvested separately, oven dried at 40 °C for 48 hours, and ground into a fine powder. For the non- inoculated plants, the leaves showing symptoms due to cross contamination were discarded. An extraction procedure outlined by Apea-Bah *et al.* (2022) was used to extract the soluble free phenolic compounds (total phenols, total flavonoids, and scavenging activity) of the plants. A 2 mL micro centrifuge tube was filled with 100 mg of each treatment and 1 mL of 80% aqueous methanol

was added, and the mixture was briefly vortexed. The treatments were stored in a thermostatic water bath at 25 °C for 45 minutes after and were covered with foil paper to prevent compounds degradation from light. This allowed the solid residues to settle and produced a supernatant that on top included free phenolic compounds. In a sterile micro centrifuge tube, the supernatant was filtered off.

ii. Determination of total phenolic content: The Folin-Ciocalteu assay method was employed to conduct the analysis for total phenol composition as described by Apea *et al.* (2022). A gallic acid standard was made up of a concentration range of 0.020 to 0.080 mg/mL so that the calibration curves could be plotted against each other to determine the overall phenolic content. A 1 mg/mL stock of each standard was created by adding 1 mL of 80% aqueous methanol to each tube holding 1 mg of the gallic acid standard and vortexing the mixture for dissolution. A series of dilutions was prepared from each stock solution in a total of 500 µL. In a microplate, a reacting solution was made from the addition of 18.2 µL of the extract/standard was pipetted, followed by a 36.4 µL of 10% aqueous Folin-Ciocalteu reagent and 145.4 µL of sodium carbonate to each well. The mixture was allowed to sit at room temperature for two hours in a dark room. The absorbance was measured using a microplate reader set to run at 760 nm wavelength.

iii. Total Flavonoids determination: The total flavonoids were quantified using the aluminium chloride method with modifications as described by Ndhlala *et al.* (2014). The mixture of the reaction was made up of 1 mL of the treatments extract and 4 mL of distilled water added to a 10 mL volumetric flask to create the reaction mixture. After adding 0.30 mL of 5% sodium nitrite to the flask and waiting five minutes, 0.3 mL of 10% aluminium chloride was combined. After

leaving the mixture for five minutes, 2 mL of 1M sodium hydroxide mixed with 10 mL of distilled water were added. A series of quercetin reference standard solutions were made. At 492 nm, the absorbance was measured.

iv. Antioxidant activity (scavenging activity) determination: The total antioxidant was carried out using the DPPH assay method (2, 2-diphenyl-1-picrylhydrazyl) as described by Apea *et al.* (2022) with minor modifications. A 50 mL centrifuge tube was weighed with 1.2 mg of DPPH and filled with 30 mL of 100% methanol to create a 60 µmol/L methanolic solution. To perform the analysis, 5 µL of the treatment extracts were poured into each microplate well. Subsequently 195 µL of the 60 µmol/L DPPH methanolic solution was added, and the plates were incubated for 60 minutes. At 517 nm, the absorbance was measured.

v. The chlorophyll content was measured following a method described by Managa *et al*. (2019). Ground treatments were weighed (0.2 g) and mixed with 2 mL of acetone and hexane at a ratio 4:6 (v/v) and extracted for 2h. The treatments were centrifuged for 10 minutes at 4 °C. The resulting supernatant was decanted, and a portion of the solution was measured at 470, 646 and 662 nm using the Biochrom Anthos Zenyth 200 Microplate Reader; SMM Instruments. The following equations were used to determine Chla, Chlb and the totchl content:

Chl a = 15.65A662 − 7.340A646

Chl b = 27.05A646 − 11.21A662.

Totchl= Chla + Chlb

3.5. Data analysis

Statistical analysis was performed to investigate the impact of various parameters on all dependent variables. To compare differences in treatment means, paired sample t-

test was performed at a 5% significance level. Sigma Stat software 2016 (ver. 4.0; San Jose, CA) was utilized to perform the statistical analysis. Graph Prism 5 was used for the formulation of the graphs.

CHAPTER 4

RESULTS AND DISCUSSION

4. Results and Discussion

4.1. Alternaria leaf spot incidence and severity on Moringa plants.

Alternaria leaf spot incidence and severity was significantly ($p \leq 0.05$) high on all inoculated Moringa plants (Table 4.1). As shown in Table 4.1, all inoculated plants displayed 100% infection at week five after inoculation. A disease incidence of 33% was recorded on non-inoculated plants which could have been due to natural infection from inoculated plants. Alternaria leaf spot severity was also significantly high on inoculated plants suggesting high susceptibility of Moringa to *A. alternata* (Table 4.1). Similarly to high disease incidence recorded, plant inoculated with *A. alternata* showed the highest disease severity of 42%, with the least observed on the non-inoculated treatment at 16%. The incidence results were in concomitant to the diseases severity that showed two-fold difference between inoculated and non-inoculated plants (Table 4.1).

