

**Efficacy of *Mondia whitei* (Hook.f.) on Management of Early Blight (*Alternaria solani*  
E.) and Late Blight (*Phytophthora infestans* Mont.) and on Growth and Yield of  
Tomato (*Solanum lycopersicum* L.)**

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**A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of  
Master of Science in Plant Health Management of Masinde Muliro University of  
Science and Technology.**

**October, 2025**

**DECLARATION**

This thesis is my original work prepared with no other than the indicated resources and support, and has not been presented elsewhere for a degree or any other award.

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**SPM/G/01-70183/2020**

**CERTIFICATION**

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## **DEDICATION**

I dedicate this work to my two beautiful daughters Maya Rosemary and Sasha Halima, my dear wife Mariam Mohamed, Grandmother Mary Auma and my parents Alfred Chitui Ashiemi and Rose Adhiambo.

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## ABSTRACT

In Kenya, tomatoes (*Solanum lycopersicum* L.) are a significant vegetable in horticulture production and consumption. Most tomato farmers use synthetic fungicides to control fungal diseases on tomato crops. However, this method has been proven to be environmentally and health hazardous, and sometimes ineffective as the pathogens develop resistance. To this effect, there is a possibility of exploiting bio-extracts from plants, as fungicides. This study that was carried out at Masinde Muliro University of Science and Technology sought to assess the impact of *Mondia whitei* Hook.f. extracts on tomato plant growth and antifungal activity both *in-vitro* and *in-vivo*, and subsequently screen for phytochemicals. *Mondia whitei* roots were harvested washed, dried, milled and extracted in methanol (MeOH), dichloromethane (DCM) and ethyl acetate (EtOA) through maceration technique. The extracts' *in-vitro* antifungal properties against *Alternaria solani* E. and *Phytophthora infestans* Mont. at concentrations of 10% and 20% were assessed using the pour plate method. Four treatments were used in a completely randomized design (CRD) for the experiment: Two levels of the extract concentrate (10% and 20%), positive (0.25% ridomil fungicide) and negative (Blank) control which were replicated three times. *In-vivo* experiment was laid in a greenhouse using CRD with five treatments: Three levels of the extract concentration (2.5%, 10% and 20%), positive and negative control which were replicated four times. Phytochemical screening was done using standard laboratory qualitative techniques. *Mondia whitei* extracts completely inhibited the *in-vitro* growth of *A. solani* and *P. infestans* at both concentrations of 10% and 20% of all the solvent extracts. Compared to the negative control, which recorded an average disease incidence of 57.86% and 80% for *A. solani* and *P. infestans*, respectively, the *in-vivo* *M. whitei* treated plants showed a significant ( $p \leq 0.05$ ) reduction in disease incidences, with 20% extract concentration recording a disease incidence of 18.5% and 35% for *A. solani* and *P. infestans*, respectively. Furthermore, compared to the negative control, which recorded disease severity index values of 8.29 and 10.42 for *A. solani* and *P. infestans*, respectively, the plants treated with 20% extract concentration had significantly lower ( $p \leq 0.05$ ) disease severity index values of 4.43 and 7.14 for *A. solani* and *P. infestans*, respectively. The experiment also showed a significant ( $p \leq 0.05$ ) increased growth rate of all growth parameters, with increase in extract concentration. 20% *M. whitei* extract concentration recorded 133.96cm and 117.71cm for height, 9.81cm and 8.44cm for leaflet size and 17 and 15 compound leaf numbers for *A. solani* and *P. infestans* respectively. Whereas, negative control recorded 93.49cm and 99.30cm for height, 7.83cm and 7.13cm for leaflet size and 10 and 8 compound leaf number for *A. solani* and *P. infestans* respectively. Secondary metabolites detected were cardiac glycosides, flavonoids, glycosides, tannins, carbohydrates, terpenoids, volatile oils, saponins, alkaloids and steroids. The results of this investigation show that *M. whitei* exhibits potent fungicidal effects against *P. infestans* and *A. solani*. The plant accumulates a diversity of phytochemicals that possibly confer the fungicidal activities. Findings from this study present *M. whitei* as a potentially safe, affordable and environmentally sound alternative to synthetic fungicides against *A. solani* and *P. infestans* in tomato. It also presents the potential use of *M. whitei* to treat early and late blights, and has provided important leads for the development of new plant-based antimicrobial fungicide.

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## LIST OF ABBREVIATIONS AND ACRONYMS

CRD	Completely Randomized Design
CNCD	Chronic non-communicable diseases
CVD	Cardiovascular diseases
FAOSTAT	Food and Agricultural Organization Statistics
ANOVA	Analysis of variance
Ppm	Parts per million
GPRS	General Packet Radio Services
WMO	World Metrological Organization
PDA	Potato Dextrose Agar
MIC	Minimum Inhibitory Concentration
rpm	Revolutions per minute
MMUST	Masinde Muliro University of Science and Technology
DCM	Dichloromethane
EtOA	Ethyl acetate
MeOH	Methanol
DMSO	Dimethyl sulfoxide
DAI	Days after Inoculation
AUDPC	Area Under Disease Progress Curve
USD	United State Dollar
LDL	low-density lipoprotein

## CHAPTER ONE: INTRODUCTION

### 1.1 Background

Tomato, *Solanum lycopersicum* L. is an important vegetable fruit crop (Sandoval-Ceballos et al., 2021). Taxonomically, it belongs to the nightshade, or *Solanaceae*, family, which is among the most valuable plant families for fruit and vegetable crops (Quinet et al., 2019). According to FAOSTAT (2022), the annual production of tomatoes in Kenya, Africa, and the world was estimated at 0.66 million metric tons, 22.9 million metric tons, and 186.1 million metric tons, respectively, and it was the most produced horticultural food crop globally and in Kenya in the same year. Additionally, the crop makes up 7% of Kenya's total horticultural goods and 14% of the nation's total vegetable production (Ochilo et al., 2019). Smallholder farmers in Kenya produce the majority of tomatoes, primarily for financial gain (Mwangi et al., 2015). According to Warum et al., (2023), Kenya ranked 58th in the world for tomato exports and 150th for tomato imports in 2022, with \$1.98 million in exports and \$34.5k in imports. In the same year, tomato was the 235<sup>th</sup> most exported and 1041<sup>st</sup> most imported product in the country. The crop is important economically not only for its profits, but also for the earnings that it brings in locally for farmers and other agricultural workers (Singh et al., 2023).

Due to its widespread use as a fundamental component in a wide range of raw, cooked, and processed dishes, the tomato's edible fruit is the most consumed vegetable in the world (Rane et al., 2024). Tomato fruits constitute a significant source of nutrition (Dawid, 2016). The fruits has a number of substances that are known to have beneficial health effects, including carbohydrates, protein, vitamins, dietary fibers and essential natural elements and antioxidants (Collins et al., 2022; Ali et al., 2020; Singh et al., 2016).

Among other human organs, the heart is thought to benefit from the fruit's ingestion (Raiola et al., 2014). Consuming tomato fruit has, in fact, been linked to a lower risk of inflammatory processes, different forms of cancer, and chronic non-communicable diseases (CNCD), which includes cardiovascular illnesses (CVD) including obesity, diabetes, hypertension, and coronary heart disease (Ali et al., 2020).

One of the strongest antioxidants found in nature is lycopene (Madia et al., 2021). According to Ali et al., (2020), this natural antioxidant can aid in the fight against a variety of cancers, such as those of the prostate, breast, lung, stomach, colorectal, oral, esophageal, pancreatic, bladder, cervical, and ovarian cancers. According to Mehrin et al., (2020), tomatoes have a high concentration of lycopene, which stays higher throughout the multiple processing steps required to produce juice or paste.

The estimated demand for tomatoes in Kenya is approximately 300,000 tons, while the actual average production is approximately 283,000 tons annually (Geoffrey et al., 2014). On the other hand, the average area harvested of tomatoes is 28,330 ha annually, which gives a proximate yield of 23.2 tons per ha (FAOSTAT, 2022). However, the potential yield is above 30.7 metric tons per ha (Ochilo et al., 2019; Anastacia et al., 2011). The yield gap is attributed to a number of yield reducing factors which include both biotic and abiotic factors and which can lead to losses of up to 100% (Mwangi et al., 2020).

Tomato diseases, are a major cause of yield gap (Anastacia et al., 2011). According to Godfray et al., (2016), fungal diseases pose a serious risk to a variety of crops, including tomato harvests. It has been reported that more than 19,000 fungi can infect crop plants globally and cause diseases (Peng et al., 2021). Furthermore, phytopathogenic fungi reduce crop yield and quality, which causes significant losses in agricultural output. According to

Panthee & Chen, (2010), early and late blight are the two primary fungi that threaten tomato yield.

Early blight, a significant foliar fungal disease of tomatoes is brought by the necrophytic fungus *Alternaria solani* E. (Panthee & Chen, 2010). *Phytophthora infestans* Mont., a filamentous fungus-like member of the Oomycota, is the cause of late blight (Moore et al., 2020). For both blights, losses in marketable crops can range from between 79.81% to 100% in an unmanaged tomato field, while the loss in managed fields can be between 12% and 65% for both blights (Amin et al., 2013).

*Alternaria solani* and *Phytophthora infestans* affect tomato leaves, fruits and stems and can severely limit yield when weather is favorable (Panthee et al., 2024; M. Singh et al., 2016). All plant components above ground become severely infected by *A. solani*. As plants age, the infection spreads upward from the older leaves (Attia et al., 2022). On the older leaves, tiny dark necrotic lesions first develop. As the lesions grow, they form concentric rings and are frequently encircled by a yellowish zone (Gulzar et al., 2018). In lesions larger than 10 mm in diameter, concentric bands of dark pigmentation are common. This lesion, known as "bullseye", is a prime example of early blight (Kumar et al., 2017). Premature defoliation also occurs that weakens the plants and set the fruit to injury from sunscald (Ojiewo et al., 2010). Partial girdling of the stems of seedlings also occurs and when the stem is completely girdled by the lesion, plant death occurs. Eventually, The infected fruits drop before attaining maturity and the fruits that reaches to maturity became unmarketable (Gulzar et al., 2018).

*Phytophthora infestans* can infect any portion of the plant that is above ground, leading to fruit rot, necrosis of the leaves and stems, and ultimately plant death (Sarkar et al., 2022;

Mugao, 2021). Early signs of infection, such as tiny lesions on plant stems and leaf tips, don't show up until three to four days after infection and, in certain situations, are barely 1 to 2 mm in diameter (Nowicki et al., 2013). Lesions of dark brown late blight may begin at a node or at the top of the stem and then spread downward. Firm, dark, oily lesions on the sides and around the stem end of green tomato fruit are often present, rendering the fruit unmarketable. Soft-rot disease can result from secondary infections invading infected tomato fruit (Nowicki et al., 2013). Normally, purple, dark brown, or black lesions soaked in water often have a pale yellowish-green border that blends in with the surrounding healthy tissue. As the pathogen spreads throughout the plant tissue, the lesions get bigger. Fluffy white sporangia can grow on the lower leaflet surface when the weather is moist (M. Singh et al., 2016). Eventually, the plant leaves wither and die as the disease develops, causing the remaining foliage to become severely defoliated (M. Singh et al., 2016).

Morphologically, *A. solani* produces conidiophores that are straight or somewhat flexuous, brown to olivaceous brown in color, and on these conidiophores, single, oblong, or ellipsoidal conidia are formed (Roopa et al., 2016). The *P. infestans* isolates exhibit a striated pattern and fluffy, cottony growth as their phenotypic feature. The pathogen's sporangia are lemon-shaped and form at the end of these sporangiophores (Raza et al., 2022).

Because synthetic fungicides are the most popular method of disease control among farmers, their usage has become a regular feature of tomato production worldwide due to the infections' frequency and potential for epidemics (Hao et al., 2019). These pesticides, on the other hand, are costly, difficult to break down, leave residues in agricultural products, and are hazardous to users. They could contaminate the environment and have a

negative impact on people's health, making them an environmental hazard (Peng et al., 2021; Lengai et al., 2017).

Furthermore, some infections have become resistant to synthetic pesticides, forcing farmers to use pesticides more frequently. Some have even turned to mixtures to ensure a presentable produce (Hao et al., 2019; Choudhury et al., 2018; Lengai et al., 2017). Chemical residues in fresh vegetables have resulted in more interceptions to profitable foreign markets (Lengai et al., 2017). To control *A. solani* and *P. infestans* on tomatoes, a safer, more economical, and ecologically friendly fungicidal options are thus required.

Plant extracts have been shown in multiple studies to possess fungicidal capabilities when tested in vitro (Gizaw et al., 2022; Choga et al., 2021; Zanna et al., 2021; Lengai et al., 2017). Particularly, it appears that plants found in the wild could be a valuable source of fungicidal metabolites (Salhi et al., 2017). Bitypic *Mondia* skeels are largely found in the humid tropical and subtropical forests of Africa (Aremu et al., 2011). Dried *Mondia* roots are also sold in open markets all over Kenya, where both men and women of various ages chew them (Oketch-Rabah, 2012). The root's extracts have been tested against nine clinical isolates and gave 47.23% inhibition against DPPH+ (Gbadamosi & Erinoso, 2015).

Therefore, the purpose of this experiment was to examine any potential antifungal activity of *Mondia whitei* (Hook.f.) skeels root extracts against *A. solani* and *P. infestans*, which are among the harmful plant's pathogens in the *Solanaceae* family that are particularly common among small-scale tomato growers.

## 1.2 Statement of the Problem

The majority of Kenya's vegetable production, including tomatoes, is done by small-scale, resource-constrained farmers. These farmers face significant threats from fungal diseases, primarily early and late blight. These fungal diseases caused by *A. solani* and *P. infestans* are economically important diseases to the tomato farming. These pathogens cause devastating foliar damage, fruit rot, and stem lesions, leading to substantial tomato yield losses of up to 100%, if not effectively managed. The diseases lead to reduced fruit quality, and even total crop failure.

The average yearly production of tomatoes in Kenya is about 283,000 tons, compared to an estimated demand of approximately 300,000 tons. However, tomatoes are harvested on an average of 28,330 hectares per year, yielding a proximate yield of 23.2 tons per hectare. But the potential output is more than 30.7 metric tons per hectare. This yield gap experienced by the farmers is attributed to the two fungal tomato diseases.

To manage these destructive diseases and ensure viable harvests, farmers rely heavily on frequent application of synthetic fungicides. However, limitations on the fungicide use are a serious concern globally, as their frequent and excessive use pose environmental and health risks to these farmers. The use of synthetic pesticides has had a detrimental effect on the environment's biotic and abiotic components. Residues contaminate soil and water, harming beneficial organisms and biodiversity. The widespread and negligent use of pesticides in agriculture has raised the risk of farmworkers and consumer exposure to residues and its associated health effects such as chronic diseases like cancer and respiratory diseases.

It has also been noted that *A. solani* and *P. infestans* have grown resistant to some of the fungicides rendering common fungicides ineffective over time. When pesticides are used improperly, they can leave residues in food and the environment that can cause harm to humans and cause pathogens to become resistant.

The core problem is therefore, balancing effective blight control to secure tomato yields with the need to reduce dependence on synthetic fungicides and mitigate their associated economic, environmental, and health risks.

### **1.3 Justification of the Study**

The severe economic losses caused by early blight and late blight in tomato cultivation necessitate effective control. Over-reliance on synthetic fungicides faces challenges like pathogen resistance, environmental contamination, and human health concerns. *M. whitei* extract presents a compelling bio fungicide alternative due to demonstrated antifungal activity. Scientific studies confirm potent inhibitory effects of *M. whitei* extracts, rich in bioactive phenolic, terpenoids, and flavonoids, against some pathogens. For instance, Dzoyem et al., (2014) and Mayunzu et al., (2012) have confirmed that *M. whitei* has significant antifungal activity against *Candida albicans* and *Aspergillus niger* fungi. Also, according to Deeh et al., (2024) *M. whitei* has a number of pharmacological advantages, such as, anti-parasitic, anti-malarial, antibacterial, antiviral, antifungal, antidiarrheal, and anticancer capabilities. In the African traditional ethnobotany, *M. whitei* is said to cure mouth thrush, tonsillitis, gums and dental related diseases, ringworms among others. Even though the plant's efficacy against some of the human pathogens is known, its investigation and use on phytopathogenesis is still novel.

Use of *M. whitei* bio fungicide may help reduced environmental and health risks. As a plant-derived product, it may offer significantly lower toxicity to non-target organisms, thereby improving soil/ecosystem health, reducing chemical residues on produce, and enhanced environmental safety compared to conventional synthetic fungicides (Godlewska et al., 2021; Li et al., 2021). Plants extracts used as bio pesticide do not pose any threats to the environment and human, therefore human exposure risk and health consequences can reduce due to the use of bio pesticides in agriculture (Kori et al., 2018). The bio products natural origin may also support sustainable agricultural practices and aligns well with integrated pest management (IPM) strategies, potentially reducing the selection pressure for resistance.

*Mondia whitei* naturally grows and can be cultivated in all tropical and sub-tropical regions of Africa, offering opportunities for cost-effective, locally-sourced disease management solutions. Its use is sustainable as only the fibrous roots are harvested leaving the taproot attached to the plant as the plant continues to grow. For that reason, *M. whitei* extracts may be used as a cost-effective and ecological substitute for managing *A. solani* and *P. infestans* in tomato farming sustainably.

Therefore, *Mondia whitei* extract warrants serious consideration and further development as an eco-friendly and effective tool for managing these devastating tomato diseases. It is therefore imperative to investigate the efficacy of *M. whitei* bio fungicides against *A. solani* and *P. infestans* in tomatoes production for a healthy ecosystem.

#### **1.4 General Objective**

To contribute to increased production of tomato through bio-management of early blight and late blight using *Mondia whitei*.

### 1.5 Specific Objectives

1. To determine the efficacy of *Mondia whitei* extract on *in vitro* growth of *Alternaria solani* and *Phytophthora infestans*.
2. To determine the secondary metabolites, present in *Mondia whitei* extracts.
3. To determine the efficacy of *Mondia whitei* on early blight and late blight in tomato.

### 1.6 Hypotheses

H<sub>01</sub>: *Mondia whitei* extracts have no efficacy on *Alternaria solani* and *Phytophthora infestans in vitro*.

H<sub>02</sub>: There are no varying secondary metabolites present in *Mondia whitei*.

H<sub>03</sub>: *Mondia whitei* extracts have no efficacy on early blight and late blight in tomato.

## CHAPTER TWO: LITERATURE REVIEW

### 2.1 Introduction

Securing sustainable tomato production necessitates effective management strategies for devastating fungal diseases such as early blight and late blight, which are major constraints causing substantial yield losses. The exploration of botanical alternatives, harnessing the inherent defense mechanisms encoded in plant bioactive compounds, presents a crucial strategy. This chapter reviews tomato crop cultivation, the key challenges in its cultivation, the scientific foundation of plant-derived bio fungicides, and specifically focuses on evaluating the *in vitro* and *in vivo* efficacy of these natural extracts against the target pathogens. A central theme is the investigation of *Mondia whitei*, an African medicinal plant renowned for its diverse bioactive profile, examining its potential as a novel and effective bio fungicide for tomato protection.

### 2.2 Tomato Production in Kenya

Tomato is a perennial plant in the nightshade family, with a weak, woody, densely hairy stem that often vines over neighboring plants (Campbell, 2025; Sarkar et al., 2022). According to Sarkar et al., (2022), tomatoes are warm-season crops that need well-drained soil, moderate to hot temperatures, and moderate to high humidity. Regardless of their growth stage, they are susceptible to frost (Ding et al., 2022). Determinate and indeterminate tomatoes are the two categories into which tomato plants can be divided according to their growth habits (Vallecillo Godoy et al., 2022). Indeterminate tomatoes keep growing and bearing fruit all season long, whereas determinate tomatoes reach a certain size, set all of their fruit at once, and then cease growing (Keleman, 2023). Proper

staking, especially for the indeterminate tomatoes and disease control are very crucial for a successful tomato crop production (Wubetie & Wubetu, 2025).

Small and medium-sized farmers in Kenya are the main producers of tomatoes. These tiny agricultural enterprises manage the majority of tomato production, while there are a few larger commercial farms (Chepchirchir et al., 2021). Kajiado, Kirinyaga, Narok, Machakos, and Kiambu are among the Kenyan counties that are well-known for producing a lot of tomatoes (Kones, 2024; Opiyo, 2022).

The best times to plant tomatoes in Kenya are March and August, with harvesting typically occurring from June to July and October to November (Muema, 2023). Nowadays, the plant is cultivated indoors or out, hydroponically or in soil, in the majority of the world's fertile regions (Campbell, 2025; Sarkar et al., 2022). Greenhouses are also used in the fields to protect the crops against high solar radiation and heavy rainfall that have the potential of destroying the vulnerable crop (Ateka et al., 2021). According to Ddamulira et al., (2021) it costs a farmer USD 309.0 to establish and manage an acres of land for tomato depending on various factors like land ownership, irrigation methods, and input costs.

### **2.3 Socio-Economic Importance of Tomatoes**

Tomato is one type of low-cost vegetable that is frequently used in meals (Goka et al., 2021). Families, gardeners, farmers, laborers, marketers, retailers, chefs, and other employees and services in the food and restaurant industries all depend on the harvest for their socioeconomic well-being (Nelson, 2008). Tomato offers a reliable source of employment and income generation to small- and medium-scale growers (Chepchirchir et al., 2021).

According to FAOSTAT (2022), Kenya, Africa, and the world produced approximately 0.66 million metric tons, 22.9 million metric tons, and 186.1 million metric tons of tomatoes annually, respectively. The vegetable fruit is the most produced horticultural food crop globally and in Kenya according to FAOSTAT, (2022). Furthermore, the crop accounts for 14% of Kenya's overall vegetable production and 7% of its total horticulture products (Ochilo et al., 2019). Smallholder farmers in Kenya produce the majority of tomatoes, primarily for financial gain (M. W. Mwangi et al., 2015). With \$1.98 million in exports and \$34.5k in imports, Kenya came in at number 58 in the world for tomato exports and 150th for tomato imports in 2022. Tomatoes were Kenya's 1041st most imported and 235th most exported product in the same year according to Warum et al., (2023). Economically, the crop is significant not only for its earnings but also for the money that farmers and other agricultural workers make locally from its production and the entire value chain (Singh et al., 2023).

According to Abu Haraira et al., (2022), tomatoes are an excellent dietary source of the antioxidant lycopene, which has been related to a lower risk of life-threatening conditions like cancer and heart attacks. Also, by removing reactive free radicals from cells, which can harm human DNA and other essential biological organelles, antioxidants slow down the aging process (Olufunmilayo et al., 2023). Additionally, tomatoes are great for liver health. In the body, tomatoes have a cleansing effect. Most likely, it is because tomatoes contain sulfur and chlorine (Xie et al., 2022). Tomato juice is also said to be a great energy drink that can help dialysis patients feel better. It also keep arteries from hardening consequently, lowering high blood pressure (Lal, 2021). Furthermore, tomatoes' lycopene

and vitamin E effectively prevent low-density lipoprotein (LDL) oxidation and because tomato juice contains a specific vitamin C, it can be used to treat sunburn (Lal, 2021).

#### **2.4 Constraints in Tomato Production**

The productivity, quality, and farmer profitability of tomato production are all greatly impacted by a number of interrelated constraints (Ddamulira et al., 2021). Land fragmentation and soil degradation, poor extension services and research linkage, post-harvest losses, market and marketing difficulties, high input costs and access, climate change and water scarcity, pests and diseases, and policy and infrastructure issues are just a few of these difficulties (Noopur et al., 2023; Nyalugwe et al., 2022; Wondim, 2021). Diseases and pests are the most destructive of these. *Tuta absoluta*, often known as the tomato leaf miner, is one of the most destructive insect pests that can cause yield losses of up to 80–100% if left unchecked. Aphids, thrips, spider mites, and whiteflies are additional pests (Muema, 2023).

Tomato originated in the Andes of South America, according to Panno et al., (2021). Because of intense selection and severe genetic bottlenecks that developed during evolution and domestication, the cultivated tomato has a low genetic diversity (Blanca et al., 2015; Bai & Lindhout, 2007). These factors make tomatoes more susceptible to a high incidence of disease, and over 200 diseases caused by various pathogens worldwide can damage them throughout production and the post-harvest period (V. K. Singh et al., 2017; King & Lively, 2012).

The production of fresh market and processed tomatoes is hampered by numerous diseases caused by fungi (*Alternaria solani*, *Botrytis cinerea*, *Cladosporium fulvum*, *Colletotrichum coccodes*, *Fusarium oxysporum*, *Fusarium clavum*, *Leveillula taurica*, *Oidium lycopersici*,

*Pseudoidium neolycopersici*, *Pyrenochaeta lycopersici*, *Rhizoctonia solani*, *Septoria lycopersici*, *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, *Stemphylium* spp., *Verticillium dahlia*, *Phytophthora infestans*, *Phytophthora nicotianae*, *Phytophthora cryptogea*, *Pythium debaryanum*, *Pythium sylvaticum*) (Gilardi et al., 2021), bacteria (*Clavibacter michiganensis* subsp. *michiganensis*, *Erwinia carotovora* subsp. *carotovora*, *Pseudomonas corrugata*, *Pseudomonas mediterranea*, *Pseudomonas syringae* pv. *tomato*, *Ralstonia solanacearum*, *Xanthomonas axonopodis* pv. *vesicatoria*) (Infantino & Loreti, 2010), phytoplasmas (*Candidatus Phytoplasma solani*) (Barba et al., 2010), and viruses (Alfalfa mosaic virus (AMV), Chickpea chlorotic dwarf virus (CpCDV), Cucumber mosaic virus (CMV), Eggplant mottled dwarf virus (EMDV), Parietaria mottle virus (PMoV), Pelargonium zonate spot virus (PZSV), Pepino mosaic virus (PepMV), Potato virus Y (PVY), Southern tomato virus (STV), Tobacco mosaic virus (TMV), Tomato brown rugose fruit virus (ToBRFV), Tomato chlorosis virus (ToCV), Tomato infectious chlorosis virus (TICV), Tomato leaf curl New Delhi virus (ToLCNDV), Tomato mosaic virus (ToMV), Tomato spotted wilt virus (TSWV), Tomato torrado virus (ToTV), Tomato yellow leaf curl virus (TYLCV), Tomato yellow leaf curl Sardinia virus (TYLCSV)) (Zaagueri et al., 2019) (Davino et al., 2018) and viroids Potato spindle tuber viroid (PSTVd), Tomato apical stunt viroid (TASVd) (Barba et al., 2010).

#### 2.4.1 Early Blight (*Alternaria solani*)

According to Matić et al., (2020), *Alternaria* species are significant plant and animal pathogens and are classified as asexual fungus. One of these, *A. solani*, is responsible for the tomato early blight disease, a significant foliar disease (Bessadat et al., 2017). On leaves, this necrotrophic fungus typically produces black, concentric patches that are

frequently encircled by a chlorotic halo. Necrotic lesions may be seen on flowers and stems as the illness progresses, and defoliation starts with older leaves and progresses to younger ones (Strandberg, n.d.). Necrotic lesions that spread out from the peduncle insertion point can also be seen in berries. The disease can cause total defoliation, which has a significant impact on the plant's photosynthetic efficiency as well as the quantity and caliber of its fruit (Lawrence et al., 2000). Frequent rainfall, high humidity, and medium-to-high temperatures (24–29 °C) are favorable for the growth and spread of *A. solani* (Chaerani & Voorrips, 2006); in cases of severe infection, yield loss can reach 80% (Adhikari et al., 2017).

All plant components above ground become severely infected by *A. solani*. As plants age, the infection spreads upward from the older leaves (Attia et al., 2022). On the older leaves, tiny dark necrotic lesions first develop. As the lesions grow, they form concentric rings and are frequently encircled by a yellowish zone (Gulzar et al., 2018). In lesions larger than 10 mm in diameter, concentric bands of dark pigmentation are common. This lesion, known as a "bullseye", is a prime example of early blight (V. Kumar et al., 2017). Premature defoliation also occurs that weakens the plants and set the fruit to injury from sunscald (Ojiewo et al., 2010). Partial girdling of the stems of seedlings occurs and when the stem is completely girdled by the lesion, plant death occurs. The infected fruits also drop before attaining maturity and the fruits that reaches to maturity became unmarketable (Gulzar et al., 2018).

Morphologically, *A. solani* yields conidiophores that are straight or somewhat flexuous, brown to olivaceous brown in color, and on these conidiophores, single, oblong, or ellipsoidal conidia are formed (Roopa et al., 2016).

As a result of the emergence of resistance strains, especially against respiration inhibitor fungicides (succinate dehydrogenase inhibitors (SDHIs) and quinone outside inhibitors (QoIs), it is essential to rotate fungicides with diverse modes of action for the effective management of this disease (Malandrakis et al., 2018). Preventive agronomic methods, such as selecting resistant cultivars, using healthy seeds or transplant material, using forecasting models, controlling above-ground plant humidity through soil-directed irrigation, rotating crops, getting rid of weeds and plant residues, and increasing plant vigor through proper fertilization management, are additional strategies to effectively manage the early blight disease (Small et al., 2015).

#### 2.4.2 Late Blight (*Phytophthora infestans*)

When tomatoes are grown in damp, chilly, rainy, and humid conditions, they are susceptible to the deadly disease known as late blight, which is brought on by *P. infestans* (Mizubuti et al., 2007). This plant pathogen is likely the most significant pathogen of potatoes and tomatoes globally, and it is one of the most infamous and destructive organisms in modern human agricultural history. It caused the tragic Irish potato (*Solanum tuberosum* L.) famine in the 1840s (Nelson, 2008).

*Phytophthora infestans* is considered a fungus-like creature rather than a real fungus (Cottyn et al., 2023). As members of the kingdom *Chromista* (*Stramenopiles* or *Straminopiles*), this pathogen is presently categorized as an *oomycete* (Schoina et al., 2021). *Saprolegniales* and *Peronosporales* are the two orders to which *oomycetes* belong. *Phytophthora* species and several other significant plant-pathogenic genera, such as the genus *Pythium*, are found in the order *Peronosporales* (Hashem et al., 2022). Morphologically, *P. infestans* isolates exhibit a striated pattern and fluffy, cottony growth

as their phenotypic feature. The pathogen's sporangia are lemon-shaped and form at the end of these sporangiophores (Raza et al., 2022).

Although *P. infestans* is found all throughout the world, the most severe outbreaks happen in places that frequently experience chilly, humid weather (Mizubuti et al., 2007). Solanaceous crops, such as potatoes, nightshade (*Solanum nigrum* L.), and tomatoes, are the primary hosts of *P. infestans* (Guha Roy et al., 2021). *Phytophthora infestans* can cause losses of up to 100% in unmanaged fields and infects all aboveground sections of vulnerable plants at any stage of plant development (Amin et al., 2013). The disease causes extensive defoliation, reduced photosynthetic leaf area, loss of plant vigor, plant death, loss of fruits and reproductive capacity, and loss of seeds (Nelson, 2008).

On the leaves of tomatoes large sections of the leaf may be covered by lesions, which start as undefined, water-soaked patches and quickly grow into pale green to brownish-black lesions (Maurya et al., 2022). Lesions on the leaf's abaxial surface may develop a gray to white moldy growth during rainy weather; this should not be confused with powdery mildew disease (Nelson, 2008). In humid weather, a ring of the pathogen's moldy development is frequently seen on the undersides of bigger lesions. The leaves turn yellow and then brown, curl, shrivel, and eventually die as the disease worsens (Mugao, 2021; Stevenson et al., 2001). Regarding tomato fruits, on green fruit, dark, oily patches appear in damp weather, a thin layer of white mycelium may also be visible (Khan et al., 2024).

According to Nelson, ( 2008) farmers are expected to use the following strategies among others to manage the pathogen effectively: Choosing a tomato variety that matures quickly, such as short-season or early-bearing varieties, growing tomato in glasshouses or other areas with controlled humidity and covered plants that are shielded from rain,

providing sufficient and additional plant nourishment to ensure rapid and strong growth of tomato plants, keeping tomato stems and branches off the ground, by staking up tomato plants, especially indeterminate varieties, growing tomato cultivars that are resistant to late blight, maintaining proper crop hygiene by routinely checking the plants for signs of late blight disease and removing any diseased material from the farm as soon as possible, practicing crop rotation, irrigating the crops early in the day, this reduces the amount of time that leaves are wet and lowers relative humidity by allowing the soil and foliage to dry out before nightfall and finally applying fungicides as needed.

Farmers spend heavily on pesticides in controlling these two tomato diseases, but inefficacy due to resistance, counterfeits, or misapplication and rising costs are major problems. Over-reliance on chemicals also raises environmental and health concerns (D. P. Singh, 2023). It is therefore imperative to have a bio-control solution which is less expensive, effective and environmental friendly.

## 2.5 “Mukhombero” (*Mondia whitei*)

*Mondia whitei* belongs to the milk-weed family; it grows in forests, bush lands and wastelands and is a deciduous canopy-climbing liane (Shitanda et al., 2007). In Africa's humid tropical and subtropical woods, bitypic *Mondia* skeels can be found. Both species are lianes with noticeable and well-exposed gynostemium, huge panicles, fringe-like interpetiolar stipular ridges, and brownish, reddish to purplish flowers (Venter et al., 2009). *Mondia whitei* is a perennial woody climber reaching 3–6 m high with twining stems which exudes white latex when cut (Aremu et al., 2011). The plant's skeels belongs to the subfamily *periplocoideae* of the *apocynaceae* (formerly the *asclepiadaceae*). Because the original name was a homonym for a genus of *ericaceous* plants, the species was later

moved to *Mondia* from its original placement in the genus *Chlorocodon*. The genus *Mondia* skeels consists of two species; *M. whitei* and *Mondia ecornuta* (N.E.Br.) Bullock (Aremu et al., 2011). The form and quantity of corona lobes are what differentiate the two species, despite their similar morphological characteristics (Venter et al., 2009).

The generic name for *M. whitei* was taken from the Zulu word, White's ginger, tonic root, or “umondi/mundi”. It's interesting to note that the species was transferred to the Royal botanical gardens in Kew, England, for documentation, and that the specific epithet "whitei" was given in honor of the collector Mr. A.S. White of Fundisweni, Natal (Ross, 1978). In Kenya, the plant is popularly known as “Mukhombero”.

#### 2.5.1 Uses of *Mondia whitei*

*Mondia whitei* is a multipurpose plant with numerous applications that have been documented or verbally down to succeeding generations. The plant is used by several African ethnic groups as a moderate laxative, to relieve nausea and stomach discomfort, and to cure bilharzia, fever, and sexual dysfunction (Crouch et al., 1998; Gelfand, 1985; Watt & Breyer-Brandwijk, 1962). In addition to historical records of *M. whitei*'s applications, ethnobotanical studies conducted more recently consistently reference the species' regional use; A root decoction of *M. whitei* has been shown to cure male infertility in Cameroon (Focho et al., 2009), eradicate worm infestation (anthelmintic) in Nigeria (Idu et al., 2010), fight malaria infection in Benin and Nigeria (Odugbemi et al., 2007; Hermans et al., 2004), and induce labor in Uganda (Ssegawa & Kasenene, 2007). According to a survey conducted in Kenya, *M. whitei* is used practically to treat a wide range of issues, including asthma, heart illness, stomach worms, ringworms, and skin conditions (McGeoch, 2004). Unspecified groups in South Africa also employ the species to help

adults who are stressed or tense (Wyk & Gericke, 2000). According to Stafford et al., (2008), *M. whitei* is used by South Africans as an aphrodisiac, to increase appetite, and to treat children's fits.

## **2.6 Plants Extracts as Disease Biocontrol *In Vitro***

It is believed that plants constitute an essential source for novel bioactive substances. Surprisingly though, little study has been conducted on the potential of therapeutic plants as a substantial source of novel treatment of plants' pathogens (Zanna et al., 2021). Research on plant extracts opens the door to the creation of natural bioactive compounds with applications in phytosanitary, along with the added advantages of a product that is both commercially and environmentally sound (Sales et al., 2016). The majority of the degradation in fruit and vegetables, tomatoes being one of them, is caused by phytopathogenic fungus (Agrios, 1991). The majority of these fungi target fruit in the fields, and they can live in the soil and in various plant cycles. They can also spread by the wind and spread through leaves, fruit, contaminated equipment, and any of their various spore forms (Cadena-Iñiguez & Arévalo-Galarza, 2008).

Scholars have evaluated natural substitutes for the management of diseases during agronomic activities and postharvest management because the use of agrochemicals has resulted in environmental problems and human toxicity. As a result, several bio-extracts have been found to exhibit antimicrobial activities in plants (Alengebawy et al., 2021).

The study conducted by (García-Ramírez et al., 2023) aimed to assess the *in vitro* inhibitory properties of black sapote fruit, neem oil extract, and cinnamon essential oil for fungus isolated from chayote fruit. After treating the extracts at 300, 350, and 400 parts per million (ppm) in petri dishes, the mycelial development of *Fusarium oxysporum* Schlecht.,

*Fusarium solani* Mart., *Goetrichum sp.*, and *Phytophthora capsici* L. was monitored for seven days. The percentage of suppression of mycelial growth per day was then computed. The investigation's findings demonstrated that cinnamon oil possesses fungicidal properties at all concentrations. Ultimately, 21.9–28.6% of the growth of all fungi was suppressed by the 400-ppm extract of black sapote. At 400 ppm, neem oil reduced the development of *F. oxysporum* by 27.8% and *F. solani* by 42.3%. It suppressed *Goetrichum sp.* mycelial growth by 20.9% and *P. capsici* mycelial growth by 53.3% at 350 ppm (García-Ramírez et al., 2023).

The effectiveness of extracts from *Citrullus colocynthis* (L.) Schrad., *Eucalyptus camaldulensis* Dehnh., and *Nerium oleander* L. against tomato bacterial spot disease was evaluated in a different investigation. The antibacterial efficacy of bio-extracts, extracted with water and ethanol was evaluated *in vitro* against an isolate of *Xanthomonas axonopodis* Dowson. *Eucalyptus camaldulensis* and *Citrullus colocynthis* showed the strongest antibacterial activity, respectively. The pathogen's development *in vitro* was affected differently by the plant extracts. When *C. colocynthis* and *N. oleander* extracts were combined at 15% concentration water (0.50 mm) and ethanol (0.59 mm) it showed the most reduction. The least level of decrease was found when *E. camaldulensis* extracts were mixed at a 15% concentration (ethanol (0.44 mm) and water (0.51)). In general, when the concentration rose from 10% to 15%, the inhibition of pathogen development increased (Abo-Elyousr et al., 2020).

Another investigation was conducted on the antimicrobial qualities and mechanism of action of several plant extracts against microorganisms that cause food spoilage and food pathogens, including *Staphylococcus aureus* R. and *Escherichia coli* M. Plant extracts

were found to have an impact on the membrane potential and cytoplasmic pH of both Gram-negative and Gram-positive *S. aureus* and *E. coli* strains. When plant extracts were added, a significant drop in cytoplasmic pH ( $P \leq 0.05$ ) was seen. The strains of *S. aureus* and *E. coli* were most significantly affected by ethanolic extracts of clove and water extracts of thyme; water extracts of rosemary, on the other hand, had the least effect (Gonelimali et al., 2018). The pH variations showed that the bacterial cell membrane had been damaged (Sánchez et al., 2010).

In a study various doses of plant extracts demonstrated encouraging potential for regulating *Alternaria solani* development *in vitro*. This was the finding of a study by Nahunnaro & Bayaso, (2012) that assessed the impact of two plant extracts from *Chromolaena odorata* L. and *Ricinus communis* L., on the management of *Alternaria solani*. The findings of radial growth showed that the lowest radial growth rates were provided by *R. communis* at 100% concentration, which were 1.43 cm, 2.00 cm, and 2.72 cm at 24, 48, and 72 hours, respectively.

The goal of a study by Zanna et al., (2021), was to investigate the antifungal properties of a few chosen medicinal herbs *in vitro*. The antifungal qualities of seven medicinal herbs against three human pathogenic fungi were examined. Polarity-based solvent extraction was used to create methanolic extracts of the plant components. The punch well method was used to investigate the antifungal qualities. The methanolic extract of *Boswellia dalzielii* Hutch. leaves (19.43 mm) against *Trichophyton rubrum* (Castell.) and *Ocimum gratissimum* L. leaves (24.3 mm) against *Trichophyton mentagrophytes* (Robin) showed strong antifungal activity. The methanolic root extract (39.55 mm) of *Candida alata* B.

inhibited *C. albicans*. The lowest MIC value (312.5 µg.ml<sup>-1</sup>) against *C. albicans* was shown by the methanolic root extract of *C. alata*.

Dzoyem et al., (2014) looked into the *in vitro* and in vivo antifungal potentials of 23 plant extracts from spices utilized in Cameroonian dietary spices, including *M. whitei*. The outcomes showed that every extract has both fungicidal and fungistatic properties. Seven extracts (30%) demonstrated moderate to high antifungal activities, inhibiting the growth of the bacteria at dosages ranging from 0.048 to 0.39 mg/ml. The most potent antifungal action was demonstrated by the *Olax subscorpioidea* L. extract, particularly against *Candida tropicalis* Cast. and *Candida albicans* C. (MIC values of 0.047 and 0.097 mg/mL, respectively). The number of colony forming units per milliliter (cfu/ml) of *C. albicans* cells in the blood decreased below the detection limit (100 cfu/ml) when *O. subscorpioidea* extract (2 g/kg of body weight) was given orally to artificially infected rats, although there was only a slight decrease in the kidney. *Aspergillus flavus* L. and *Penicillium spp.* mycelia growth were significantly altered by the essential oil extracted from *M. whitei* roots. *Penicillium spp.* and *A. flavus* conidia germination were markedly suppressed by the essential oil derived from *M. whitei* (Youassi et al., 2019).

Furthermore, in a study by Mayunzu et al., (2012), the powdered root bark of *M. whitei* inhibited growth of several human bacteria and fungi, such as *Aspergillus niger* Van., *Bacillus subtilis* Coh., *Candida albicans* (C.-P. Robin), *Escherichia coli* M., and *Staphylococcus aureus* R. The root bark powder's water, methanol, and ethanol extracts were tested for its ability to suppress the aforementioned bacterial and fungal species in vitro. The water extract demonstrated the best activity against *S. aureus*, but it did not demonstrate significant activity against other species. The ethanol extract, on the other

hand, MIC values of 14.65 µg/ml and 58.59 µg/ml, respectively, demonstrated significant activity against *A. niger* and *C. albicans*. *Aspergillus niger* and *E. coli* had high MIC values of less than 14.65 and 14.7 µg/ml for methanol extract, respectively (Mayunzu et al., 2012).

In the review above, on plants' extracts as disease biocontrol, several plant extracts have been effectively employed to combat human infections, food pathogens, and phytopathogens—microorganisms that cause food to decay. Plant extracts have a clear chance to be used as environmentally acceptable bio pesticides. According to this review, *M. white* is also employed in ethnobotany to treat illnesses and stop the spread of infections in humans. This indicates a gap in the use of *M. whitei* as an economical and environmentally friendly alternative for controlling tomato blights, which are economically significant diseases in tomato production, among farmers. It also offers the possibility of using the plant's extract to control diseases of other plants. Therefore, the research discussed above support the potential use of plants to control diseases caused by dangerous pathogens and have provided important leads for the development of new plant-based antimicrobial medicines.

## **2.7 Plants Secondary Metabolites**

As sessile organisms, plants produce a variety of secondary metabolites with significant physiological and ecological consequences to protect themselves from external abiotic and biotic stresses (Guerriero et al., 2018). Because they are toxic to herbivores and microbes, and repellent to them, secondary metabolites in plants play a significant role in defense against microbial diseases and predators (Naboulsi et al., 2018). Terpenoids, phenolic

compounds, alkaloids, and chemicals containing sulfur are the four main types into which plant secondary metabolites can be divided (Guerriero et al., 2018).

Biologically active substances, secondary plant metabolites are organic chemicals that are produced from primary metabolites (Šernaitė, 2017). Terpenes or terpenoids are the most abundant and diverse class of naturally occurring chemicals. Mono, di, tri, tetra, and sesquiterpenes are the groups into which they are divided according to isoprene units (5-carbon base) (Eslahi et al., 2017). Plants are the primary source of secondary metabolites, which are the primary component of plant essential oils. Terpenes are an important and diverse class of naturally occurring chemicals that offer medicinal benefits to living creatures. Terpenes are commonly found in plants such as tea, thyme, citrus fruits, Spanish sage, and cannabis. Terpenes have a variety of therapeutic applications, but its antiplasmodial effect is particularly noteworthy because it functions similarly to the widely used antimalarial medication chloroquine (Cox-Georgian et al., 2019).