Moringa is a host plant of *A. alternata* (Mridha and Barakah, 2015), which permits the successful entry and establishment of the pathogen into the cells of the plant (Manohararchary and Kunwar, 2014). Alternaria conidia can be primarily dispersed by wind in the environment from infected plants to susceptible hosts. Alternaria species are known to cause serious damage to susceptible host plants resulting in significant yield losses (Al-lami *et al*., 2020). The disease severity may be dependent on the infection time and crop growth stage at which the pathogen infects the plant (Gitonga and Githae, 2021). Differences in disease severity among the two treatments were significantly high ($p \leq 0.001$). There is a strong relationship between disease severity

and the processes that affect the growth, development, and yield of a plant (Huber *et al*., 2012). A high severity level due to *A. alternata* inoculation resulted in a significant decrease in the growth and yield attributes of Moringa as discussed in 4.2. As discussed in 4.3, there is a significant change in the physiological attributes of Moringa plant due to high severity of Alternaria leaf spot as inoculated with *A. alternata*. As the physiological processes were significantly affected by the high severity level of Alternaria leaf spot from the inoculated treatment compared to the non-inoculated treatment, there was a significant change in the colour attributes of Moringa leaves as a result. The biochemical processes, as discussed below were affected by a high disease severity as inoculated with *A. alternata*. The rate at which the disease affects plant processes depends on its severity.

Table 4.1. An overall average of disease incidence and disease severity of *A. alternata* on the inoculated and non-inoculated treatment grown in the greenhouse over a period of five weeks following inoculation.

	Inoculated treatment	Non-inoculated treatment	p- value
Disease incidence	100%***	33%	p=0.000
Disease severity	42%***	16%	p=0.000

Superscripts mean significant differences at the probability level of *** (p≤0.001).

4.2. The effect of *A. alternata* on growth and yield attributes of Moringa.

Inoculation of Moringa plants with *A. alternata* had a significant effect on plant growth and yield parameters (Figure 4.2 (A and B) and Table 4.2). There was significant difference (p≤0.05) between the inoculated and non-inoculated plants on growth (plant

height) and yield (fresh shoot mass) of Moringa. *Alternaria alternata* inoculation resulted in a stimulatory effect on inoculated plants height from week two to three at 25.67-45.98 cm. Non-inoculated control plants displayed a shorter height (22.77-39.23 cm) during the same growth period. There was however a reduction in plant height in inoculated plants from week five, with non-inoculated plants showing an increase in growth pattern (Figure 4.2A). In addition, non-inoculated plants showed higher fresh shoot mass (1.8g) in comparison to their counterpart (inoculated: 1.2 g). The stem diameter and root mass showed no significant difference between the two studied treatments as illustrated on Figure 4.2B and table 4.2.

The significant difference may be attributed to the effect of the pathogen inoculum on the development of the plant. Cohen *et al*. (1997) reported an initial increase in the growth of Cistus plants inoculated with *Botyrosphaeria stevensii* followed by a cessation of growth, then a sharp decrease thereafter. Marraiki *et al.* (2022) reported a leaf spot disease caused by *A. alternata* which caused a significant reduction in spinach leaf quality and yield. Agamy *et al.* (2013) provided support to the findings by describing the destructive necrotic symptoms of the pathogen, which affect plants at every stage of growth and result in losses in production and growth. According to Abd El-Hai (2015), Alternaria leaf spot is a disease that seriously impaired the growth and yield of Faba bean plants due to fluctuations in temperature promoting the infection. Because of their mutual influence, there was a comparable trend in both the fresh shoot mass and the plant height. While the plant's apical growth accounts for the majority of plant height, the plant's lateral growth and apical growth combine to form fresh shoot mass. Plant height is determined by the length and number of internodes, which are particularly significant because of their effect on plant yield. Shorter plants frequently give up on yield-related characteristics since they have fewer nodes (Clark *et al.,*

2023). Because internodes are produced as the plant height increases, there is an increased stem development and leaf production which increases fresh shoot mass of the plant, resulting in a positive trend between the plant height and fresh shoot mass.