Among the many different types of natural goods are plant steroids. They are produced biosynthetically from S-squalene-2,3-epoxide by the acetate-mevalonate route. Numerous biological functions of steroids have been reported (Gunaherath & Gunatilaka, 2006). Alkaloids are beneficial phytochemicals that can also be poisonous to one another. They are typically found in poisonous plants. These substances work well against fungus, bacteria, and parasites (Ain et al., 2016).

A phenolic compound group is made up of several substances that have at least one hydroxyl substituent and an aromatic ring. Tannins, flavonoids and phenolic acids belong to this group of phytochemicals (Šernaitė, 2017). Phenolic compounds have been proven to show antioxidant and antimicrobial activities. Additionally, isolated groups of phenolic

compounds (tannins, phenolic acids and flavonoids) are known to have fungicidal properties (Babenko et al., 2019; Lin et al., 2016; Maqsood et al., 2014).

In one work, yellow senescent mangrove leaves were used to isolate secondary metabolites in broth ethyl acetate extracts (BEAE) and mycelial ethyl acetate extracts (MEAE) of *Salisapilia tartarea*. These extracts were then tested using thin layer chromatography (TLC). Both BEAE and MEAE included anthrones, flavonoids, phenols, triterpenes, and anthraquinones, according to the TLC. While not found in MEAE, coumarins were found in BEAE. MEAE contains lower total phenolic and flavonoid contents than BEAE, as determined by quantifying the extracts' total phenolic and total flavonoid contents in terms of gallic acid and quercetin equivalents, respectively (Marcelo et al., 2018).

Zanna et al.'s study from 2021 looked at a few medicinal plants to identify the large class of bioactive chemicals that underlie their effects. They looked into seven therapeutic plants. The presence of alkaloids, anthraquinones, flavonoids, and tannins was revealed by phytochemical analyses of the active extracts. Significant anti-fungal capabilities were found in the methanolic extract of the leaves of *B. dalzielii* (19.43 mm) against *T. rubrum* and *O. gratissimum* (24.3 mm) against *T. mentagrophyte*, indicating that the plant extracts were also effective against the human fungi that were tested. The methanolic root extract (39.55 mm) of *Candida alata* inhibited *Candida albicans*. The lowest MIC value (312.5  $\mu\text{g}\cdot\text{ml}^{-1}$ ) against *C. albicans* was shown by the methanolic root extract of *C. alata*.

The results for the qualitative and quantitative analysis of *M. whitei* extracts from a study by (Onohuean et al., 2022) were as follows; Phenolic compounds, flavonoids, tannins, triterpenoids, reducing sugars, and cardiac glycoside were present in any of the aqueous, ethanolic, and chloroform fractions of the extract. While saponins and terpenoids were

only present in aqueous and ethanolic fractions, alkaloids were only present in chloroform fractions, and then steroids and anthraquinones were conspicuously absent in all three fractions of the extracts. Interestingly, alkaloid was only present in the chloroform fractions, whereas on the quantitative analysis, the aqueous fraction (AQ) of *M. whitei* extract produced the highest amount of total phenol, followed by the ethanolic fraction (ET), and the ethanolic fraction gave the maximum quantity of total proanthocyanidin, flavonoids, and flavonols followed by chloroform fraction (CH).

According to this review, secondary metabolites have been found to exist in plants extracts and have demonstrated either synergistic or single-acting antimicrobial activity. Also, both qualitative and quantitative analyses of *M. whitei* extracts have revealed the presence of the same active secondary metabolites. Therefore, there is a clear chance that *M. whitei* extracts could be employed as a bio pesticide. This opens up the possibility of using *M. whitei's* extract to manage other plant diseases, particularly the pathogens that cause tomato blights, which is the focus of this work. Additionally, the above analysis supports the potential use of plants extracts to control diseases, and has provided important leads for the development of new plant-based antimicrobial pesticide.

## **2.8 Plants Extracts as Disease Biocontrol *In vivo*.**

Plant extracts may have antifungal properties that are superior to some commercially available synthetic fungicides (Choudhury et al., 2018). A study was conducted to assess the efficacy of bio-extracts of *Nerium oleander*, *Eucalyptus chamadulonsis*, and *Citrullus colocynthis* against tomato bacterial spot disease and to investigate the induction of resistance by tomato in order to support a sustainable management system. Using isolates of *Xanthomonas axonopodis*, the *in vivo* antibacterial activity of *N. oleander* and *E.*

*chamadulonsis* water and ethanol bio-extracts was assessed. The ethanol extracts of *N. oleander* and *E. chamadulonsis* were more effective than water extracts in reducing the number of pathogens on tomato leaves. When tomato plants were treated with plant extracts at a 15% (v/v) concentration in a greenhouse, the severity of the disease was much decreased, and the "*Super Marmande*" tomato variety's shoot weight increased (Abo-Elyousr et al., 2020).

The results also indicated that applying 15% concentration of plant extracts reduced the number of bacteria in tomato leaves when compared to the infected control. *C. colocynthis* had the greatest reduction in colony forming units (CFU) per gram of leaf tissue, while the ethanol extracts of *N. oleander* (5.0 CFU/g) and *E. chamadulonsis* (5.1 CFU/g) differed little from each other. The least amount of growth was seen in the aqueous extracts of *E. chamadulonsis* (7.7 CFU/g) and *N. oleander* (6.2 CFU/g), which were similar. The findings presupposed that the administration of plant extracts decreased the amount of bacterial infections by making the pathogen more hazardous within its cytoplasm (Abo-Elyousr et al., 2020).

A study on the antifungal properties of the plant against the mold associated with yams (*Dioscorea rotundata* Poir.) that causes tuber rot disease found that the concentration of *M. whitei* oil boosted the prevention of yam tuber rot necrosis. In the preventative test, *Aspergillus flavus* showed a 47.41% suppression of necrosis at 2500 µl/l, while *Penicillium spp.* showed a 31.8 % inhibition of necrosis at 2625 µl/l. In the curative test, 72% suppression of *A. flavus* necrosis was seen at 2500 µl/l. Conversely, at 2625 µl/l, *Penicillium spp.* produced 43.17% of the necrosis inhibition (Youassi et al., 2019).

In a different study, the following plant extracts were tested to see if they could shield wheat crops from the *Puccinia triticina* Erikss. pathogen that causes leaf rust disease: Lantana, henna, pomegranate, chinaberry, and acalypha. Compared to the untreated control, which displayed an average coefficient of infection of 75.00, the plant extracts dramatically decreased it to between 7.50 and 20.00. The most efficient plant extract was found to be lantana, with an efficiency of 88.88%, which was comparable to the fungicide diniconazole, which had an efficiency of 89.92%. Second place went to henna extract (80.00%), then chinaberry (76.00%), acalypha (72.0%), and pomegranate (68.0%) (Draz et al., 2019).

An investigation on the impact of applying different extracts from medicinal herbs on the occurrence of mosaic disease on a plantation of *Capsicum annuum* L. caused by infection with the Cucumber Mosaic Virus (CMV). The administration of plant extracts decreased the severity of the CMV-caused illness, according to the results. The results of the experiment showed that plant extracts from *Datura stramonium* and *Annona muricata* were the most successful in reducing the severity of the virus-caused sickness, with respective percentages of 8.33% and 1.42%. These percentages were significantly higher than those of the control and several other treatments. While it was discovered that they differ very little from *Azadiracta indica* (50%) and control (50%) and not at all from *Piper bitle* (15.27%), *Cymbopogon citrates* (12.5%), and *Curcuma domestica* (24.98%) (Hamidson et al., 2018).

In this review, a number of plant extracts have been successfully used to fight phytopathogens, which cause plant diseases. The potential for using plant extracts as bio pesticides is therefore obvious. This review reveals that *M. white* is also used in

ethnobotany to heal ailments and prevent the spread of infections in people. This suggests that farmers can use *M. whitei* as a cost-effective and sustainable substitute for managing tomato pathogens including blights, which are economically important diseases in tomato production.

## CHAPTER THREE: MATERIALS AND METHODS

### 3.1 Study Location

The Masinde Muliro University of Science and Technology (MMUST) microbiology lab and university farm greenhouse, respectively, were the sites for the *in vitro* and *in vivo* research. The *in vitro* experiment was conducted in January and February of 2023, while the *in vivo* trial was conducted as from March to June of the same year.

Masinde Muliro University of Science and Technology is located in the western region of Kenya, Kakamega County, Lurambi Sub-County, with GPRS coordinates of 0.28885170727398324 N, 34.76370529438328 E (Google Maps, 2024). Over the year the temperature typically varies from 14°C to 29°C with diurnal temperature ranges for afternoon averages at around 26°C, while nights average at around 14°C and 78% average relative humidity. The town receives an average annual rainfall ranging from 1280.1 mm to 2214.1 mm (WMO, 2023). The town has varied soil types with varying degrees of fertility. Dark brown sandy soils are found in the northern, central and southern parts while yellow-red loamy soils are found in the western and north-western parts. The southern and eastern parts are mainly made up of dark, red friable soils (Khayesi, 1993).

### 3.2 Extraction of *Mondia whitei* Plant Crude Extract

*Mondia whitei* plant roots were harvested from Kakamega forest (0.2913° N, 34.8565° E). A total of 5 Kgs of fresh roots were manually harvested using a sterilized hand hoe and a knife. The roots were then packed into a clean sample bag and transported to the MMUST Microbiology laboratory immediately.

After cleaning with flowing tap water, the root samples were rinsed with sterile distilled water. After being cut off from the vascular cambium, the root bark was allowed to air dry for eight weeks at room temperature in a shaded area before being ground into a fine powder. Using a modified procedure by (Kritzinger, 2006), crude extracts from samples of roots of the *M. whitei* plant were extracted. Extractions of the bio-extracts were performed on 900 g of the finely milled material. The extraction on *M. whitei* powder was performed using maceration technique by soaking in absolute dichloromethane (DCM), Ethyl acetate (EtOA) and Methanol (MeOH) solvents for 72 hours at a ratio of 1:10 (w/v). The mixtures were then properly covered with aluminum foil and labeled. An orbital shaker was used to shake the mixture at a speed between 100-300 rpm for 6 hours. The solvents were then filtered using the process of vacuum filtration with a vacuum pump.

Thereafter, the filtrates obtained were concentrated in a vacuum at each solvent respective boiling points (DCM-39.6 °C, EtOA- 77.1 °C and MeOH- 64.7 °C) using a rotary evaporator bio-based equipment (EYELA Digital water bath SB-1000-Oasis Scientific, Inc.). The *M. whitei* extracts were then kept chilled at 4°C in a refrigerator until they were needed.

### **3.3 Preparation of a Working Solution from a Stock Solution**

Stock solutions with concentrations of 100% were prepared from each solvent extracts using Dimethyl sulfoxide (DMSO), thereafter a working solution preparation formula;  $C_1V_1 = C_2V_2$  by Bassa & Uppu, (2022) was used to prepared working solutions at 2.5 %, 10% and 20% of each of the solvent extract.

### 3.4 *In vitro* Efficacy Trial of *Mondia whitei* Extracts against *Alternaria solani* and *Phytophthora infestans*

#### 3.4.1 Experimental Design

A completely randomized design (CRD) was used to set up the laboratory experiment, with three replicates and four treatments; two different concentration levels of *M. whitei*-extract, and positive and negative controls were fungicide (ridomil gold) and blank, respectively (**Table 3.1**).

**Table 3.1:** Summary table of *Mondia whitei* treatments

Treatments	
10% <i>Mondia whitei</i>	T1
20% <i>Mondia whitei</i>	T2
Fungicide-ridomil Gold 0.25% (+ control)	T4
Blank (- Control)	T5

#### 3.4.2 Pathogen Isolation of *Alternaria solani* and *Phytophthora infestans*

Samples of tomato plants exhibiting symptoms of both early blight (*A. solani*) and late blight (*P. infestans*) were gathered from a farm located in Lurambi Sub-County. The samples were surface disinfected in 70% alcohol (MeOH) for one minute then immediately rinsed three times in different beakers containing sterilized distilled water, then cultured on an autoclaved potato dextrose agar (PDA) media, in petri dishes under continuous ultraviolet light (laminar flow). The PDA was amended with streptomycin antibiotic at a concentration of 2 mg/3 ml of distilled water to hinder proliferation of opportunistic microbes like bacteria. The cultures were then incubated for seventy-two hours at 28°C.

Then using a stereomicroscope (Model BS-3090-BestScope), *A. solani* and *P. infestans* cultures were identified morphologically; *Alternaria solani* yielded conidiophores that are straight or somewhat flexuous, brown to olivaceous brown in color, and on these conidiophores, single, oblong, or ellipsoidal conidia were formed. *Phytophthora infestans* isolates exhibited a striated pattern and fluffy, cottony growth as their phenotypic feature. The pathogen's sporangia were lemon-shaped and formed at the end of those sporangiophores. The pathogens' culture was sub cultured, and maintained at 4°C in a refrigerator.

### **3.4.3 Pathogen Inoculation and Inhibition of Fungi Mycelium Growth**

Pour plate method was used in screening *M. whitei* extracts, for their fungicidal property. For each pathogen, mycelia growth was evaluated in 60mm petri dishes containing PDA medium that has been amended with either 10% or 20% aqueous extracts from each solvent extracts of *M. whitei*, 0.25% ridomil and non-treated control. Using a sterile cork borer, 5 mm diameter fungal plugs of *A. solani* and *P. infestans* from seven-day-old pure fungal cultures were positioned in the center of the petri plates. After that, the infected petri dishes were then wrapped with parafilm and kept in an incubator at 28°C for seven days. Observations on growth of the fungus were recorded at three and seven days after inoculation (DAI). Mycelial growth was observed as an indicator of the antifungal activity of each extract.

### **3.5 Phytochemical Screening of *Mondia whitei* Extracts**

The presence or lack of phytochemicals such as saponins, phenols, flavonoids, tannins, alkaloids, and glucosides in each plant extract was examined using a number of common

laboratory qualitative procedures in accordance with the protocol established by (Harborne, 1998).

### **3.5.1 Test for Tannins**

After combining 10 millilitres of distilled water with 0.5 grams of each bio-extract, the mixture was filtered. Two millilitres of each filtrate were mixed with two drops of 1% ferric chloride solution. Tannins were present when a blue-black or blue-green precipitate appeared.

### **3.5.2 Test for Steroids and Terpenoids**

Two millilitres of chloroform, 0.2 g of each bio-extract, and two drops of concentrated  $H_2SO_4$  were added. After giving the mixture a good shake, it was left to stand for a while. The presence of triterpenoids were shown by yellow colour, but the presence of steroids confirmed by the presence of red colour in the lower layer.

### **3.5.3 Test for Glycosides**

Each plant extract in a test tube received 5 ml of concentrated  $H_2SO_4$ . Fehling solutions A and B were added, and the mixture was heated to boiling. After 15 minutes of heating on boiling water, the presence of glycosides was revealed by the appearance of brick-red precipitate.

### **3.5.4 Test for Anthraquinones**

Each bio-extract was weighed out at 0.2 g, shaken with 10 ml of benzene, and then filtered. The filtrate was then mixed with 0.5 ml of 1% ammonia solution and shaken again. The presence of anthraquinones was depicted by the ammoniacal (lower phase) exhibiting a pink red or violet appearance.

### **3.5.5 Test for Saponins**

One gram of each bio-extract, in 5 ml of distilled water was boiled then filtered. After adding 3 ml of distilled water to each filter, the mixture was rapidly shaken for approximately 5 minutes. Saponins were present on the persistence of foaming upon heat.

### **3.5.6 Test for Alkaloids**

After treating each plant bio-extract with three drops of Dragendroff's reagent, it was acidified with 1% HCL. The presence of alkaloids was detected by an orange-brown precipitate known as Dragenroff's.

### **3.5.7 Test for Terpenoids**

One milliliter of each bio-extract, 0.5 milliliter of acetic anhydride, and two drops of concentrated  $H_2SO_4$  were added. The presence of terpenoids was evidenced by a blue green precipitate.

### **3.5.8 Test for Carbohydrate (Fehling's test for reducing sugar)**

To two milliliters of each bio-extract in a test tube, a combination of 5 milliliters each of Fehling's solutions A and B was added. Two minutes were spent boiling the resulting mixture. An observable crimson precipitate of copper (I) oxide signified a successful test for sugar reduction.

### **3.5.9 Test for Flavonoids**

Each bio-extract was diluted with 5 drops of NaOH. Flavonoids were present in a yellow solution that became colorless upon the addition of concentrated HCL.

### **3.5.10 Test for Cardiac Glucosides**

A half a gram of each bio-extract was dissolved in 2 ml glacial acetic acid containing 1 drop of ferric chloride solution. This was underplayed with 2 ml of concentrated  $H_2SO_4$ ; a

brown ring formation at the inter-phase indicated the presence of deoxy sugar characteristics of cardiac glucosides.

### 3.5.11 Test for Volatile Oils

One gram of the bio-extract was agitated using 0.1M HCL and diluted with NaOH. The presence of volatile oils was shown by the development of white precipitate.

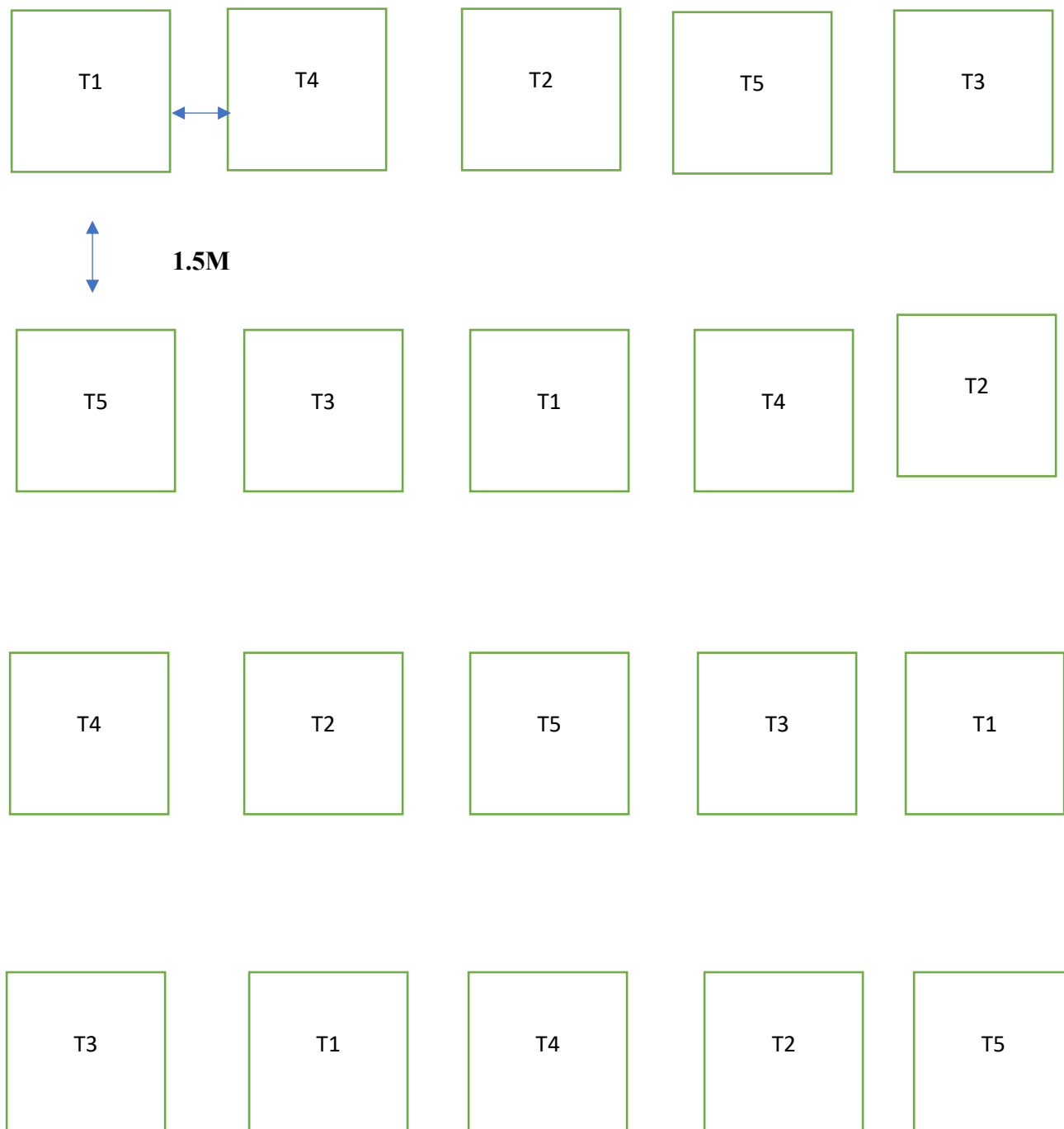
## 3.6 Effects of *Mondia whitei* Extracts on Early Blight and Late Blight

### 3.6.1 Experimental Design

The greenhouse experiment (in vivo) employed a Completely Randomized Design (CRD) with five treatments. The treatments were three levels of bio-extracts and a positive and negative control which was replicated four times giving a total of forty plots. The levels for *M. whitei* extract concentration were 2.5%, 10% and 20%, while the positive control was applied at the rate of 0.25% ridomil. Negative control treatment was blank application (Table 3.2). Plants were established in pots, with five plants per plot. There were two hundred plants, one hundred for each pathogen experiment.

**Table 3.2: *Mondia whitei* In vivo Treatments**

Treatments	
Blank (-Control)	T1
Fungicide-ridomil gold 0.25% (+ Control)	T2
2.5% <i>Mondia whitei</i>	T3
10% <i>Mondia whitei</i>	T4
20% <i>Mondia whitei</i>	T5



**Figure 3.1: Experimental design plots**

**Legend:**

**T:** Treatment, **R:** Replicate and **M:** Meters

### 3.6.2 Crop Establishment and Treatment Application

Top soil was collected from Kakamega forest at a depth of 10 cm and sterilized through solarization. Certified seeds; “*Moneymaker*” variety were grown in a nursery in the MMUST greenhouse on the sterilized soil. Two hundred sanitized polythene bags measuring 35 cm in height and 20 cm in top diameter were filled to a height of 30 cm with sterilized soil and supplemented with well-decomposed farm yard manure at a ratio of 3:1 of the soil and manure. Three weeks old healthy tomato seedlings were carefully selected then transplanted in the perforated black polythene bags with the sterile soil. After one week of recovering from transplanting stress, tomato plants in 100 bags were foliar inoculated with *A. solani* while, tomato plants in the remaining 100 bags were also foliar inoculated with *P. infestans*. The conidia were isolated using pour plate technique and thereafter by using hemocytometer (Superior Marienfeld, Germany) under the binocular light microscope (Labomed cxl mono) (400x) the conidia were adjusted to the concentration of  $1 \times 10^8$  and  $1 \times 10^9$  mL<sup>-1</sup> conidia and used for inoculation. The greenhouse was demarcated into two by a barrier polythene sheet to separate the two pathogens’ experiments.