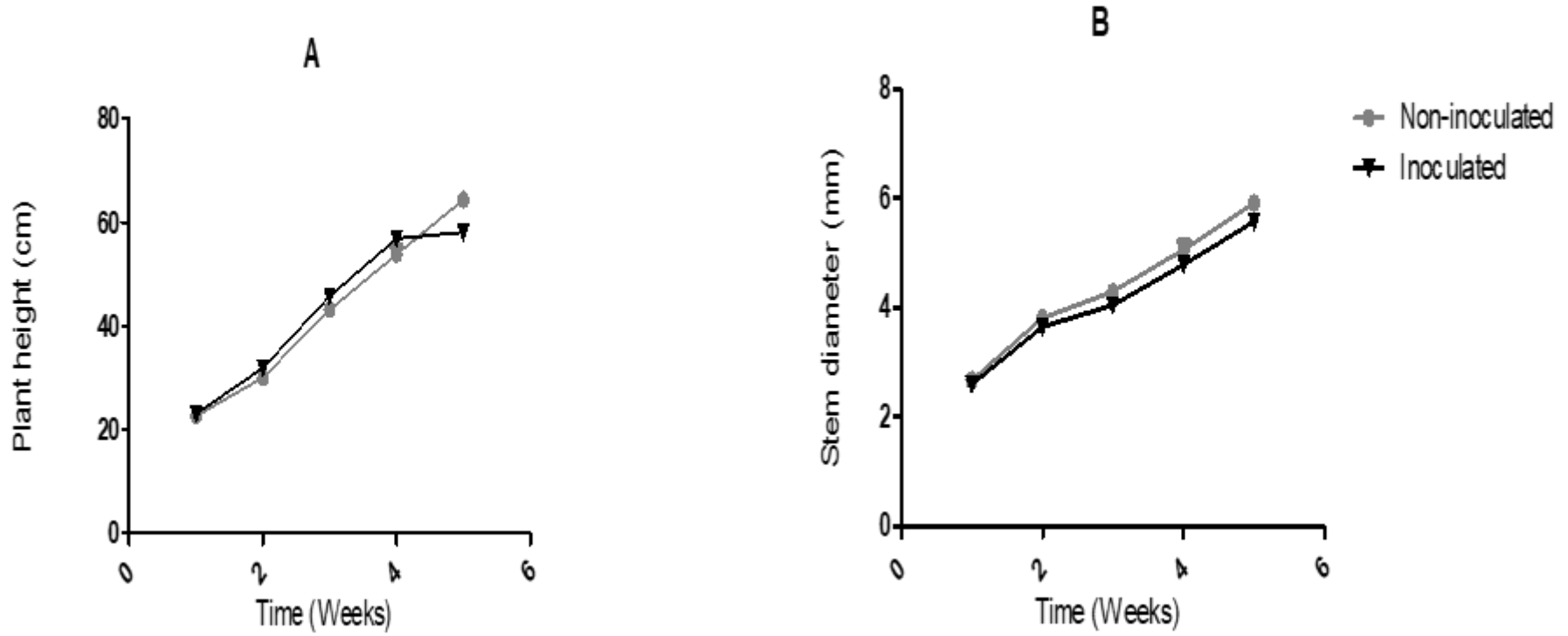

Figure 4.2. The effects of *A. alternata* on plant height (**A**) and stem diameter (**B**) from week 1 to 5 respectively after inoculation under greenhouse conditions. Results are expressed as the mean values ± standard error (*n* =6)

Table 4.2. The effects of *A. alternata* on yield parameters of Moringa at five weeks after inoculation.

Treatments	Root length (cm)	Fresh shoot mass (g)
Non-Inoculated	6.600±0.792^{ns}	1.800±0.335*
Inoculated	6.050±0.433	1.200±0.188
P-value	0.951	0.041

Values are expressed as means ± standard error (n=6). For all the values within a column, superscripts mean significant differences * ($p \leq 0.05$) and ^{ns} ($p > 0.05$).

4.3. The effect of *A. alternata* on the physiological attributes of Moringa.

Table 4.3 shows the effects of *A. alternata* inoculation on the physiological attributes namely: photosythentic rate, stomatal conductance, Ci-intercellular CO_2 concentration (Ci CO_2), transpiration rate (TR) and water use efficiency (WUE) of Moringa. There was significant difference ($p<0.05$) between inoculated and the non-inoculated plants on the Photosynthetic rate, TR, and WUE of the studied Moringa. *Alternaria altenata* inoculation resulted in a reduction effect on inoculated plants photosynthetic rate, and stomatal conductance, by 4%, and 17%, at harvest as compared to those of the non-inoculated treatments, respectively (Table 4.3). The *A. alternata* inoculation had a stimulatory effect on the water-use efficiency of the inoculated treatment by 10% higher than those of the non-inoculated plants as shown in Table 4.3. The photosynthetic rate decreased (22.932) because of the *A. alternata* pathogen