The plants were then monitored for infection and disease progression. Thereafter, the different concentrations of bio-extract treatments were then sprayed on the tomato plants once the disease symptoms were first observed and after every one week until the symptoms subsided. Throughout the entire season, three spray dosages of the fungicide; ridomil were sprayed at 14-day intervals as per the manufacturer’s instructions. Utilizing a

calibrated hand sprayer set to discharge at a rate of 100 liters per hectare, the bio-extract solution was sprayed on the foliage of the tomato plants as part of the treatment process.

Normal agronomic and tomato crop maintenance practices were applied as was required.

### **3.7 Data Collection**

#### **3.7.1 In vitro Laboratory Experiment**

##### **1. Morphological Identification:**

Observing and recording the physical and morphological appearance of the *A. solani* and *P. infestans* under microscope

##### **2. Radial Growth:**

The diameter of the radial growth was measured at the third and seventh day using a Vernier caliper.

##### **3. Percent Inhibition:**

Growth inhibition zones were calculated using the formula:

$$\% \text{ Inhibition} = \frac{dc-dt}{dc} \times 100$$

Used by Taskeen-Un-Nisa et al., (2011). Where dt is the average diameter of fungal colonies growing in the presence of the plant extract, and dc is the average diameter of the fungal colony of the negative control.

#### **3.7.2 Greenhouse Assessment of *M. whitei* Extracts**

Collection of data commenced on the first day of application of treatments, and thereafter weekly. Plants were tagged for consistency in data collection. Data was collected from all plants.

- 1. Disease Incidence:** Disease incidence was assessed on all plants per treatment. Incidence of blight was assessed by counting the number of infected plants per treatment and expressed as percentage of total plants in the same treatment. Disease incidence was calculated using the following formula:

$$DI\% = \left( \frac{NI}{TNP} \right) \times 100$$

Where DI is Disease incidence, NI is Number of infected plants and TNP is total number of plants (Amin et al., 2013).

- 2. Disease Severity:** From each treatment twelve compound leaves of each plant was used to determine the disease severity. This was based on the proportion of the leaves infected, with the aid of the modified Horsfall–Barrat rating scale of 1–12 index (1 = 0%, 12 = 100% disease severity) (Choga, 2021).
- 3. Plant Height (cm):** The height of the plants was measured and recorded, from the ground to the tip using a tape measure.
- 4. Leaflet Size and Compound Leaves Number:** The length of leaflet in a selected and marked compound leaf was measured from the apex to the point where it joins the stalk and recorded per treatment. The number of the compound leaves was counted per plant and recorded per treatment.
- 5. Flower Trusses:** The number of flower trusses in each plant was counted and recorded per treatment.
- 6. Yield:**

The fruits were selected for harvesting by observing the external maturity index which was skin color. The fruits were harvested when the skin turned to pink color. They were harvested when the said color was observed on three quarter of the fruit. All the fruits were harvested including the damaged ones.

### **Total Yield**

- i) Fruits were collected from each plant in week four, five, six, seven and eight of the experiment and the numbers recorded.
- ii) The harvested fruits from each treatment were weighed using a weighing balance and the weight was recorded.

### **Marketable Yield**

- i) Fruits were collected from each treatment and a group of respondents who were already sensitized on quality/marketability attributes of tomato fruits were asked to grade their satisfaction levels with fruits, using indicator of a five-level of satisfaction Likert scale (Tansakul & Yenradee, 2020).

7. **Total Dry Biomass:** At the end of the season individual plants of each treatment were uprooted carefully, soil and debris washed off with running water and wrapped in a foil. The plants were then dried in an oven at a temperature of 70°C for 24 hours and weighed using a scale and the weight recorded.

### 3.8 Data analysis

Data collected was subjected to Analysis of Variance (ANOVA) and mean separated using the Tukey's Test at  $P \leq 0.05$ . The CRD model that was fitted for experiment is as shown below:

$$Y_{ijk} = \mu + \rho_i + \alpha_j + \epsilon_{ij}$$

$I = 1, 2, 3, 4$   $j = 1, 2, 3, 4, 5$  (for field/greenhouse) or  $1, 2, 3, 4$  (for lab)

Where;  $Y_{ijk}$  – tomato response

$\mu$  -grand mean,

$\rho_i$  - $i^{\text{th}}$  replication effect,

$\alpha_j$  –  $j^{\text{th}}$  treatment effect in the  $i^{\text{th}}$  replicate

$\epsilon_{ij}$  – random error component

## CHAPTER FOUR: RESULTS

### 4.1 Introduction

This chapter presents the experimental findings evaluating the efficacy of *M. whitei* extracts against early blight (*Alternaria solani*) and late blight (*Phytophthora infestans*), and their effects on the growth and yield of tomato. Following the methodology in chapter three, results include pathogen morphology and appearance, in vitro pathogen mycelium radial growth, quantitative data on disease incidences, severity, plant growth parameters (plant height, leaflet size, compound leaf number, and number of flower trusses), yield components (fruit number, weight, and total dry biomass), and comparisons to control treatments. Statistical significance was determined using ANOVA followed by post-hoc tests (Tukey's HSD), which were rigorously applied to determine the significance of observed differences between treatment. Data are presented objectively in tables, plattes and figures, forming the empirical basis for subsequent discussion and conclusions.

### 4.2 *In vitro* Efficacy Trial of *Mondia whitei* Extracts for *Alternaria solani* and *Phytophthora infestans* Antifungal Activities

Mycelial growth was not observed and this was an indicator of the antifungal activity of each solvent extract (10% and 20% *M. whitei*). There was no growth (0.0mm) on the petri dishes treated with both concentrations of *M. whitei* extracts of all the solvents (MeOH, DCM and EtOA) for both *A. solani* and *P. infestans* experiments. Petri dishes treated with positive control (0.25% ridomil) also exhibited no growth of the two pathogens in both experiments (**Table 4.1 and Plate 4.1**). A significantly high ( $p \leq 0.05$ ) mean radial growth of 29.5 mm, 29.4 mm and 31.1 mm were recorded in non-treated media (- control) for MeOH, DCM and EtOA extract solvents respectively, in *A. solani* experiment. While a

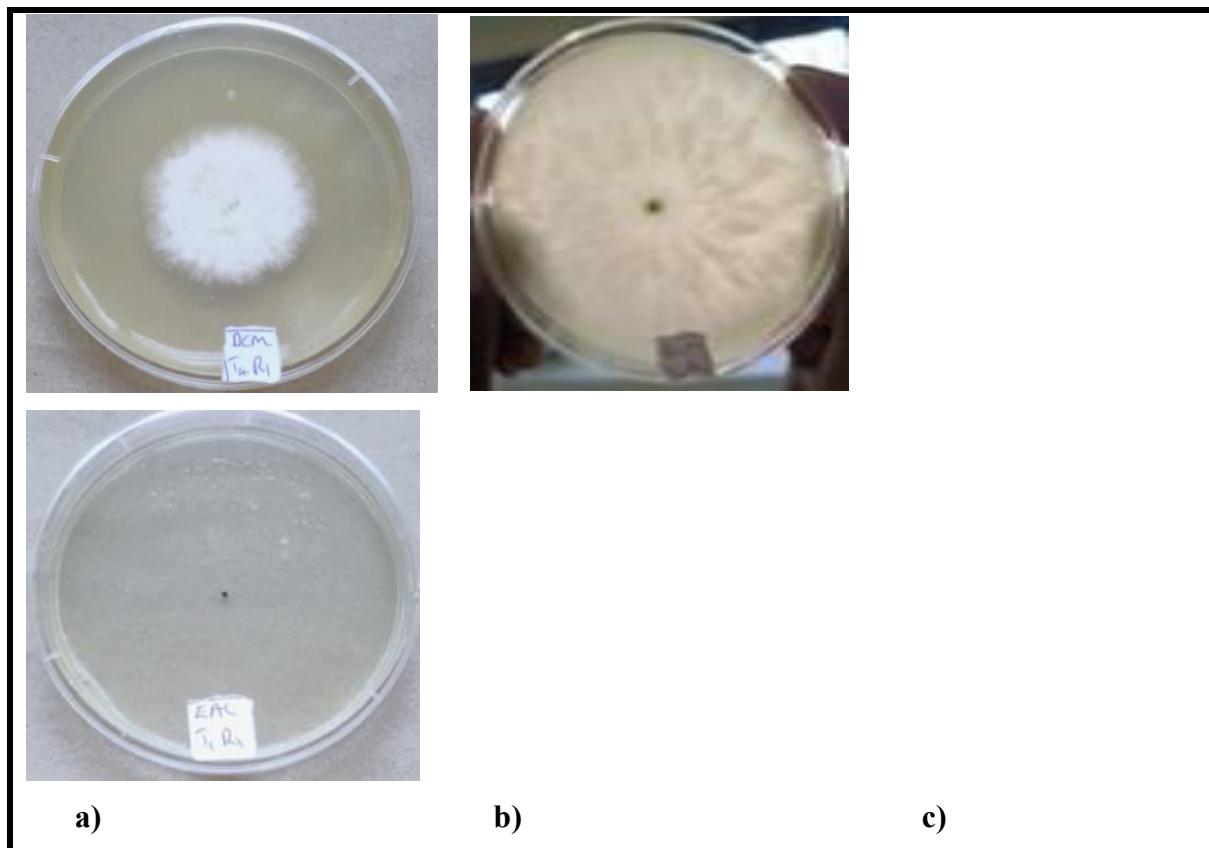
significantly high ( $p \leq 0.05$ ) mean radial growth of 60.0mm, 60.0mm and 60.0mm was recorded in non-treated media (- control) for MeOH, DCM and EtOA extract solvents respectively in *P. infestans* experiment. **Table 4.1** displays the specifics of the outcomes.

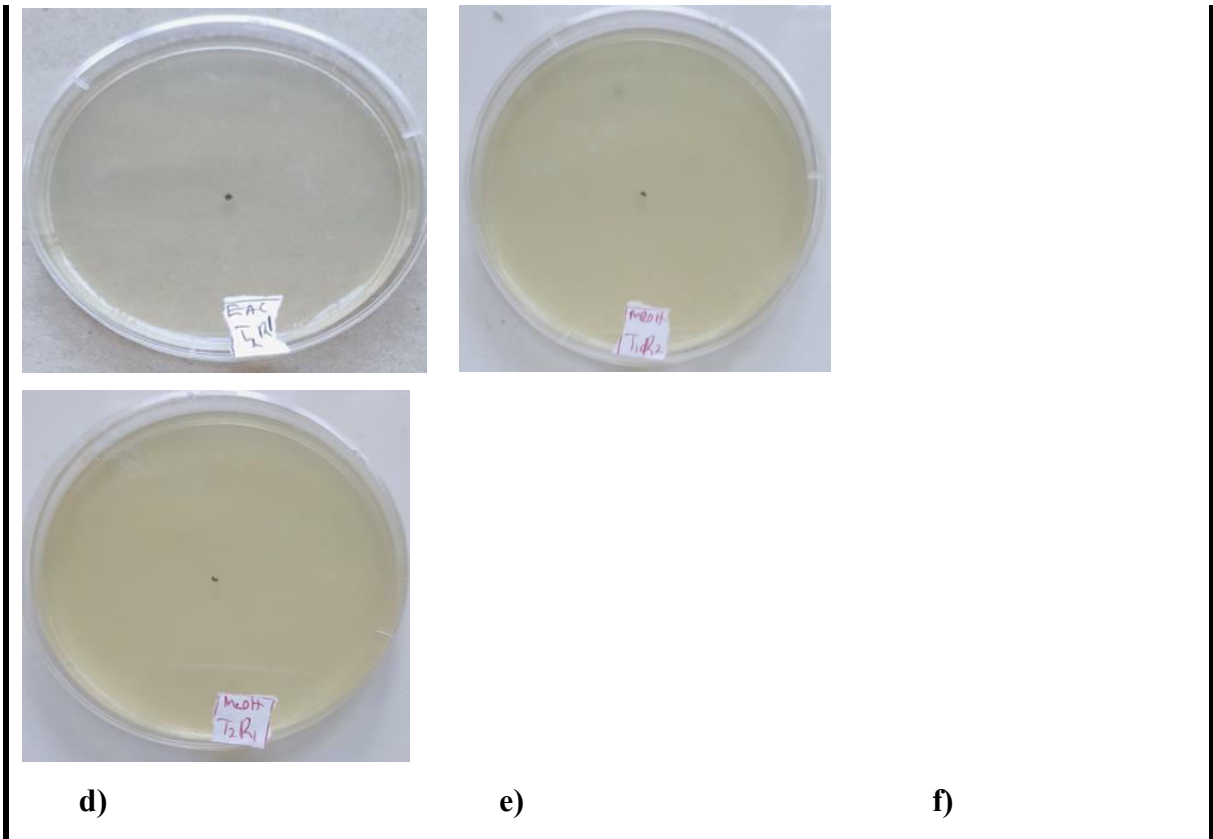
**Table 4. 1: Antifungal activity screening of *Mondia whitei* roots extracts against *Alternaria solani* and *Phytophthora infestans***

(mm)	Plant Extract	Concentration	Mean Radial growth	
			<i>A. solani</i>	<i>P. infestans</i>
b	MeOH	10%	0.0 b	0.0
b	MeOH	20%	0.0 b	0.0
b	Ridomil	0.25%	0.0 b	0.0
a	Control	non-treated media	29.5 a	60.0
b	DCM	10%	0.0 b	0.0
b	DCM	20%	0.0 b	0.0
b	Ridomil	0.25%	0.0 b	0.0
a	Control	non-treated media	29.4 a	60.0

EtOA	10%	0.0 b	0.0
b			
EtOA	20%	0.0 b	0.0
b			
Ridomil	0.25%	0.0 b	0.0
b			
Control	non-treated media	31.1a	60.0
a			

\*Values connected by the same letter within a column are not significantly different according to Tukey HSD test at  $p \leq 0.05$ .





**Plate 4.1:** Effect of *Mondia whitei* extracts on mycelium growth.

a) and b) *A. solani* and *P. infestans* respectively under negative control, c) and d) under 10% and 20% of extracts concentration respectively and e) and f) under positive control.

#### 4.3 Morphological Identification of *Alternaria solani* and *Phytophthora infestans*

Here are the results for the morphological identification of *Alternaria solani* and *Phytophthora infestans* based on laboratory observations both macroscopic on agar plates and microscopic:

##### 4.3.1 *Alternaria solani* (Agent of Early Blight)

**Macroscopic Observation (Petri Dish):** Colonies of the isolated fungus grew rapidly in negative control petri dishes after 7 days of incubation. The colony exhibited a dense, woolly or cottony aerial mycelium. The mycelium was initially white or greyish, becoming

olivaceous-black to dark grey or black with age due to prolific sporulation. The reverse side of the colony appeared dark brown to black. Distinct concentric rings of sporulation were frequently observed within the colony (**Plate 4.2 a**).

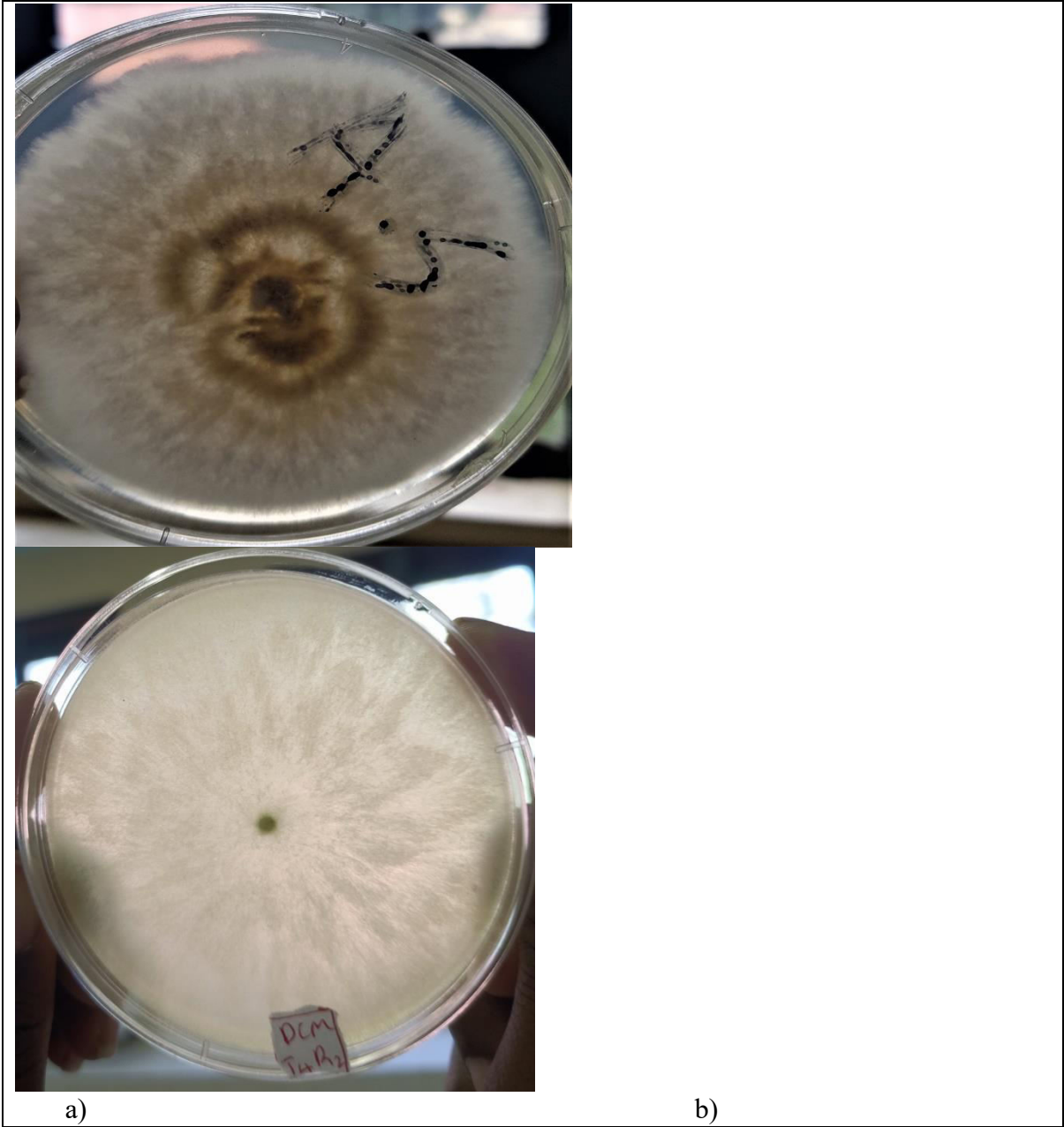
**Microscopic Observation:** Microscopic examination revealed the presence of characteristic conidiophores and conidia. Conidiophores emerged singly or in small groups, arising laterally or terminally from hyphae. They were erect, straight or slightly curved geniculate (zig-zag) near the apex, pale to mid brown, and septate. Conidia were produced singly or in short chains (typically 2-5), though often breaking apart. Conidia were large, obclavate (club-shaped, broadest near the base, tapering towards the apex) to muriform (having both transverse and longitudinal septa). Conidia possessed a distinct, elongated, beak-like apical cell which was often pale and attenuated. The conidial body was dark brown, smooth-walled or slightly verruculose, with transverse and longitudinal septa dividing it into multiple cells, typically 8-15 transverse septa and 1-5 longitudinal septa. The morphological characteristics observed; rapid growth, dark colony with concentric rings, geniculate brown conidiophores, and large obclavate, muriform conidia with a prominent long beak, are diagnostic for *A. solani* (**Plate 4.3 a**).

#### 4.3.2 *Phytophthora infestans* (Agent of Late Blight)

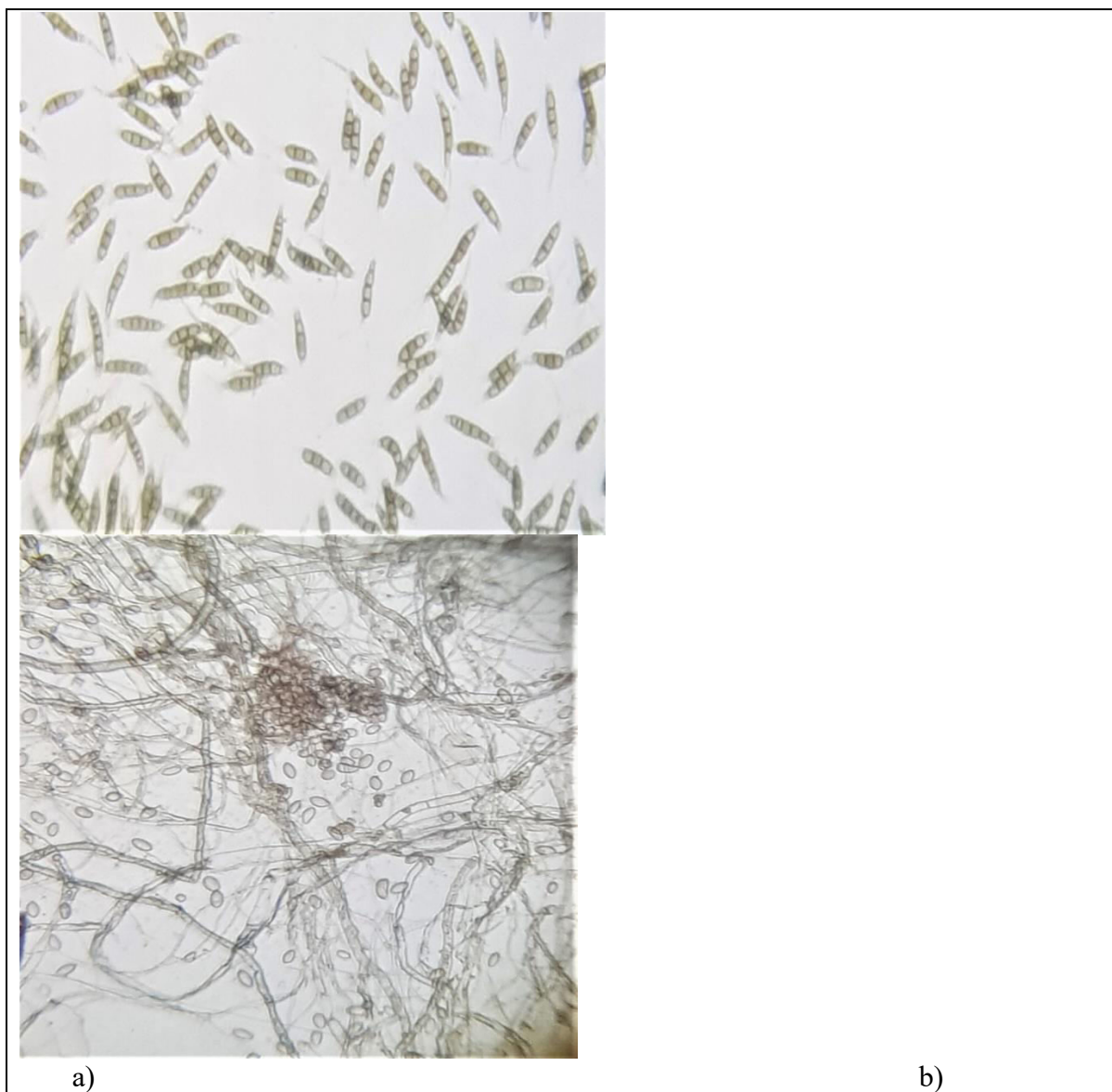
**Macroscopic Observation (Petri Dish):** Colonies of the isolated oomycete grew rapidly, after 7 days of incubation. The colony morphology was typically stellate (star-like) or petaloid with limited aerial mycelium, appearing flat and appressed to the agar surface. The aerial mycelium was very fine, sparse, and white. The colony color was predominantly white to colorless, with subtle radiating patterns. A distinctive feature observed was the

production of sporangia directly at the colony margin, visible as a faint white fringe or halo under low magnification (**Plate 4.2 b**).