inoculation in relation to the non-inoculated treatments (24.182). According to a report by Soleimani and Kirk (2012), leaf spot reduces the area of photosynthetic leaves and increases the imbalance between the supply and demand of nutrients, which results in yield losses. This may be explained by the phytotoxic chemical secreted by the fungus diffusing into the surrounding tissue around each lesion, creating an invincible halo. According to Yang *et al.* (2018), an *A. alternata* infection inhibited photosynthesis by lowering photosysytem II photochemical activity and compromising the integrity of the cell membranes, decreased photosynthesis rate. The same author reported that the decrease was attributed to either the pathogen-to the esterification and alcoholization of chlorophyll, or the breakdown of chlorophyll structure and function, which in turn caused a decrease in chlorophyll contents. According to Wang *et al*. (2015), a pathogen infection can impact the physiology of water and photosynthesis in higher plants. The infection-induced closure of stomata, which disrupts photosynthesis' metabolic pathways which is the cause of a decreased net photosynthesis. Photosynthetic capacity of cucumber (*Cucumis sativus* L.) plants because of an infection by *Fusarium oxysporum* f. sp. *lycopersici* was decreased (Wang *et al*., 2015). The reports are in support with the findings from the study. Biotic foliar pathogens can also have an impact because they can penetrate plant stomata and/or increase the permeability of water in cell membranes by producing lytic enzymes or toxins (Lindenthal *et al.,* 2005).

The effect of the pathogen on the stomatal conductance (SC) varied significantly ($P \leq 0.05$) for both treatments. The Stomatal conductance decreased (0.060) as an effect of the *A. alternata* inoculation on inoculated Moringa plants compared to the non-inoculated plants (0.084). According to Melotto *et al*. (2017) and Xiang *et al.* (2021), the plant may have developed mechanisms to block the stomata in response

to the detection of microbe associated molecular patterns (MAMPs), which could explain the variation in the SC. The inoculation with *A. alternata* stimulated an increase in the rate of transpiration of Moringa plants. According to Hirsch (2014), *Uromyces fabae*, a rust pathogen results in the formation of haustorical mother cells after the hyphae contacts a host mesophyll cell. A mature haustorium that promotes the passage of hexose sugars, amino acids, and water into the host is formed when the haustorial mother cell infiltrates the host plasma membrane through the plant cell wall. The result becomes a considerable decrease in the average transpiration rate and average stomatal aperture of bean leaves during the fleck stage of the disease development. According to reports, defoliation, stomatal closure, and obstruction of xylem components and stomata are all possible contributors to the decrease in the transpiration rate. The *A. alternata* inoculation resulted in a significant increase in the water use efficiency (WUE) (400.525) of Moringa plants compared to the non-inoculated treatments (325.661). This could be the consequence of the pathogen interfering with the cuticular or stomatal regulation of transpiration by breaking the cuticle or entering the stomatal pores, or it could take the form of pathogen-producing spore-bearing structures that burst through the epidermis or stomatal openings, rupturing the cuticle and influencing the regulation of water (Grimmer *et al*., 2012). When visible disease symptoms appear, plant pathogens either temporarily or permanently increase the rate of water loss by creating infection structures on the surface of the plant that increase evaporation by damaging the cuticle and causing cell death leading to an uncontrollably high loss of water (Lindenthal *et al*., 2005).

Table 4.3. The effect of *A. alternata* inoculation on physiology of Moringa

Treatments	Photosynthetic rate (µmol CO2 m^{-2} s^{-1})	Stomatal conductance (SC) (H2O m^{-2} s^{-1})	Ci Intercellular CO_2 concentration (Ci CO_2 conc)(µmol mol^{-1})	Transpiration rate (TR) (mmol m^{-2} s^{-1})	Water use efficiency (WUE) (mmolCO2.m^{-1} H2O)
Inoculated	22.932±1.226	0.060±0.004	246.097±12.129	1.830±0.165	400.525±22.059***
Non- Inoculated	24.182±0.511**	0.084±0.012***	269.132±14.845ns	1.981±0.220**	325.661±52.244
P-value	0.003	0.000	0.724	0.007	0.000

Values are expressed as means ± standard error (n=6). For all the values within column different superscripts mean significant differences at ** (p≤0.01) ***, (p≤0.001) and ns (p>0.05)

4.4. The effect of *A. alternata* on the colour attributes of Moringa plants.

In the study of plants, leaf colour is an important indicator because it reflects certain strong physiological processes and the health of the plant in response to biotic or abiotic stimuli (Clement *et al*., 2015). Colour is an important component associated to quality characteristics including sensory, nutritional, and visual or non-visual defects (Pathare *et al*., 2013). It is an indicator of the changes in the biochemical processes of the plant (Pathare *et al*., 2013). The study examined the colour of the leaves on Moringa plants to comprehend the relationship between the physiological and biochemical processes brought about by the *A. alternata* inoculation that ultimately exhibit on the growth and yield attributes. The colour of Moringa plants were affected by the *A. alternata* pathogen. Table 4.1. summarizes the effect of *A. alternata* inoculation on the changes in colour parameters, including L*, a*, b*, c*and h. There was a significant difference between the two treatments based on the colour coordinates' L* (Lightness value) a* (red-green axis), b* (yellow-blue axis) and c* value ($p<0.05$). There was no significant difference between the studied treatments on the h (hue) angle. The representation of the colour space shows that the a* value ranges from green (-a*) to red (+a*), the b value from blue (-b*) to yellow (+b*), and the L* value varies from black (L*=0) to white (L*=100) (Ontiveros *et al*., 2022). There was a minimal increase in the L* value of Moringa plants due to the inoculation with *A. alternata* (44.050) in comparison to the non-inoculated treatment (43.043). Similarly, *A. alternata* inoculation induced an increase in the a* value of the inoculated to the non- inoculated Moringa plants (-10.178: -12.552). There was a significant increase due to *A. alternata* inoculation (12.653 and 17.842) when related to the non-inoculated treatment (9.045 and 13.635) on the b* value and c* respectively. The more positive b* value on the inoculated treatment signifies that Moringa plant was yellowish (Chwa

et al., 2023), in comparison to the non-inoculated treatment, meaning the inoculation had the ability to decrease the blueness of the Moringa plant. The non- inoculated treatment was bluish, which is more desirable. When compared to the non- infected treatment, the inoculated treatment high L* value suggests a lighter coloration. A negative a* readings represent a green colour of the Moringa leaves. The inoculation decreased the greenness of the plant, as evident on the non-inoculated treatment with a more negative rating. The c* value of the inoculated treatment indicated a bright-toned and the one of the non-inoculated treatment indicated a light toned colour. The findings on the colour of the plants can be explained by the physiological processes that occurred as mentioned by Clement *et al*. (2015). It is possible to link the yellowish (b value) coloration of the infected treatment and loss of greenness (a value) to a decreased rate of photosynthesis and chlorophyll degradation. As seen in Table 4.3. and Figure 4.4., the photosynthetic rate and the chlorophyll content of the inoculated treatment were low. The chlorophyll concentration is reflected in leaf greenness, which may provide an understanding of the physiological processes occurring in the plant. Additionally, photosynthesis and leaf greenness are intimately associated since chlorophylls are abundant in plants and absorb light energy that powers carbon-fixing activities (Nagaraj *et al*., 2002).

Table 4.4. Effect of *A. alternata* inoculation on the leaf surface colour

Treatments	L*	a*	b*	c*	H
Inoculated	44.050±0.560*	-10.178±0.575**	12.653±1.013*	17.842±1.108**	135.127±1.253
Non-Inoculated	43.043±1.263	-12.552±0.574	9.045±0.880	13.635±1.003	139.915±1.483^{ns}
P-value	0.025	0.005	0.040	0.010	0.260

Values are expressed as means ± standard error (n=6). For all the values within a column, different superscripts mean significant differences * ($p \leq 0.05$), ** ($p \leq 0.01$) and ^{ns} ($p > 0.05$).

4.5. The effect of *A. alternata* on the biochemical compounds of Moringa

The effects of *A. alternata* inoculation on biochemical compounds including total flavonoids, total phenols, antioxidant activity (scavenging activity) and chlorophyll (a, b and total chlorophyll) contents of Moringa was tested and showed significant difference ($p<0.05$) between the two studied treatment. The *A. alternata* infection significantly ($p<0.05$) increased the total flavonoids, phenols and antioxidant activity by 69%, 4% and 4% respectively in comparison to the non-inoculated treatment. As shown in Figure 4.3, the radical scavenging activity of the inoculated treatments (10.212) were greater than those of the non-inoculated treatment (9.501). These results indicate that the scavenging potential of the treated Moringa plants were higher compared to that of the non- treated plant.

Furthermore, the chlorophyll content decreased significantly in *A. alternata* inoculated plants. Clhorophyll a (chl a), chlorophyll b (chl b) and total chlorophyll (totchl) has been significantly decreased ($p\leq0.01$) as follows: chl a by 48%, chl b by 43% and the totchl by 44% by the infection with the pathogen. Chlorophyll is made up of a significant amount of vitamins with antibacterial, antioxidant and anti-inflammatory properties. Due to the antioxidant properties of chlorophyll content, oxidation processes that produce free radicals can be inhibited, preventing damage to bodily cells (Ngcobo *et al*., 2023). The decreased level in the quantity of chlorophyll in produce causes fruits and vegetables to lose their pigmentation, which lowers the market value of the crop (Ngcobo *et al*., 2023). Studies show that when exposed to biotic stress, plants react in a variety of ways at the molecular and cellular levels. The biochemical components of the plants, whether they were present before the stress or were induced, have been linked to plants resistance (Mallick *et al*., 2015). When pathogens or their toxic metabolites are exposed to plants, it disrupts their physiological processes and causes a significant alteration in their biochemical component. This includes the production of