**Microscopic Observation:** Microscopic examination revealed aseptate (coenocytic), hyaline (colorless), branching hyphae. Sporangia were borne on specialized, distinct sporangiophores. Sporangiophores emerged from the hyphae, typically showing sympodial branching (growing past a sporangium then bending to the side to form the next one), giving a characteristic swollen or knotted appearance at branching points. Sporangiophores were indeterminate, hyaline, and relatively thick. Sporangia were formed at the tips and subsequent points on the sporangiophore. They were lemon-shaped (papillate and ovoid) to ellipsoid. A distinct apical papilla (a nipple-like projection) was clearly visible on most mature sporangia. Sporangia were hyaline and deciduous, readily detaching from the sporangiophore at maturity. The morphological characteristics observed; white, appressed stellate colony with marginal sporulation, sympodial branched sporangiophores, and lemon-shaped, papillate, deciduous sporangia capable of releasing zoospores are diagnostic for *P. infestans* (**Plate 4.3 b**).



**Plate 4.2:** a) *A. solani* and b) *P. infestans* petri dish physical appearance



**Plate 4.3:** a) *A. solani* and b) *P. infestans* morphological appearance under microscope

#### **4.4 Phytochemical Screening**

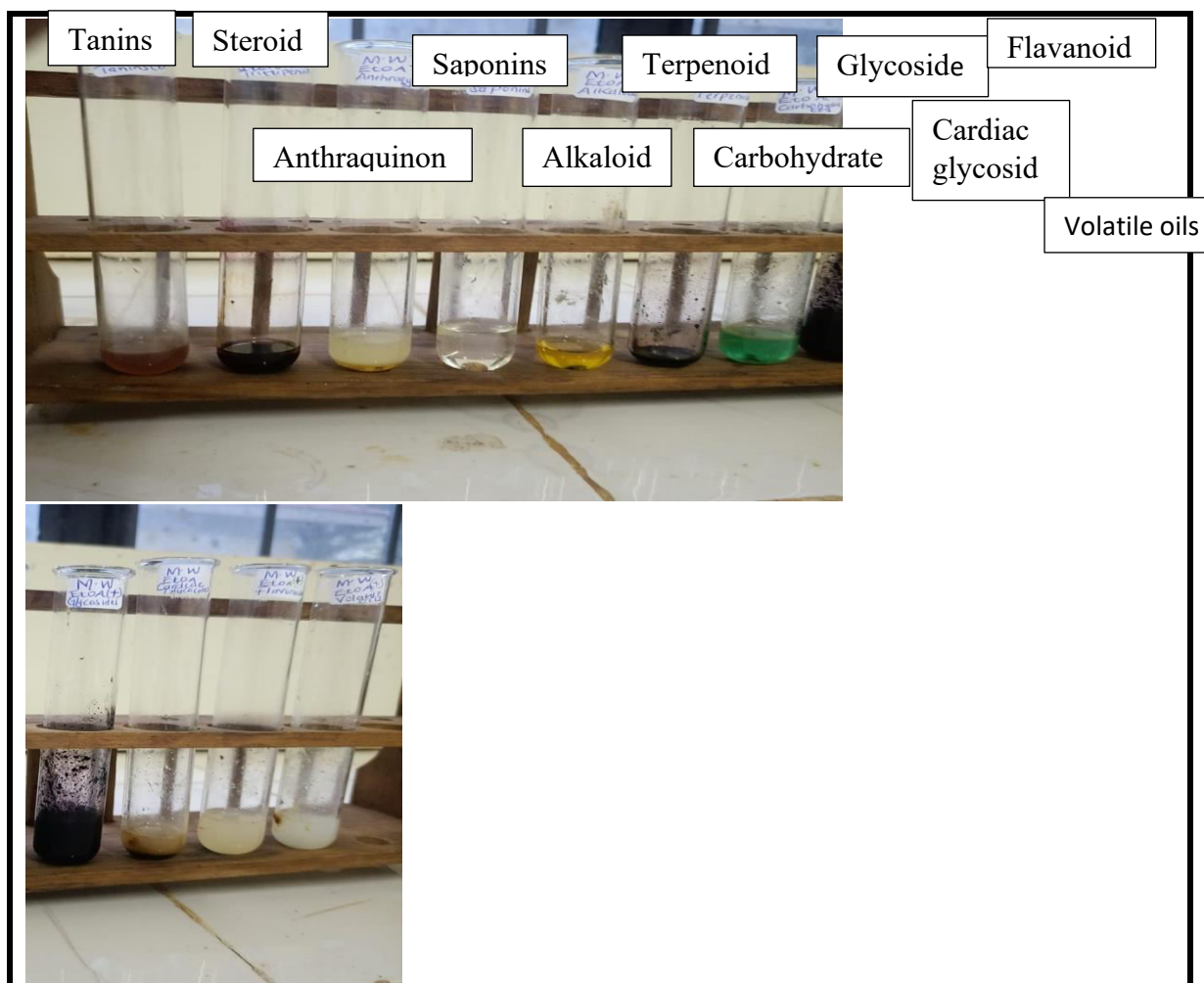
*Mondia whitei* extracts were found to have various tested secondary metabolites, except for triterpenoids and anthraquinones. Methanol extract was found to have steroids, cardiac glycosides, carbohydrates, alkaloids, saponins, terpenoids and volatile oils, while dichloromethane extract had tannins, steroids, glycosides, flavonoids, cardiac glycosides,

alkaloids and volatile oils. Ethyl-acetate extract had tannins, steroids, glycosides, flavonoids, cardiac glycosides, alkaloids and volatile oils (**Table 4.2**).

**Table 4. 2: Phytochemical Screening tests of MeOH, DCM and EtOA**

Secondary Metabolites	Plant Solvent Extracts		
	MeOH	DCM	EtOA
Tannins	-	+	+
Steroids	+	+	+
Terpenoids	+	-	-
Triterpenoids	-	-	-
Saponins	+	-	-
Flavonoids	-	+	+
Volatile oils	+	+	+
Anthraquinones	-	-	-
Cardiac glycosides	+	+	+
Glycosides	-	+	+
Alkaloids	+	+	+
Carbohydrates (Reducing sugar)	+	-	-

NB: - and + represents absence and presence of the secondary metabolites respectively



**Plate 4.4:** Phytochemical screening of *Mondia whitei* extract

#### **4.5 In vivo Efficacy Trial of *Mondia whitei* Extracts for Early Blight and Late Blight Antifungal Activities on Tomato Plant**

##### **4.5.1 Disease Incidences**

While the negative control recorded average disease incidences of 57.86% and 80% for early blight and late blight respectively, ridomil 0.25%, *M. whitei* extracts concentrations of 2.5%, 10% and 20% recorded disease incidences of 20%, 30%, 25% and 18.57% respectively for early blight and 30%, 52.14%, 40% and 35% respectively for late blight over the experiment period.

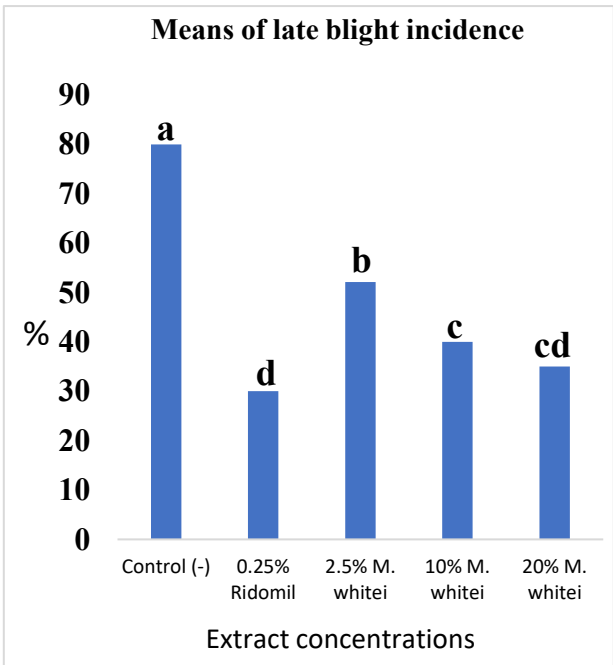
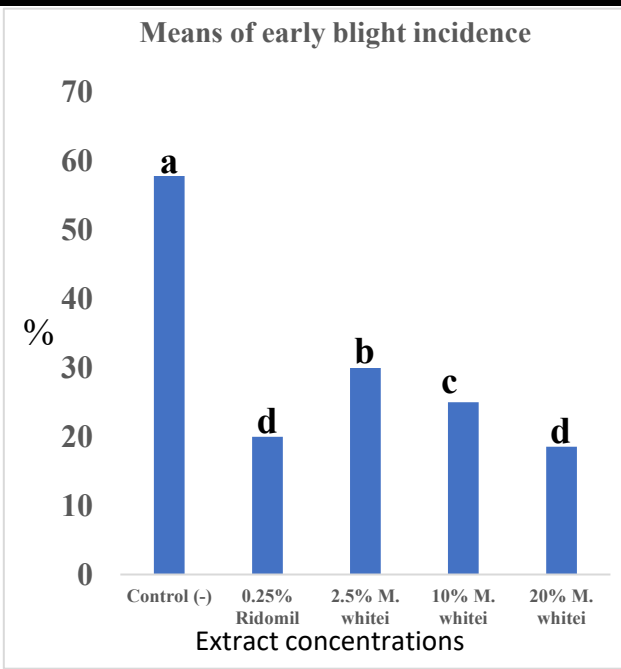
There was no significant difference ( $p \leq 0.05$ ) in early blight disease incidence between plants treated with 20% extract concentration that recorded 18.57% and those treated with 0.25% ridomil that recorded 20% disease incidences. Similarly, there was no significant difference ( $p \leq 0.05$ ) in late blight disease incidences between plants treated with 20% extract concentration that recorded 35% and those treated with 0.25% ridomil that recorded 30% disease incidences.

There was a significant difference ( $p \leq 0.05$ ) in early blight disease incidences between two treatments when plants were treated with 2.5% extract concentration which recorded 30% disease incidence and 25% in 10% extract treatment concentration (**Figure 4.2 a**). Similarly, late blight disease incidences in plants treated with 2.5% and 10% extract concentration were significantly different ( $p \leq 0.05$ ), with the former recording 52.14% and the later recording 40% disease incidences (**Figure 4.2 b**).

Nevertheless, no statistically significant difference ( $p \leq 0.05$ ) was found between late blight inoculated plants treated with extract concentration of 10% and 20% that recorded disease incidences of 40% and 35% respectively (**Figure 4.2 b**). In addition, disease incidences were significantly higher ( $p \leq 0.05$ ) in both pathogen experiments, on tomato plants treated with negative control (57.86% and 80% for early blight and late blight respectively) compared to the rest of the treatments (**Figure 4.2 a and 4.2 b**).

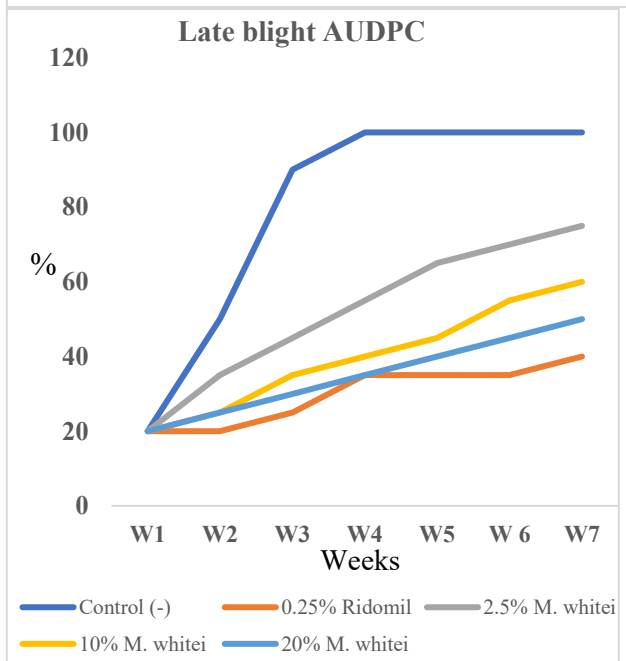
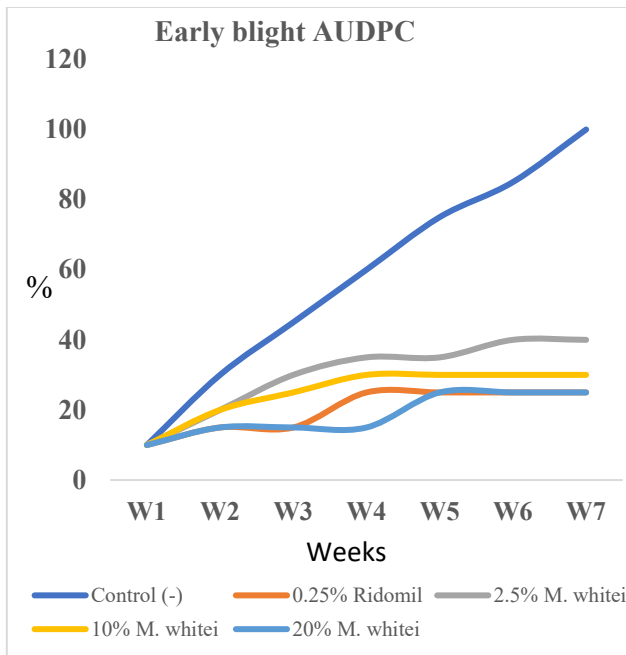
Overall, as the number of weeks progressed from week one to week seven, *M. whitei* extracts significantly reduced disease incidences of both pathogens, as show in the area under disease progress curve (AUDPC) (**Figure 4.2 c and 4.2 d**). Furthermore, increasing extract concentration from 2.5% to 20% significantly reduced disease incidences from 30% to 18.57% and 52.14% to 35% in early blight and late blight inoculated plants respectively.

For early blight, disease incidences were increasing exponentially in the negatively controlled plants from week one to week seven that recorded 100% disease incidences. While, plants inoculated with late blight and treated with negative control recorded an exponential increase in disease incidences between week one and week four which recorded disease incidences of 100%.



a)

b)



c)

d)

**Figure 4.2:** Effect of *Mondia whitei* extracts on incidences of early blight and late blight of tomato.

According to Tukey's HSD, levels that are not connected by the same letter are statistically different at ( $p \leq 0.05$ ).



**Plate 4.5:** a) Early blight and b) late blight disease incidences on negative controlled tomato plant.

#### 4.5.2 Disease Severity

Disease severity was significantly higher ( $p \leq 0.05$ ) in tomato plants treated with negative control compared to treatments with *M. whitei* extracts and ridomil in both pathogen experiments. The negative controls recorded an average disease severity index of 8.29 and 10.42 for early blight and late blight respectively. Ridomil at 0.25%, *M. whitei* extracts 2.5%, 10% and 20% recorded severity index of 4.57, 6.0, 4.71 and 4.43 respectively for early blight and severity indices of 6.57, 8.57, 7.57 and 7.14 respectively for late blight.

Early blight inoculated plants disease severity was significantly lower ( $p \leq 0.05$ ) in plants treated with 10% extract concentration which recorded severity index of 4.71, 20% extract concentration which recorded severity index of 4.43 and 0.25% ridomil which recorded severity index of 4.57 even though, all the three treatments were not significantly different from each other (**Figure 4.3 a**).

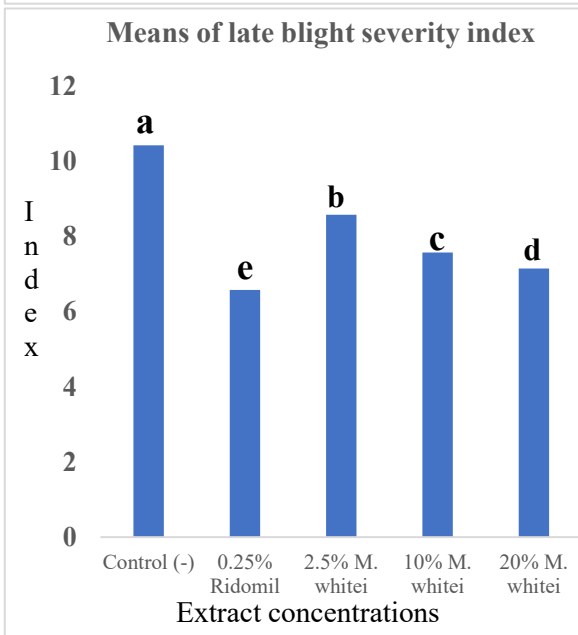
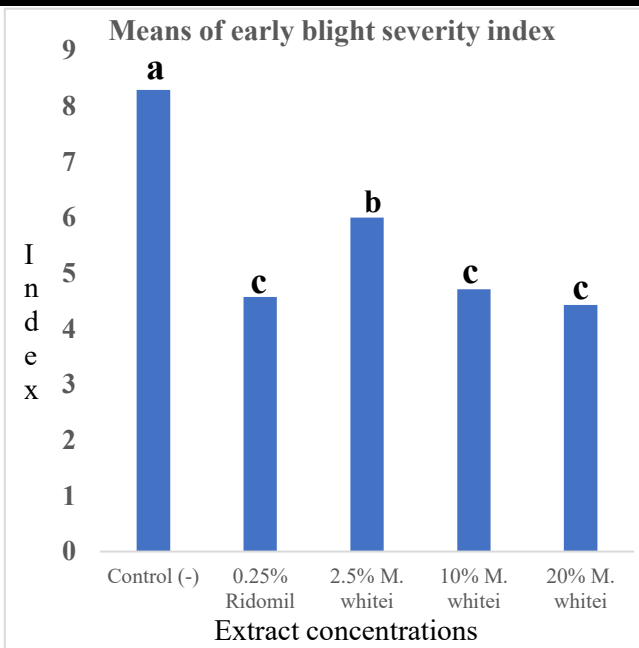
Plants treated with 2.5% extract concentration had a significantly higher ( $p \leq 0.05$ ) disease severity index of 6.00 as compared to those treated with 10%, 20% extract concentration and 0.25% ridomil which recorded severity indices of 4.71, 4.43 and 4.57 respectively in the early blight inoculated plants.

However, disease severity was significantly higher ( $p \leq 0.05$ ) in tomato plants treated with negative control which recorded a severity index of 8.29 compared to the rest of the treatments in the early blight inoculated plants (**Figure 4.3 a**).

Similarly, in the late blight experiment, disease severity was significantly higher ( $p \leq 0.05$ ) in negative control treatment which recorded a severity index of 10.42, compared to the rest of the treatments which recorded severity index of 6.57, 8.57, 7.57 and 7.14 for 0.25%

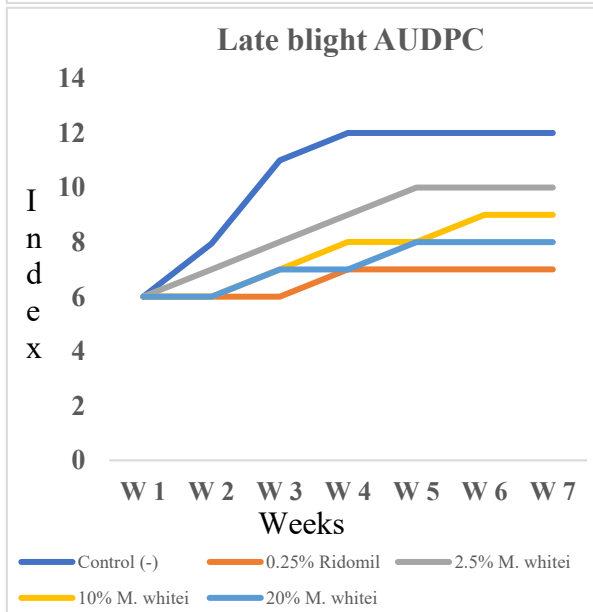
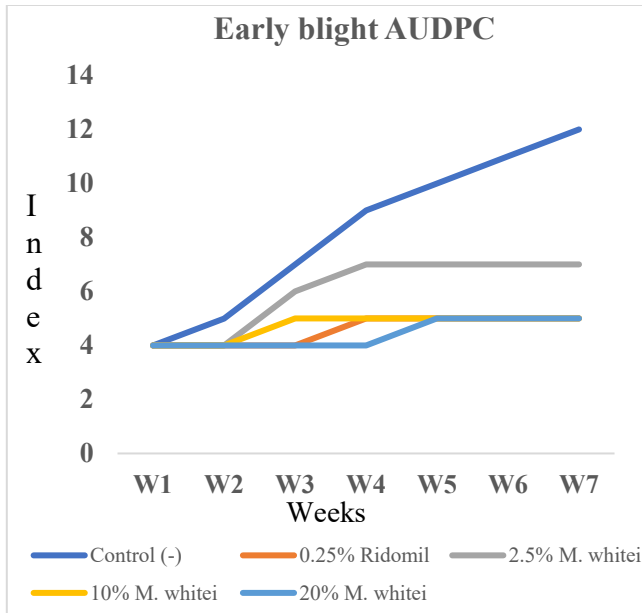
ridomil, 2.5%, 10% and 20% extract concentrations respectively. While plants treated with 0.25% ridomil recorded a significantly low ( $p \leq 0.05$ ) disease severity which was recorded at 6.57, compared to other treatments in the plants inoculated with late blight (**Figure 4.3 b**).

As weeks progress, the disease severity reduced in plants treated with extract concentrates and 0.25% ridomil as compared to the negative control for both pathogens experiment. Plants treated with negative control recorded an exponential increase in disease severity from 4.00 in week one to 12.00 in week seven in the early blight inoculated plants (**Figure 4.3 b**). Whereas, plants inoculated with late blight and treated with negative control recorded an exponential increase in disease severity from 6.00 in week one and 12.00 in week four (**Figure 4.3 d**).



a)

b)



c)

d)

**Figure 4.3:** Effect of *Mondia whitei* extracts on severity of early blight and late blight of tomato

c) and d) Area under disease progress curve (AUDPC). According to Tukey's HSD, levels that are not connected by the same letter are statistically different at ( $p \leq 0.05$ ).



**Plate 4.6:** Late blight disease severity on negative controlled tomato plant.

#### **4.5.3 Plant Height**

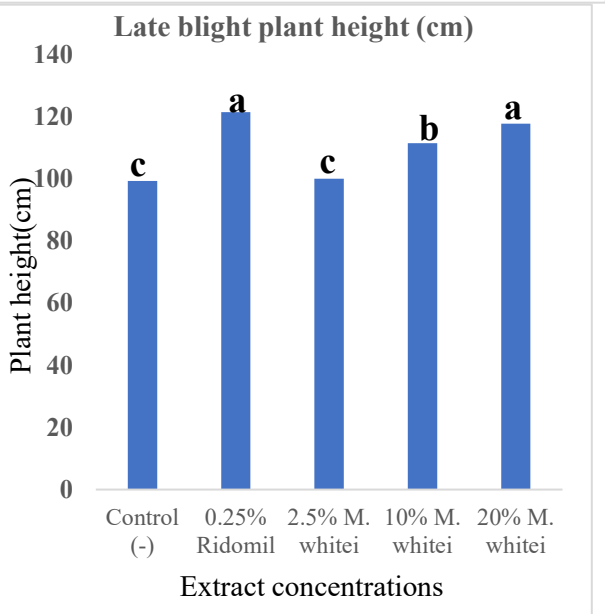
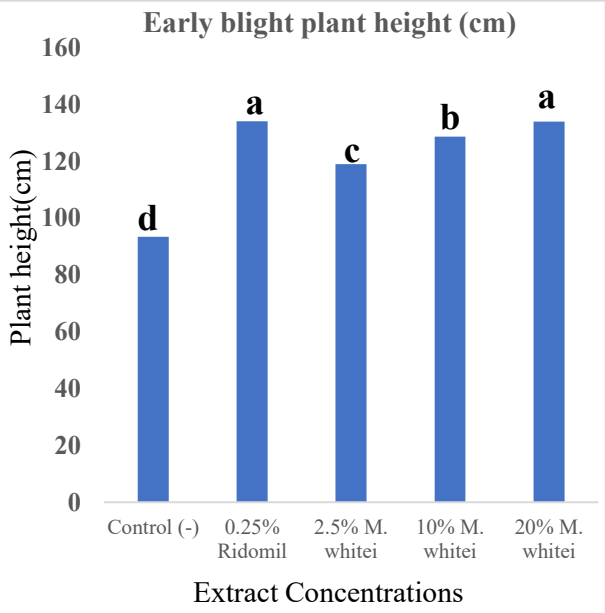
The 20% extract concentration and 0.25% ridomil recorded an average height of 133.96 cm and 134.07 cm respectively in plants inoculated with early blight, whereas 20% extract concentration and 0.25% ridomil recorded an average height of 117.71 cm and 121.41 cm respectively in plants inoculated with late blight.

Significantly high ( $p \leq 0.05$ ) plants heights were obtained from plants that were treated with 20% extract concentration and 0.25% ridomil compared with all other treatments, for both diseases treatments. The two treatments exhibited that they were statistically at par ( $p \leq 0.05$ ) with each other on the early blight inoculated plants and the same was also seen on late blight inoculated plants (**Figure 4.4 a and b**).

The average heights recorded for the negative control experiments for early blight and late blight inoculated plants were 93.49 cm and 99.30 cm respectively. For both diseases, the

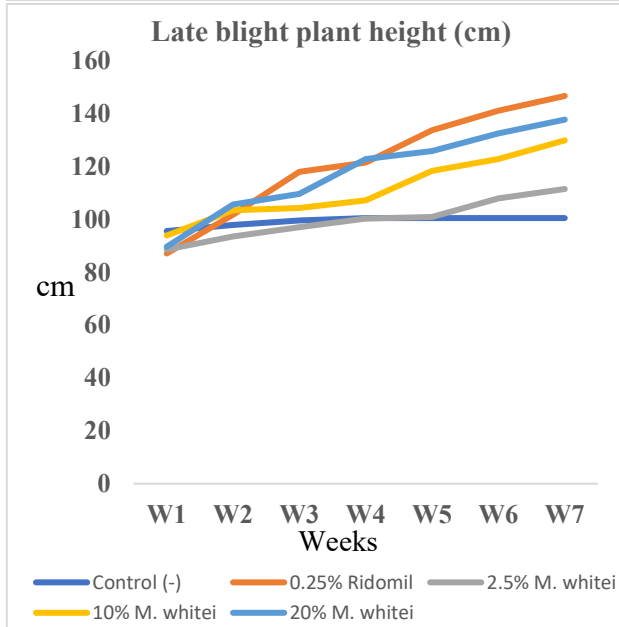
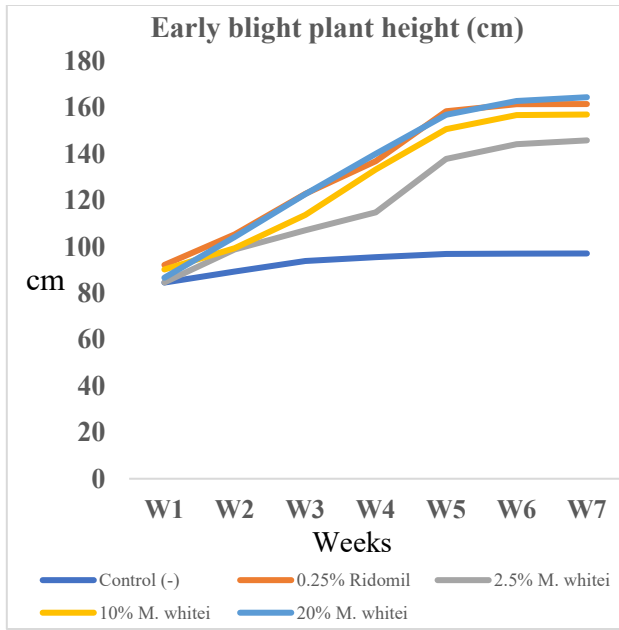
negative control treated plants recorded a significantly low ( $p \leq 0.05$ ) height compared to all other treatments (**Figure 4.4 a and b**).

In all treatments, plants height was observed to be increasing as weeks progressed, except for negative control treated plants that had very low growth rate in both early blight and late blight inoculated plants (**Figure 4.4 c and d**).



a)

b)



c)

d)

**Figure 4.4:** Effect of *Mondia whitei* extracts on tomato plant height

According to Tukey's HSD, levels that are not connected by the same letter are statistically different at ( $p \leq 0.05$ ).

#### 4.5.4 Plants Leaflet Size

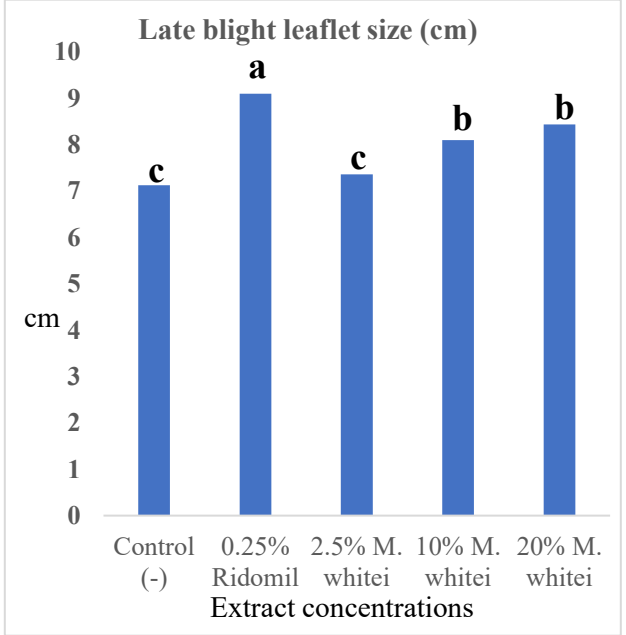
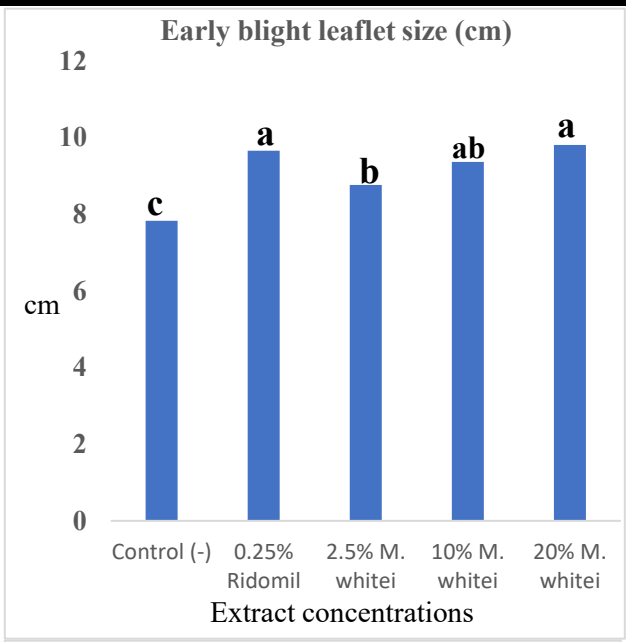
The average leaflet size recorded for plants treated with 20% and 0.25% ridomil were 9.81 cm and 9.66 cm respectively for the early blight inoculated plants and 8.44cm and 9.10 cm respectively for the late blight inoculated plants.

Significantly large ( $p \leq 0.05$ ) leaflet size was recorded where plants were treated with 20% extract concentrations and 0.25 ridomil for plants inoculated with early blight; the two treatments were significantly difference. Plants treated with 10% extract concentration recorded an average leaflet size of 9.37 cm for early blight inoculated plants. This was not significantly different ( $p \leq 0.05$ ) from average size of leaflets recorded from plants that were treated with 20% extract concentration, 0.25% ridomil and 2.5% extract concentration; which recorded an average length of 9.81, 9.66 and 8.77 cm respectively. Negative control recorded an average leaflet size of 7.83 cm, which, in comparison to other treatments in the early blight experiment, was significantly smaller ( $p \leq 0.05$ ) (**Figure 4.5 a**).

In the late blight experiment, there was a significant difference ( $p \leq 0.05$ ) in the size of the plant's leaves treated with 20% extract concentration and 0.25% ridomil, as the latter recorded a significantly larger ( $p \leq 0.05$ ) leaf size of 9.10 cm compared to the former which recorded 8.44 cm (**Figure 4.5 b**). In addition, plants treated with 10% extract concentration recorded an average leaflet size of 8.10 cm which was statistically at par ( $p \leq 0.05$ ) with the leaflet size of plants that were treated with 20% extract concentration. However, the former treatment's plants average leaflet size had substantially bigger size ( $p \leq 0.05$ ) than leaves of plants treated with 2.5% extract concentration that recorded an average leaflet size of 7.36 cm. The negative control recorded an average leaflet size of 7.13 cm, which was to have a significantly smaller ( $p \leq 0.05$ ) than other treatments in the late blight experiment. However,

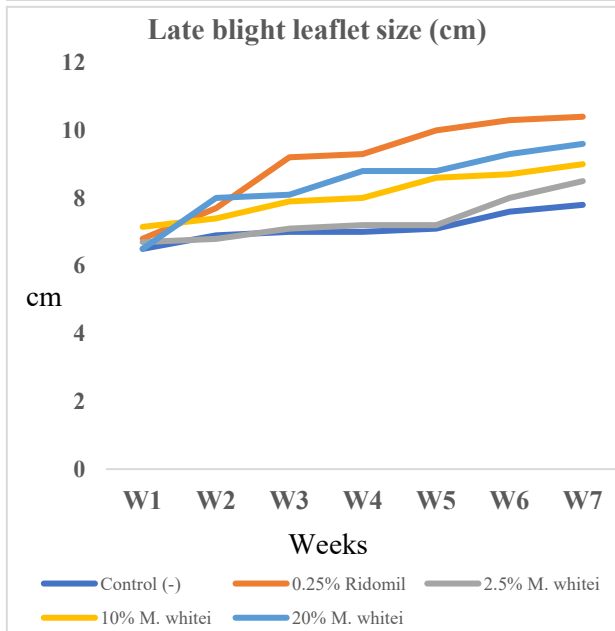
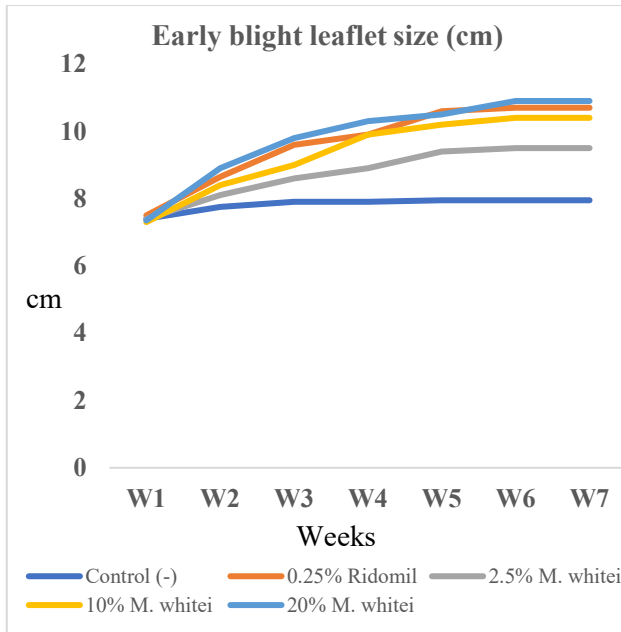
the leaflet size recorded for control treatment was statistically at par with the average leaflet size recorded from plants that were treated with 2.5% extract concentration (**Figure 4.5 b**).

In all treatments, plants leaflet size was observed to be increasing as weeks progressed, except for negative control treated plants that had very low growth rate in both early blight and late blight inoculated plants (**Figure 4.5 c and d**).



a)

b)



c)

d)

**Figure 4.5:** Effect of *Mondia whitei* extracts on tomato plant leaflet size

According to Tukey's HSD, levels that are not connected by the same letter are statistically different at ( $p \leq 0.05$ ).

#### 4.5.5 Effect on Number of Compound Leaves

The plants treated with 20% extract concentration and 0.25% ridomil recorded an average compound leaf number of 17.30 and 16.92 respectively for the early blight inoculated plants and 15.43 and 15.76 respectively for late blight inoculated plants.

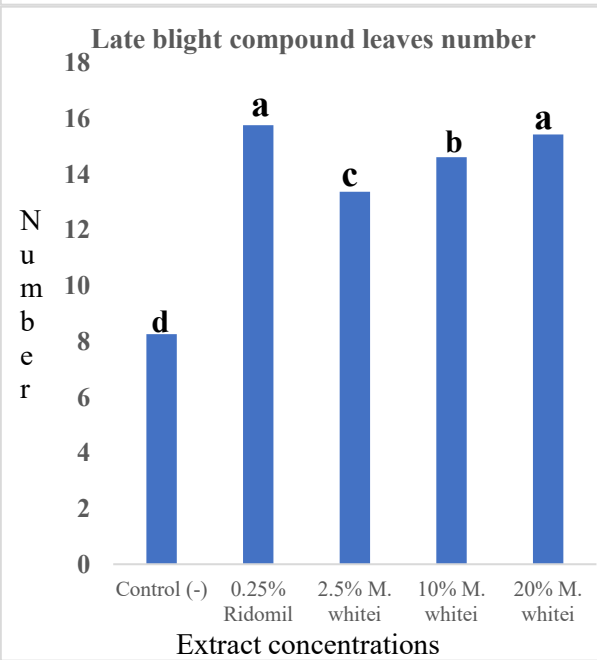
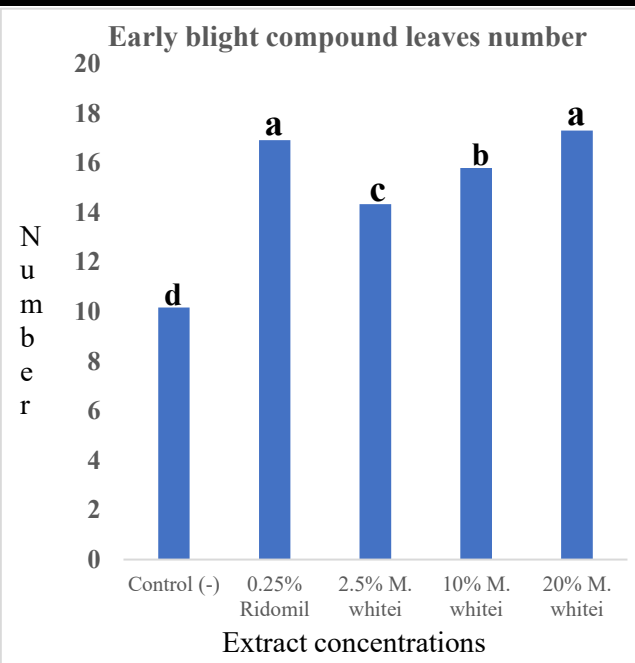
Significantly high ( $p \leq 0.05$ ) compound leaves number were recorded from plants that were treated with 20% extract concentration, which recorded 17.30 and 15.43 compound leaves for early blight and late blight inoculated plants respectively. Similarly, 0.25% ridomil treated plants recorded 16.92 and 15.76 compound leaves numbers for early blight and late blight respectively, which are significantly high ( $p \leq 0.05$ ) compound leaves numbers in the experiments. The two treatments were not significantly ( $p \leq 0.05$ ) different from each other on the early blight inoculated plants and the same was also seen on late blight inoculated plants (**Figure 4.6 a and b**).

The plants that were treated with 10% extract concentration recorded a significantly higher ( $p \leq 0.05$ ) average compound leaves number of 15.79, compared to plants inoculated with 2.5% extract concentration, which recorded 14.33 compound leaves. The plants that were treated with negative control recorded 10.17 compound leaves, which was significantly low ( $p \leq 0.05$ ) number of compound leaves compared to all other treatments in the early blight inoculated plants (**Figure 4.6 a**).

The late blight inoculated plants which were treated with 10% extracts concentration recorded an average compound leaves number of 14.61 which is significantly higher ( $p \leq 0.05$ ) than the 13.37 recorded in plants treated with 2.5% extract concentration. The plants which were treated with negative control recorded 8.26 compound leaves, which

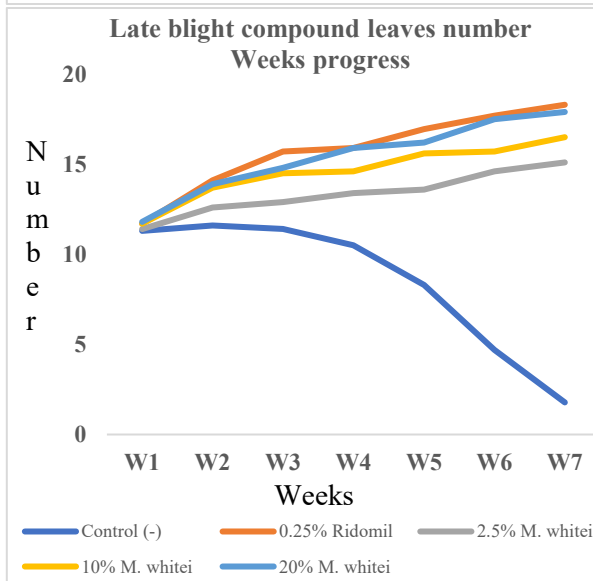
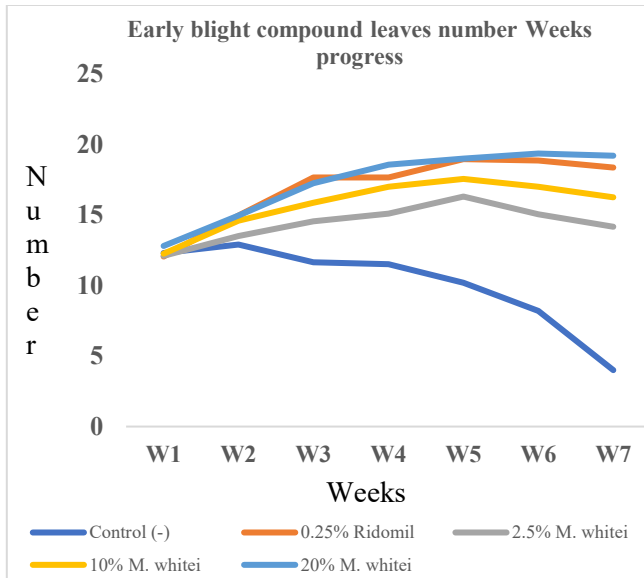
was significantly low ( $p \leq 0.05$ ) number of compound leaves compared to all other treatments in the late blight inoculated plants (**Figure 4.6 b**).

Extracts from *M. white* revealed a rise that was concentration-dependent of the compound leaves number as weeks progressed from week one to the seventh week for both pathogens' experiments. Compound leaves number in plants treated with negative control, reduced exponentially from 12.30 to 4.00 and 11.30 to 1.78 in week one to seven for early blight and late blight inoculated plants (**Figure 4.6 c and d**).



a)

b)



c)

d)

**Figure 4.6:** Effect of *M. whitei* extracts on tomato plant leaves number

According to Tukey's HSD, levels that are not connected by the same letter are statistically different at ( $p \leq 0.05$ ).