oxidative defence enzymes and the build-up of specific metabolites (Meena *et al*., 2016). As observed from the results, there was a significant increase in the total flavonoids, phenols and antioxidant activity on the inoculated treatment as a response to *A. alternata* interacting with the host plant and causing an infection. A build-up of metabolites was observed in the inoculated treatment as a result. Plants produce compounds called flavonoids as secondary metabolites, and they appear to have a variety of biological purposes, including stress prevention. According to Lin *et al*. (2018), Moringa is abundant in phenolic acids and flavonoids that play an antioxidant effect in the plant (Saeed *et al*., 2012). The studied Moringa plant that was under *A. alternata* inoculation stress produced more flavonoids compared to the control. The production of the flavonoids in a case of pathogen attack may be to regulate the polar movement of auxin, a plant growth regulator that aids in controlling stomatal opening and resource allocation under poor-growth conditions (Winkel, 2002). A rise in the flavonoid content of infected Indian mustard leaves was observed by Macioszek *et al*. (2020), in a manner that was dependent on the leaf position, supporting the study. According to a hypothesis presented by Agati *et al*. (2012), a shift in the homeostasis of a cellular redox potential activates the biosynthesis of flavonoids specifically the metabolism of flavonol. The transcription factors responsible for the biosynthesis, myeloblastosis, are regulated by alterations in cellular redox potential. The infection by the inoculum resulted in a shift in the homeostasis equilibrium of the plant, which triggered the response and defence system of the plant to maintain the equilibrium balance by increasing the flavonoids content in the plant. In higher plants, phenolic compounds are widely dispersed and play a major part in the defense of the plant against biotic and abiotic stressors, in particular, phytopathogenic fungi (El-Nagar *et al*., 2020). Regardless of the genotype response to Alternaria, Ventakesh *et al*. (2016) found that healthy leaves had a higher phenol content than infected leaves which was

in contrast with the results of the study. In general, plants phenolic content increases due to pathogenic infections. Koc and Üstün (2012) supported the findings of the study by reporting a maximal rise in phenolics found on the susceptible leaves and stems of pepper on day six after infection with Phytophthora capsici. Khan *et al*. (2001) reported an increase followed by a decrease of total phenols content four days after inoculation, however, the concentration of the phenols was higher on the diseased plant than on healthy plants, supporting the study. Pathogen infections trigger an increased production of phenols as a way of fighting disease progress. Chauhan *et al*. (2022) showed that, when compared to the uninoculated treatment, the phenolic content of the infected plants on the moderately resistant cultivar was higher due to *A. alternata* infection. Agamy *et al*. (2013) observed a reduction in chlorophyll a and b content in an Alternaria-affected tomato plant. The conclusions of the study were corroborated by Sa. (2022), who found that when A. cucumerina infected cucumber seedlings, chl a, chl b, and the total chl decreased. Alternaria alternata produces toxins that inhibit QA and QB, which lowers photosystem II(PSII) activity and increases ROS content, both of which have a negative effect on chlorophyll content (Sa, 2022). This may be the cause of the decrease in chlorophyll content. Meena *et al*. (2016) observed that when tomato plants were exposed to toxins (TeA, AOH, and AME) isolated from the *A. alternata* pathogen, the chla, chlb, and tot chl content decreased. In a report by Kyseláková *et al*. (2011), it is indicated photosynthesis and chlorophyll decrease during an infection due to starch and soluble sugars accumulating in infected cells as a result of physiological response, indicating a shift in the metabolism of the cells from sink to source metabolism. Free radicals can cause irreversible alterations to proteins, lipids, and nucleic acids within the cell due to their ability to binding metal ions and donating hydrogen atom electrons (Ndhlala *et al*., 2024). In this study, the antioxidant activities of the inoculated plants were significantly higher. According to Anthony *et al*.

(2017), when a defence system of a plant is activated, it produces antioxidant compounds that alter the antioxidant characteristics of the infected plant by reducing and repairing the damage caused by reactive oxygen species (ROS) in response to invasion. By scavenging ROS, the antioxidant molecules aid the plant in its defence (Afzal *et al*., 2014). Anthony *et al*. (2017) reported a significant increase in the antioxidant activity in the root, stem, leaf and fruit of Banana (Musa balbisiana L.) trees as infected with Fusarium.

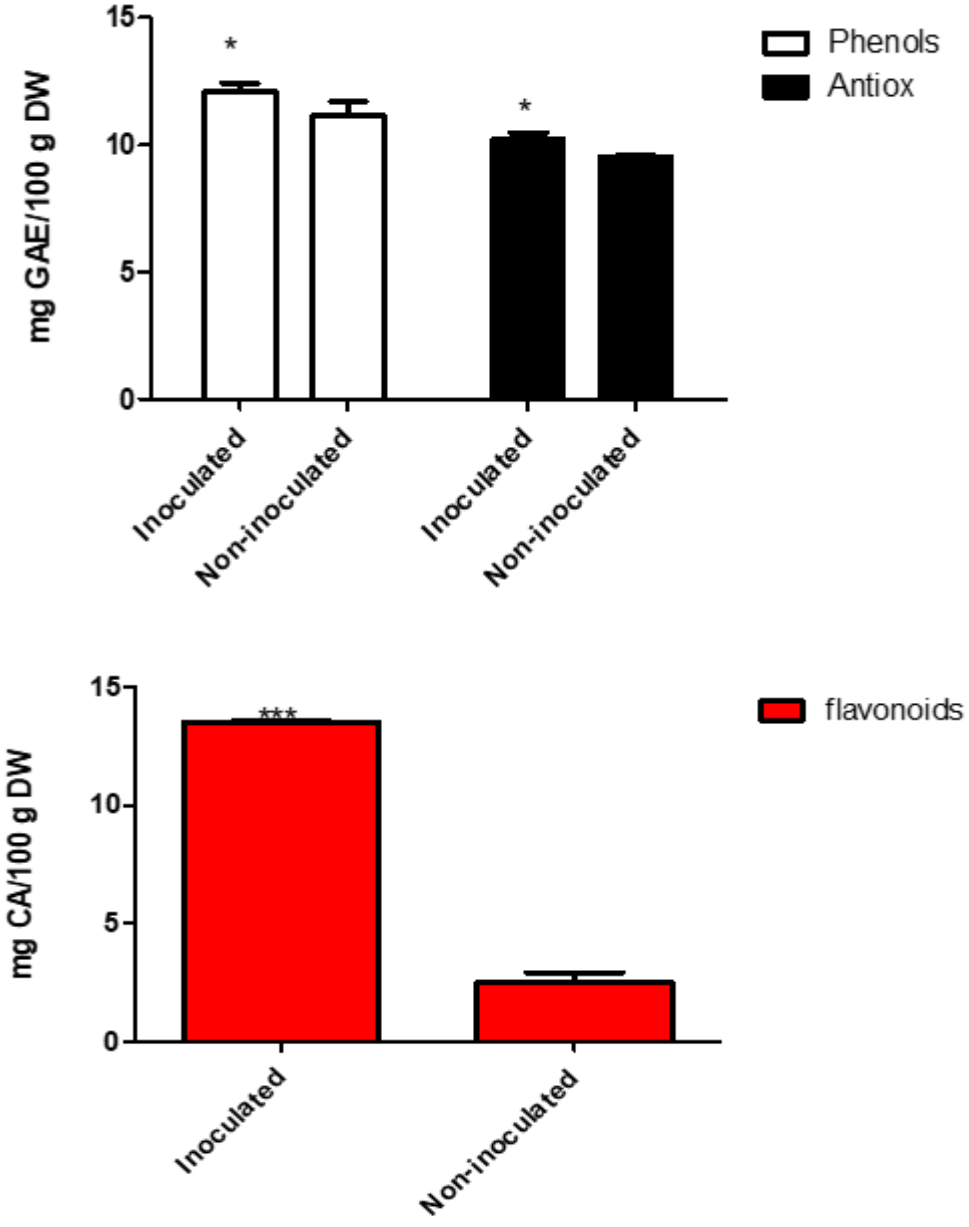

Figure 4.3. The effect of *A. alternata* inoculation of the total flavonoids (Flavonoids), total phenols (Phen) and antioxidant activities (Antiox) of Moringa plants under greenhouse conditions. Results are expressed as the mean values ± standard error (n=4). Different superscripts mean significant differences * (p≤0.05) and *** (p≤0.001).