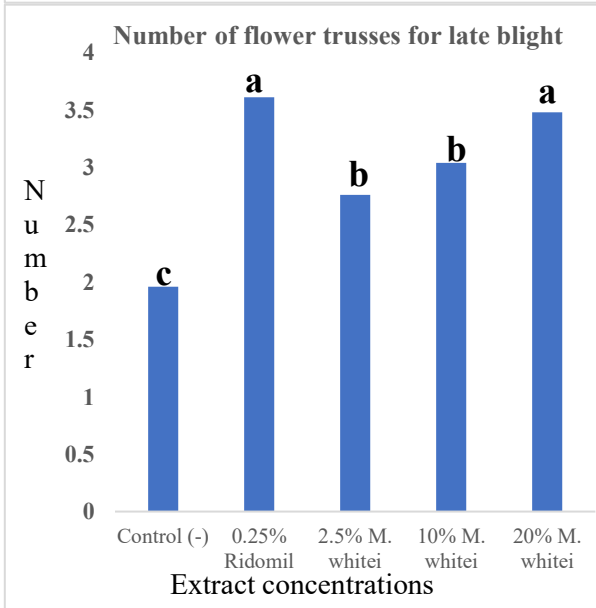
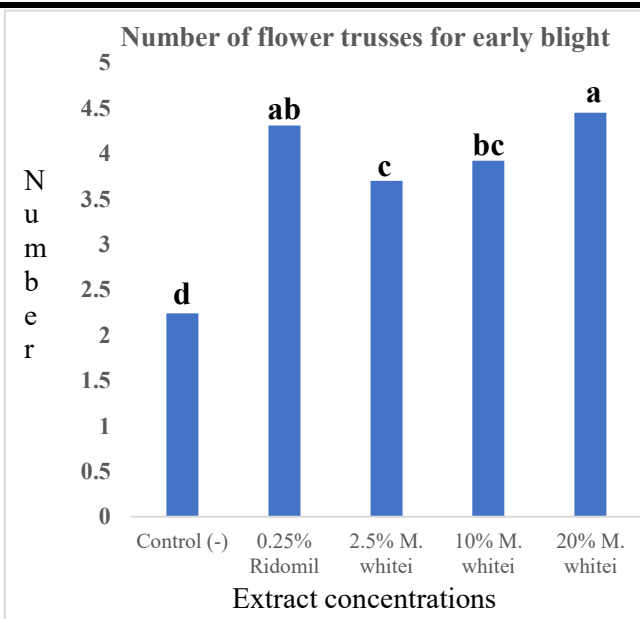
#### 4.5.6 Number of Flower Trusses

Early blight inoculated plants treated with 20% extracts concentration recorded an average of 4.45 flower trusses, which was significantly high ( $P \leq 0.05$ ). This record was not

significantly different ( $P \leq 0.05$ ) from the number of flower trusses recorded from plants that were treated with 0.25% ridomil, which was recorded at 4.31. Early blight inoculated plants treated with 10% extracts concentration recorded an average of 3.92 flower trusses which was statistically at par ( $P \leq 0.05$ ) with 4.31 and 3.70 flower trusses in 0.25% ridomil and 2.5% extracts concentration respectively. The plants that were treated with negative control recorded an average of 2.24 flower trusses, which is a significantly lower than all other treatments in the early blight inoculated plants (**Figure 4.7 a**).

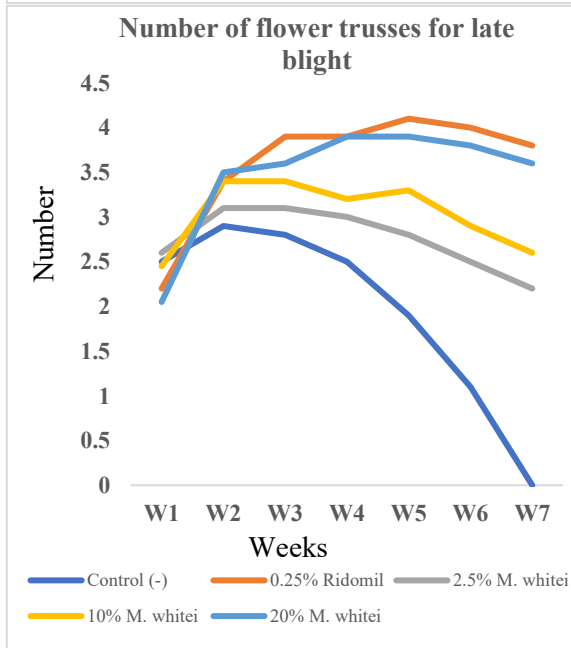
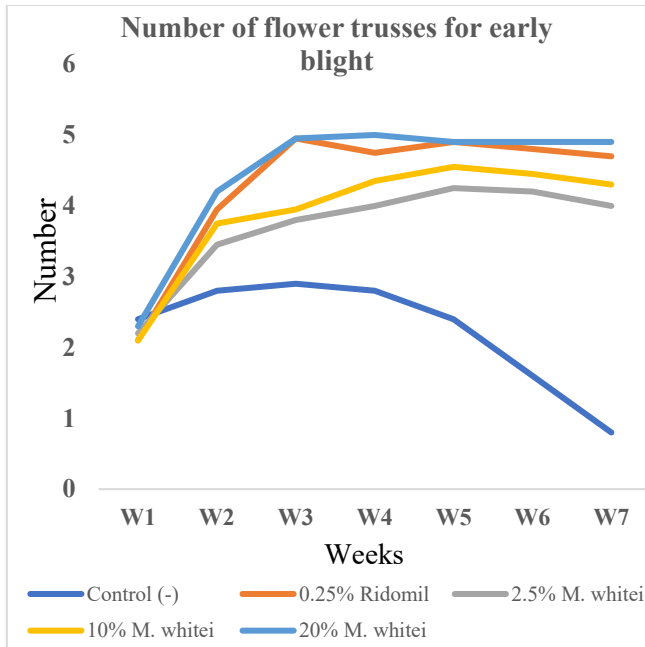
Late blight inoculated plants that were treated with 0.25% ridomil and 20% extracts concentration recorded 3.61 and 3.48 number of flower trusses which were both statistically at par with each other ( $P \leq 0.05$ ) and both significantly higher ( $P \leq 0.05$ ) than the other treatments. Similarly, late blight inoculated plants treated with 10% and 2.5% extracts concentration treatments recorded 3.04 and 2.76 flower trusses respectively, which had no significant difference ( $P \leq 0.05$ ). However, negative control treatment recorded an average of 1.96 flower trusses which was significantly low ( $P \leq 0.05$ ) compared to all the other treatments in the late blight inoculated plants (**Figure 4.7 b**).

Generally, for all the treatments, flower trusses number increased as weeks progressed from week one to week seven, except for negative control treated plants that had a decline in the compound leaves numbers in both early blight and late blight inoculated plants (**Figure 4.7 c and d**).



a)

b)



c)

d)

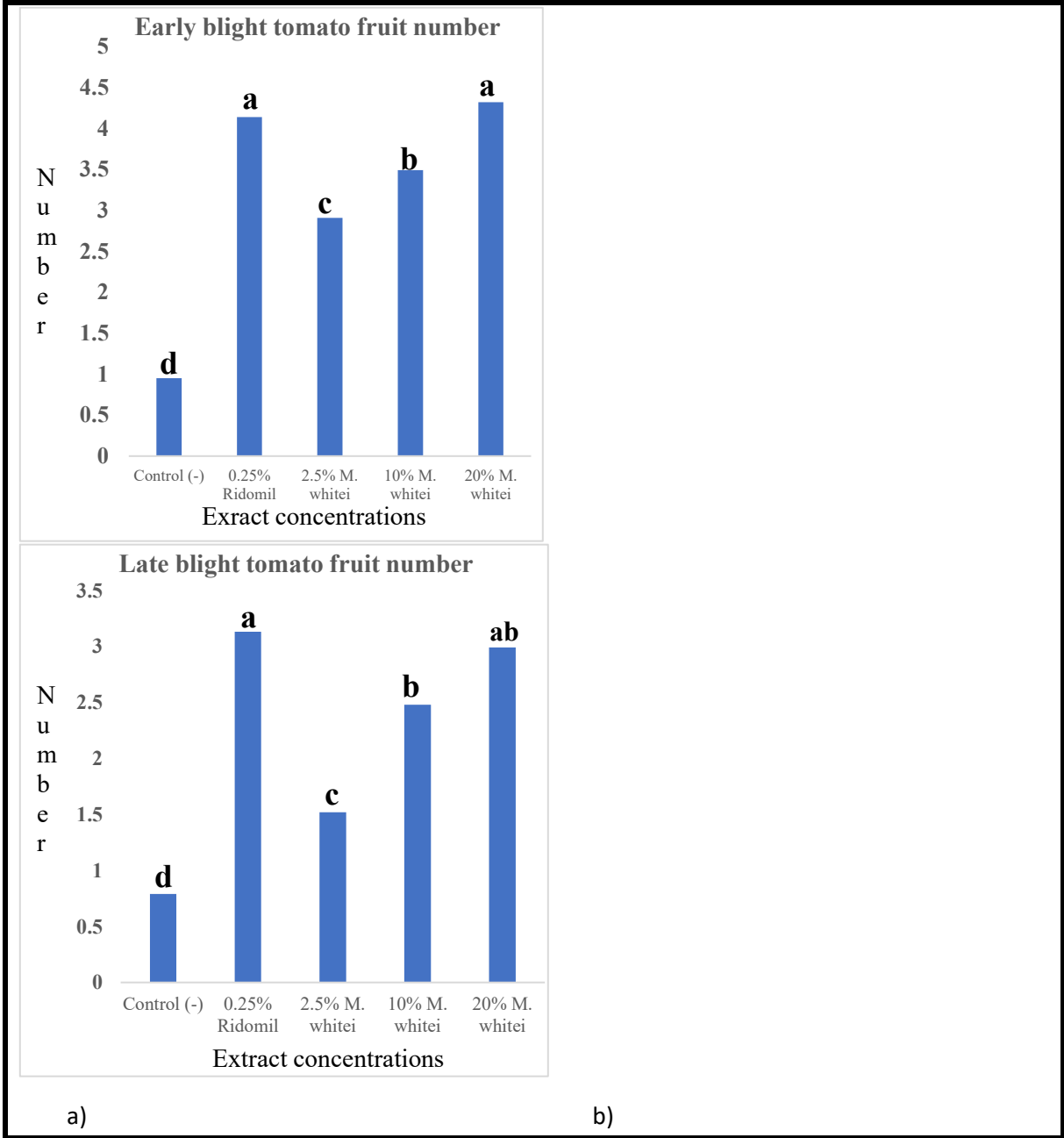
**Figure 4.7:** Effect of *Mondia whitei* extracts on tomato plants number of flower trusses

According to Tukey's HSD, levels that are not connected by the same letter are statistically different at ( $p \leq 0.05$ ).

#### 4.5.7 Number of Fruits

Early blight inoculated plants that recorded the highest number of fruits 4.32 and 4.14 were those treated with 20% extract concentration and 0.25% ridomil respectively. These two treatments had no significant difference ( $P \leq 0.05$ ) and recorded a significantly higher number of fruits compared to the rest of the treatments in the experiment. Early blight inoculated plants treated with 10% extract concentration recorded an average of 3.49 fruits and was significantly higher ( $P \leq 0.05$ ) compared to plants treated with 2.5% extract concentration which recorded an average of 2.91 fruits. Negative control treatment recorded an average of 0.95 fruits which was significantly smaller ( $P \leq 0.05$ ) than other treatments in the early blight inoculated plants (**Figure 4.8 a**).

Late blight inoculated plants recorded an average of 3.13 and 2.99 fruits where plants were treated with 0.25% ridomil and 20% extract concentration respectively. These two treatments did not have any significant difference ( $P \leq 0.05$ ) and recorded significantly high ( $P \leq 0.05$ ) number of fruits compared to the other treatments in the experiment. Late blight inoculated plants treated with 10% extract concentration recorded an average of 2.48 fruits which was not different from the plants treated with 20% extract concentration. However, it was significantly higher in number than plants treated with 2.5% extract concentration which recorded an average of 1.52 fruits. Additionally, negative control recorded an average of 0.79 fruits which is significantly smaller ( $P \leq 0.05$ ) than the other treatments in the experiment (**Figure 4.8 b**).



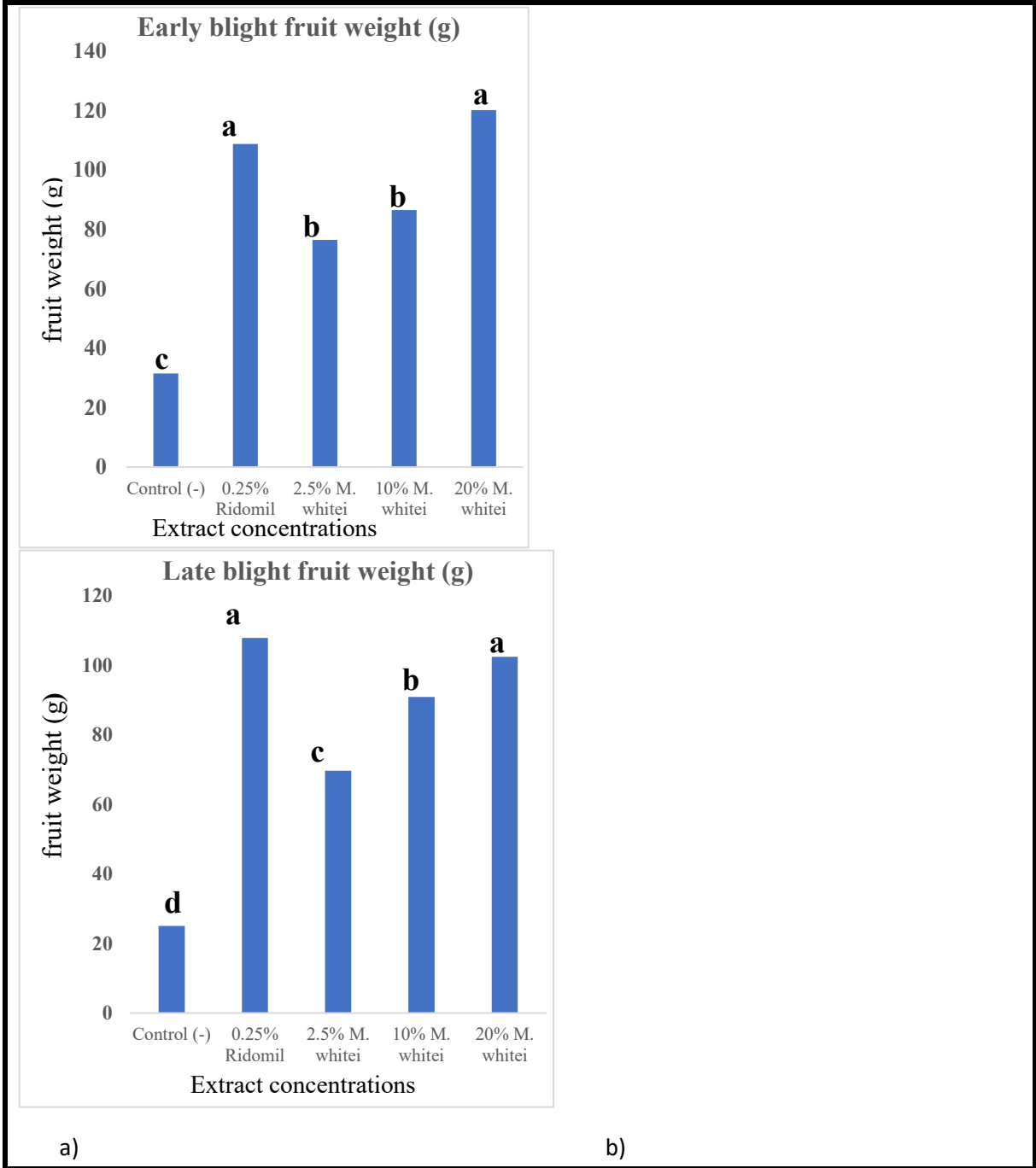
**Figure 4.8:** Effect of *Mondia whitei* extracts on the number of tomato fruits.

According to Tukey’s HSD, levels connected by the same letter are not different at ( $p \leq 0.05$ ).

#### 4.5.8 Fruit Weight

Early blight inoculated plants treated with 20% extract concentration and 0.25% ridomil recorded high weight of 120.15g and 108.78 g respectively. The two treatments recorded significantly high ( $P \leq 0.05$ ) average fruit weight compared to the negative treatment that recorded an average weight of 31.50g, however, the two treatments were not significantly different ( $P \leq 0.05$ ) from each other. Early blight inoculated plants treated with 10% and 2.5% extract concentration recorded average weight of 86.55g and 76.48g respectively. The two treatments recorded a significantly high ( $P \leq 0.05$ ) fruit number compared to the negative control. However, they were not significantly different ( $P \leq 0.05$ ) from each other (**Figure 4.9 a**).

In late blight inoculated plants, an average weight of 107.78g and 102.41g was recorded where plants were treated with 0.25% ridomil and 20% extract concentration respectively. The two treatments recorded significantly high ( $P \leq 0.05$ ) average fruit weight compared to all other treatments but were statistically at par ( $P \leq 0.05$ ) with each other. Late blight inoculated plants treated with 10% extract concentration recorded an average weight of 90.84g which was significantly high ( $P \leq 0.05$ ) compared to plants treated with 2.5% extract concentration which recorded an average weight of 69.60g. Plants treated with negative control recorded an average weight of 25.04g which was significantly lower ( $P \leq 0.05$ ) compared to all the other treatments in the late blight experiment (**Figure 4.9 b**).



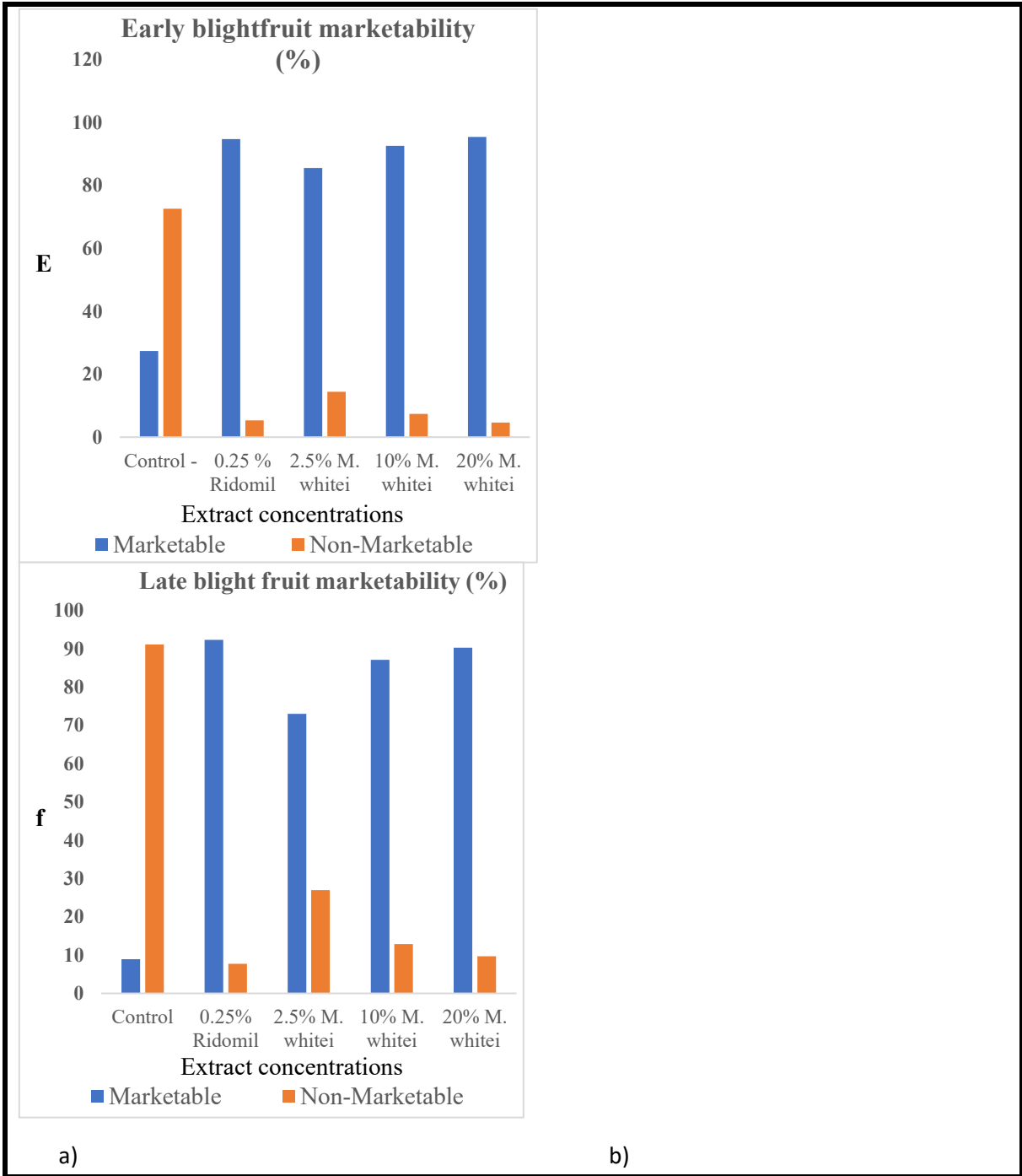
**Figure 4.9:** Effect of *Mondia whitei* extracts on tomato fruit weight.

According to Tukey’s HSD, levels connected by the same letter are not different at ( $p \leq 0.05$ ).

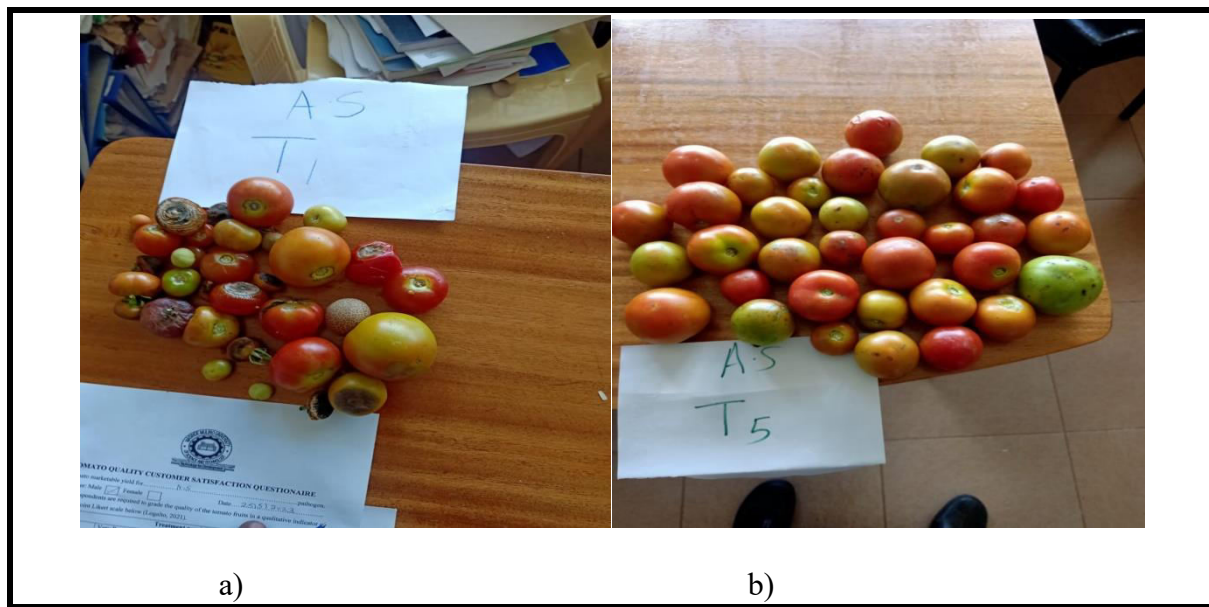
#### 4.5.9 Fruit Marketability

Early blight inoculated plants treated with 20%, 10% and 2.5% extracts concentration recorded 95.4% marketable and 4.6% non-marketable fruits, 92.6% marketable and 7.4% non-marketable fruits, 85.6% marketable and 14.4% non-marketable fruits respectively. On the other hand, those treated with 0.25% ridomil and negative control recorded 94.7% marketable and 5.3% non-marketable fruits and 27.4% marketable and 72.6% non-marketable fruits respectively (**Figure 4.10 a**).

Additionally, late blight inoculated plants that were treated with 20%, 10% and 2.5% extracts concentration recorded 90.3% marketable and 9.7% non-marketable fruits, 87.1% marketable and 12.9% non-marketable fruits, 73.0% marketable and 27.0% non-marketable fruits respectively, whereas those that were treated with 0.25% ridomil and negative control recorded 92.3% marketable and 7.7% non-marketable fruits and 8.9% marketable 91.1% non-marketable fruits respectively (**Figure 4.10 b**).



**Figure 4.10:** Effect of *Mondia whitei* extracts on marketability of tomato fruits.



**Plate 4.7:** Quality of tomato fruits harvested from a) – control and b) 20% *M. whitei* extracts respectively inoculated with *A. solani*.

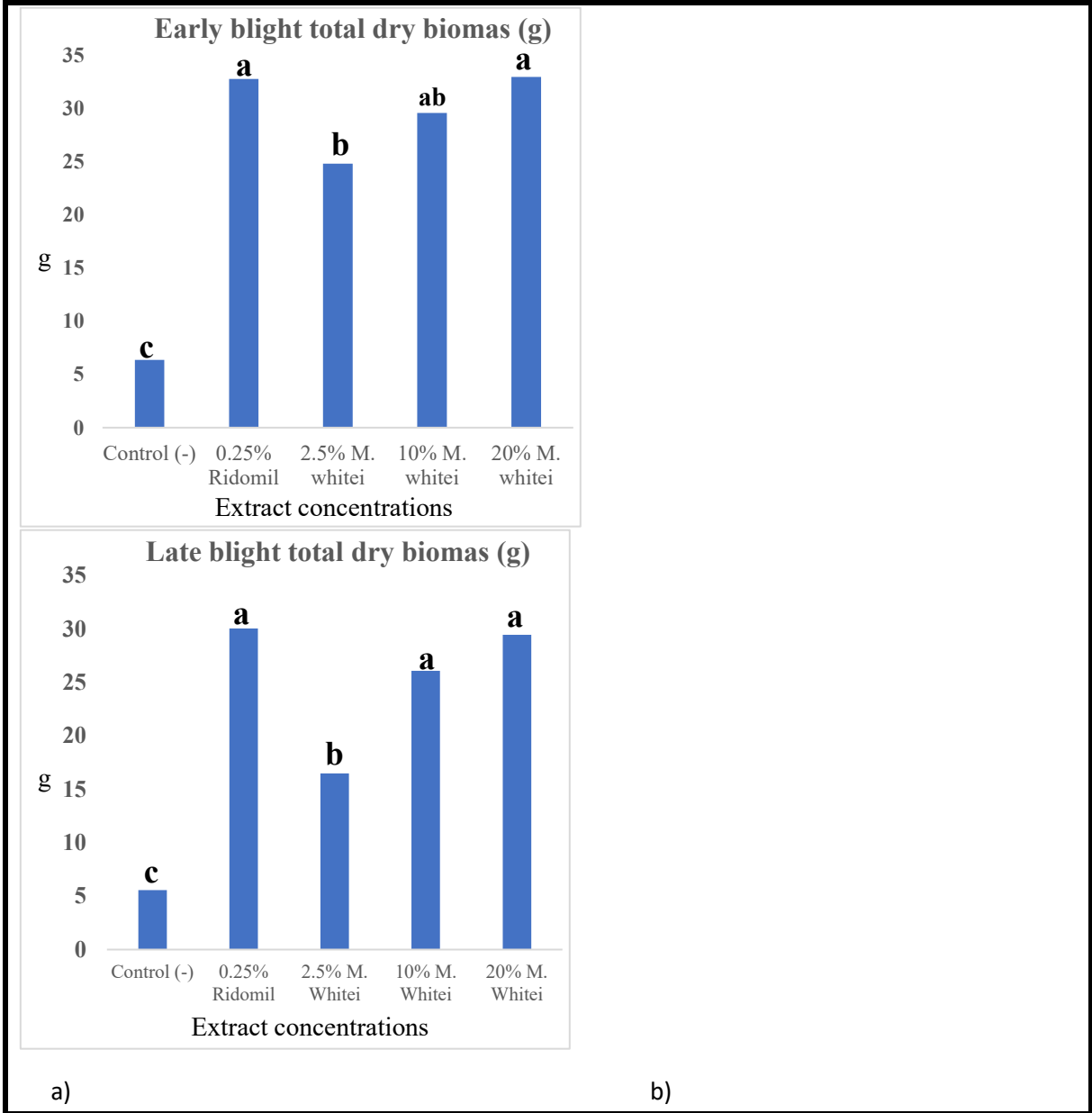
#### 4.5.10 Total Dry Biomass

Plants treated with 20% extract concentration and 0.25% ridomil recorded an average weight of 32.98 g and 32.78 g respectively. The two treatments recorded a significantly high ( $P \leq 0.05$ ) total dry biomass compared to all other treatments in the early blight experiment, but were not significantly different ( $P \leq 0.05$ ) from each other.

Late blight inoculated plants treated with 10% and 2.5% extract concentration recorded average weight of 29.58g and 24.83g respectively. However, they were not significantly different ( $P \leq 0.05$ ) from each other. Also, the average total dry biomass of plants treated with 10% extract concentration did not have significant difference ( $P \leq 0.05$ ) with plants treated with 20% extract concentration and 0.25% ridomil. Plants treated with negative control recorded an average total dry biomass of 6.36 g which was significantly lower than all the other treatments in early blight inoculated plants (**Figure 4.11 a**).

Late blight inoculated plants recorded an average dry biomass of 30.01 g and 29.44 g where plants were treated with 0.25% ridomil and 20% extract concentration respectively. The two treatments recorded significantly high ( $P \leq 0.05$ ) average total dry biomass compared to all other treatments in the experiment, but were not significantly different ( $P \leq 0.05$ ) from each other.

Late blight inoculated plants treated with 10% extract concentration recorded an average weight of 26.07 g which was not significantly different from plants treated with 20% extract concentration and 0.25% ridomil, and also recorded a significantly high ( $P \leq 0.05$ ) average total dry biomass compared to plants treated with 2.5% extract concentration which recorded an average dry biomass of 16.47 g. Plants treated with negative control recorded an average total dry biomass of 5.57 g which was significantly lower ( $P \leq 0.05$ ) compared to all the other treatments in the experiment (**Figure 4.11 b**).



**Figure 4.11:** Effect of *Mondia whitei* extracts on dry biomass of tomato plants amend the y titles as above

According to Tukey’s HSD, levels that are not connected by the same letter are statistically different at ( $p \leq 0.05$ ).



## CHAPTER FIVE: DISCUSSION

### 5.1 Introduction

Building on the experimental results, this section discusses the potential of *Mondia whitei* extracts as a novel botanical intervention for the critical tomato pathogens causing early blight and late blight of tomato. The analysis focuses on the observed levels of disease suppression, the concomitant effects on plant growth and yield enhancement, and the significance of these findings for developing alternative, sustainable management strategies.

### 5.2 Appearance of the Pathogens

The physical characteristics observed for *A. solani* in this study were rapid growth of mycelium; which looked dense, woolly or cottony and was initially white or greyish, and after four days became olivaceous-black to dark grey or black due to prolific sporulation. Similar appearance had also been observed in a study by Rahmatzai et al., (2016) where the fungus at first produced cottony growth which was dark, ranging from grey to black with tints of brown or olive. In the study, colonies of the fungus were spreading hairy and grey brown to black, the whole texture of the fungus was similar to cotton. In the current study, the mycelium also looked dark in colony with concentric rings. A similar observation had been made by MOHSIN, (2013) who noted that the *A. solani* colony shapes were irregular, and grows forming a pattern of concentric rings. The current study also concluded that *A. solani* under microscope had geniculate brown conidiophores, and large obclavate, muriform conidia with a prominent long beak. Similarly, a study by Alhussaen, (2012) indicated that the conidiophores were formed singly or in groups or

flexuous brown to olivaceous brown, the conidia were solitary straight or slightly flexuous or muriform or ellipsoidal tapering to beak, pale and sometimes branched.

In the current study, the morphological characteristics that were observed in *P. infestans* fungus were white mycelium, and appressed stellate colony with marginal sporulation. These observations are similar to what Mugao, (2021) observed where the fungus had white fluffy mycelia with beige tones caused by the presence of sporangia. The mycelia were white in colour and aseptate with long sporangiophores, and ellipsoidal sporangia. In the current study, under microscope the fungus had branched sporangiophores, and lemon-shaped, papillate, deciduous sporangia capable of releasing zoospores. This is similar to what Raza et al., (2022) found where the sporangiophores and sporangia of the pathogen appeared to be of lemon shaped and developed at the end of these sporangiophores.

### **5.3 In vitro Efficacy of *Mondia whitei***

In the in vitro experiment, *M. whitei* extracts were found to be highly efficient at controlling *A. solani* and *P. infestans* from growing at 10% and 20% extract concentrations. All the solvent extracts showed antifungal activity against the two pathogens with 100% inhibition at concentrations of 10% and 20%. This is a clear indication that *M. whitei* plant extracts has fungicidal properties against the two pathogens. Similar outcomes were also observed in the study where aqueous extracts of the plants *Asphodelus tenuifolius*, *Cotula cinerea*, *Artemisia herba alba*, and *Euphorbia guyoniana* all demonstrated growth-inhibiting properties against *Fusarium graminearum* and *Fusarium sporotrichioides* mycelium at 10% and 20% levels of dilution (Salhi et al., 2017). This is a clear indication that plants extract can serve as a source of bio pesticides. The current study therefore revealed that extracts from *M. whitei* have antifungal qualities

against the fungus *P. infestans* and *A. solani* in vitro. Like in the current study, Mayunzu et al., (2012) had also found that extracts from *M. whitei* root barks have inhibitory effects against *Escherichia coli* which are bacteria.

#### **5.4 Bio Active Compounds in Plants in Controlling Pathogens**

The current investigation found that the *M. whitei* extracts contained tannins, alkaloids, flavonoids, saponins, steroids, phenols, and glycosides. The existence of active secondary metabolites may have contributed to the inhibitory capacity of *M. whitei* extracts. In the current study, the secondary metabolites detected in *M. whitei* have been reported to have antibiotic activities (Roy et al., 2022; Othman et al., 2019) and this could confer the inhibitory effect in *M. whitei*. In a similar study by Onohuean et al., (2022), the results for the qualitative and quantitative analysis of *M. whitei* extracts were as follows; Phenolic compounds, flavonoids, tannins, triterpenoids, reducing sugars, and cardiac glycoside were present in any of the aqueous, ethanolic, and chloroform fractions of the extract. While saponins and terpenoids were only present in aqueous and ethanolic fractions, alkaloids were only present in chloroform fractions, and then steroids and anthraquinones were conspicuously absent in all three fractions of the extracts. Interestingly, alkaloid was only present in the chloroform fractions. These results are similar to the findings of this study and are a clear indication that *M. whitei* possess secondary metabolites.

The current study revealed the ability of *M. whitei* extracts to inhibit growth of the two tomato pathogens. The presence of the active secondary metabolites may have contributed to the inhibitory ability of the plant's extracts because the secondary metabolites detected in *M. whitei* have been reported to have antimicrobial activities (Roy et al., 2022; Othman et al., 2019). In other similar studies such as one by Zhu et al., (2019), tannins acted against

*Penicillium digitatum* fungi by inhibiting its mycelial growth and spore germination. In the study, in vivo tests showed that tannins significantly decrease the disease symptoms caused by *P. digitatum* fungi in an inoculated citrus fruit. In the current study, tannin was present; this could have also contributed in inhibiting growth of *A. solani* and *P. infestans*. According to Zhu et al., (2019), tannins compromise the membrane's permeability and the cell wall's structural integrity in their antifungal function. Leakage of intracellular contents, including sugars, results from breakdown of the cell wall and the plasma membrane. This could be attributed to the results observed in the current study as tannins were found to be present in the *M. whitei* extracts.

Alkaloids are chemical substances with a variety of forms that are said to have antibacterial qualities by preventing infections' enzyme activity (Patra, 2012). Plant secondary metabolites known as flavonoids have a variety of structural properties. They have been shown to prevent the growth of fungi by damaging plasma membranes, causing malfunctions in the mitochondria, and lowering the production of cell walls, cell division, RNA (ribonucleic acid), protein synthesis, and the efflux mediated pumping system (Aboody & Mickymaray, 2020). These traits of alkaloids and flavonoids could have led to the antifungal attribute of *M. whitei* as the secondary metabolites were found present in the plant's extracts. Methanol extract was found to have saponins. Saponins are a special kind of glycosides that have strong antifungal and soapy qualities (Barros Cota et al., 2021; Morcia et al., 2022). Presence of Saponins too, could have synergistically led to the antifungal ability of *M. whitei*.

Also, among these screened secondary metabolites, all the solvent extracts had steroids. Certain mixtures of the steroids and other compounds inhibited the growth of fungi

synergistically (Meli Sonkoue et al., 2023). Plants produce a class of secondary metabolites called phenols, which are employed as antimicrobial treatments because they can inhibit certain electron transport enzymes and cause non-specific degradation of membrane structural integrity (Bhattacharya et al., 2010). In this study, the extracts were all effective in *A. solani* and *P. infestans* fungal growth inhibition, this could be attributed to singular or synergetic action of the present compounds (Jubair et al., 2021).

### **5.5 Effect of Plant Extracts on Diseases Incidences and Severity**

In the greenhouse experiment, extracts from *M. whitei* effectively reduced disease incidences and severity of early and late blight at 2.5%, 10%, and 20% extract concentrations in tomato plants. Analogous findings were also documented in investigations assessing the induction of protection against *Puccinia triticina*-caused leaf rust disease infection in wheat through the application of extracts from *Lawsonia inermis*, *Acalypha wilkesiana*, *Melia azedarach*, *Punica granatum*, and *Lantana camara*. It was discovered that every concentration of plant extract tested was effective against the infection of leaf rust disease. In contrast to the untreated control group, which had an ACI of 75.00, they considerably lowered the ACI to between 7.50 and 20.00. The most effective was lantana extract, with an efficiency of 88.88%, which was quite similar to the fungicide "diniconazole," which was utilized, with an efficiency of 89.92%. Henna extract ranked second at 80.00% efficiency, followed by chinaberry at 76.00% efficiency, acalypha at 72.00% efficiency, and pomegranate at 68.00% efficiency (Draz et al., 2019). These results are similar to the finding in the current study where *M. whitei* plant extract at all concentrations early and late blight diseases incidences and severity in tomato plants.

In a different study, the acetone extract from the *Melia azedarach* plant demonstrated strong antifungal activity, showing 97% inhibition against the *F. proliferatum* pathogen. In contrast, the combined acetone extracts from the plants Combretum, erythrophyllum, and *Quercus acutissima* showed 96%, 67%, and 56% inhibition against the *F. verticilloides*, *F. proliferatum*, and *F. solani* microbes, respectively (Seepe et al., 2020). This control ability of *M. whitei* extracts against tomato early and late blight diseases and the efficacy of the plants discussed above against the said diseases could be attributed to presence of active secondary metabolites that degrade the cell wall of the pathogens cells resulting in inhibition of the pathogens' growth.

#### **5.6 Plants Extracts Effect on Growth and Yield Increase**

*Mondia whitei* extracts were effective in improving tomato plant growth and yield as the bio-extracts showed an extract-dependent increase in the plants' height, leaflet size, compound leaf number, number of flower trusses, number of fruits, fruits weight and total biomass. This could be attributed to reduction of the early and late blight incidences and severity, thereby enhancing plant growth parameters. Such results were also obtained by Mkindi et al., (2020) where Smallholder farmers using pesticidal plants in common bean; *Phaseolus vulgaris* realized that the treatment with plant extracts with pesticidal properties increases the development of plants. In the investigation, 10% w/v extracts of *Tephrosia vogelii* and *Tithonia diversifolia* were made and just like in the current study, the results of this investigation demonstrated that the number of pods per plant was greatly boosted by the plant extracts, chlorophyll content and overall seed yield was also improved. In the current study and also the discussed study above, it is evident that through effective control of diseases, it allows for optimum crop performance.

The increased growth rate and yield in plants as a result of application of plant extracts could also be attributed to metabolic processes being induced through the application of plant extracts (Mkindi et al., 2020). As seen in the current experiment, infection was significantly ( $P \leq 0.05$ ) reduced in tomato plants treated with *M. whitei* extracts concentrations of 20%, 10% and 2.5%.

*Mondia whitei* extracts were also effective in increasing marketability of the harvested tomato fruits as a result of the extracts controlling the two pathogens' severity. Such outcomes were also noted in a study conducted in a greenhouse with the best results for zucchini plants in terms of leaf area, number of fruits per plant, yield per plant, and total yield (marketable and non-marketable) following treatments with *T. viride* and *E. camaldulensis* LE (4000 mg/L) and *P. fluorescens* + *T. viride*. (Hassan et al., 2021). These abilities in the plant extracts increasing yield and improving marketability could be attributed to presence of active secondary metabolites that confer antifungal ability to *M. whitei* extract to reduce disease severity and incidences. Also, the abilities in the plant extracts improving marketability could be attributed to presence of antioxidant compounds that enhance the fruit preservation properties of edible coatings (Bajaj et al., 2023)

### **5.7 Importance of Bio-pesticide on Environmental Pollution and Human Health**

Both the biotic and abiotic elements of the environment have been negatively impacted by the usage of synthetic chemical pesticides. Human exposure risk and health consequences have increased due to the extensive and careless use of pesticides in agriculture (Kori et al., 2018). When pesticides are used improperly, they can leave residues in food and the environment that can damage humans and cause pests to become resistant. Both people and the ecosystem are negatively impacted by pesticides (Hou & Wu, 2010). However,

there is a slight risk to human health and the environment from biopesticides. They are generally less harmful than chemical pesticides, frequently specific, have negligible or no aftereffects, and can be used in organic farming (Rana et al., 2019). Utilizing bio-pesticides in agricultural and public health initiatives has a number of potential advantages. One of the biggest concerns for consumers, particularly with regard to fruit and vegetables, is waste, which bio-pesticides do not have. Bio-pesticides can be just as effective as traditional pesticides when used as part of MIP, especially for crops like fruits, vegetables, nuts, and flowers (S. Kumar, 2012). With the flexibility of minimal application limits and the potential for better resistance management, bio-pesticides combine environmental safety and performance in an efficient manner. The benefits of products that are (i) inherently less hazardous and safer for the environment, (ii) have a specific goal, (iii) frequently effective in very tiny doses, (iv) naturally and rapidly decompose, and (v) useable as a component of IPM are what have sparked interest in bio-pesticides (Rana et al., 2019).

## CHAPTER SIX: CONCLUSIONS AND RECOMENDATIONS

### 6.1 Conclusions

This study's findings imply that *M. whitei* extracts are effective in vitro in controlling *A. solani* and *P. infestans* pathogens causing early and late blights respectively.

The study also concludes that *M. whitei* possesses ten secondary metabolites. The antifungal effects of extracts of *M. whitei* can therefore be attributed to the presence of these different phytochemicals that either act independently or in synergy with the others.

This study also implies that *M. whitei* extracts can be a safe alternative fungicide against early blight and late blight diseases of tomato plants.

The effectiveness of the *M. whitei* extracts in tomato plant growth may be attributed to the extracts reducing disease incidences and severity. Thus, its usage lessens the use for both artificial fungicide and in a sustainable way reducing prevalence of early and late blight in tomato crops and at the same time protecting the environment.

### 6.2 Recommendation

This study recommends isolation of the bioactive compounds from *M. whitei* plant and establishing of mechanism of action of the bioactive compounds against the two pathogens.

### 6.3 Innovation Points

To the best of our knowledge, this is the initial report on the fungistatic effect of *M. whitei* against tomato plant phytopathogens. The work is already protected under “the industrial property act. 2001” Application number KE/P/2023/4587.

The possible use of *M. whitei* extracts from medicinal plants as a substitute bio-fungicide to protect tomato plants against *A. solani* and *P. infestans* pathogens was established in this study.

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## APPENDICES

Appendix 1: Modified Horsfall–Barrat rating scale of late blight disease

<b>Index</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>
<b>% Leaf area affected</b>	<b>0</b>	<b>0-3</b>	<b>3-6</b>	<b>6-12</b>	<b>12-25</b>	<b>25-50</b>	<b>50-75</b>	<b>75-87</b>	<b>87-94</b>	<b>94-97</b>	<b>97-100</b>	<b>100</b>