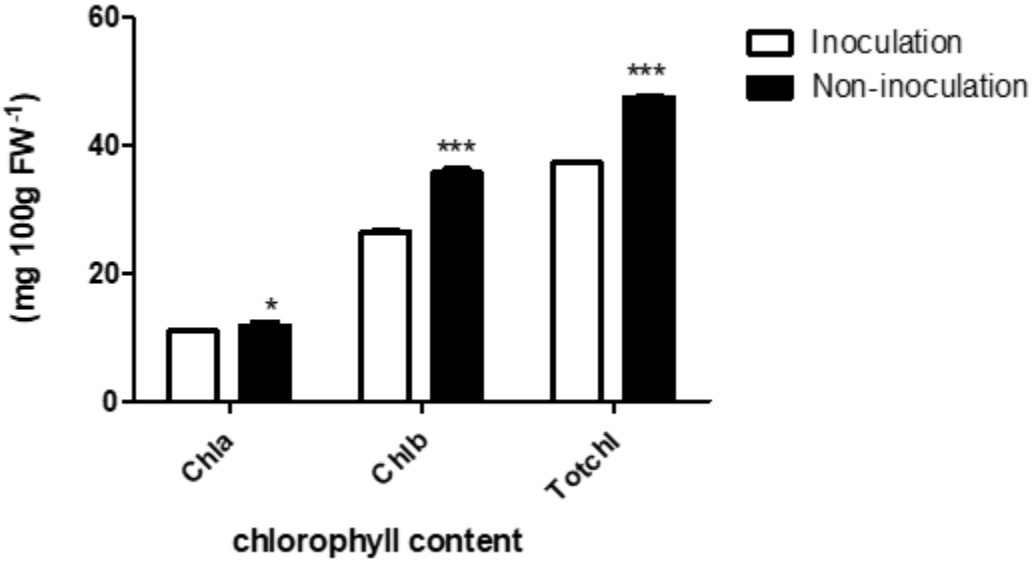

Figure 4.4. The effect of *A. alternata* inoculation on the chlorophyll a (Chla), chlorophyll b (Chlb) and total chlorophyll (Totchl) content of Moringa plants under greenhouse conditions. Results are expressed as the mean values ± standard error (n=4). Different superscripts mean significant differences * for p≤0.05, ** for p≤0.01 and -*** for p≤0.001.

CHAPTER 5:

SUMMARY OF FINDINGS, SIGNIFICANCE, CONCLUSIONS AND RECOMMENDATIONS

5.

5.1. Summary of findings

The findings of this research provided new information on the effects of *Alternaria alternata* on the growth, yield and phytochemical contents of a PKM1 cultivar of *Moringa oleifera* grown under greenhouse conditions. The *A. alternata* induced Alternaria leaf spot symptoms on the inoculated leaves of Moringa and caused a severity level of 42%. The research indicated that infection of Moringa trees by *A. alternata* results in a significant reduction in plant height (cm) and fresh shoot mass (g), and no significant effect on the stem diameter (mm), root length (cm) after 5 weeks of infection. The physiological attributes were significantly affected by an infection by *A. alternata*. The photosynthetic rate, the stomatal conductance and the transpiration rate of the infected plant was significantly reduced. The water use efficiency was significantly increased by the infection with *A. alternata*. The infection had no significant influence on the Ci intercellular CO_2 concentration of the plant. The attributes of colour were significantly affected by the infection. The inoculation significantly increased the L* value, a* value, b* value, c* value and had no significance effect on the h value of the colour meter. On the biochemical compounds of Moringa, the treatments significantly increased the total flavonoids, phenols and antioxidant activity by 69% ,4% and 4%, and significantly reduced the chl a by 48%, chl b by 43% and the totchl by 44%.

5.2. Significance

The findings of the study demonstrate how significantly Alternaria leaf spot impacts the physiology, quality, and biochemical composition of Moringa. As a result, the data will be recorded, used to better understand how plant diseases affect Moringa, and to increase the production of Moringa which is safe for human use. Small-scale Moringa growers and farmers will receive the information to better inform them of the impact Alternaria leaf spot has on the plant. Increased output and high-quality harvest are what farmers require, whereas leaf spot gives the reverse. As a result, the data can be utilised to maximise output through continuing research on disease control that is both economical and ecologically benign. The data may also necessitate the investigation on how the pathogen *A. alternata* affects the distribution of biochemical components in the plant at various phases of growth.

5.3. Conclusions and recommendations

In conclusion, the introduction of *A. alternata* into the PKM1 cultivar of *M. oleifera* influenced its growth and development. The significance of the information lies in its potential to fill the knowledge gap in science on the undocumented effects of the pathogen on Moringa development and quality. Farmers may find the material helpful to understand the effects of the disease on plants and to take preventative and control actions to maximise yield.

The results of this study suggest that preventive measures be taken to prevent *A. alternata* infection. This will optimise the growth, yield, colour attributes, and physiological attributes of Moringa, as the infection has a detrimental impact on these parameters. The infection inhibits the physiological processes of the plant, which are responsible for the growth and yield of Moringa. As a result, the growth, yield, and

quality are impacted. When a plant becomes infected by a pathogen management strategies should be implemented to control the disease spread and impact on the plant.

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