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M.Sc. in Biochemistry Science and Technology

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ISOLATION AND CHARACTERIZATION OF *Macrophomina Phaseolina* FROM THE SOIL SAMPLE OF INFECTED MAIZE FIELDS

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Momen Adel NURAZ

ABSTRACT

ISOLATION AND CHARACTERIZATION OF *Macrophomia Phaseolina* FROM THE SOIL SAMPLE OF INFECTED MAIZE FIELDS

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M.Sc. in Biochemistry Science and Technology
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Maize, a warm-season cereal crop grown annually, its yield is affected by climate change and pathogens. *Macrophomina phaseolina* (MP), a fungus, continuously mangle the corn crop by surviving in soil for years as multicellular sclerotia, which are generated in host tissues and discharged into the soil as the tissues decompose. For this purpose, the soil sampling method was adopted. The research was conducted on a soil sample withdrawn from the corn cultivated in twenty districts of Türkiye in 2022. The field number, tag number (isolate name), and GPS data were collected for each sample. Each sample was divided into sub-samples. Data of count for radial growth and severity of the disease was made from a soil survey. During this study, it was observed that the radial growth of MP in Adana, Osmaniye, Diyarbakır, Gaziantep, Mardin, and Kahramanmaraş exhibited the lowest number andless severity of disease. In contrast the highest number and severity were defined in Diyarbakır and Şanlıurfa provinces. The determination of variability among *M. phaseolina* isolates is fundamental to guide the development of appropriate strategies for disease management according to different districts.

Key Words: Climate change, diseases, *Macrophomina phaseol*ina, soil, microsclerotia.

ÖZET

İNFEKTELI MISIR TARLALARI TOPRAK ÖRNEKLERINDEN *Macrophomina Phaseolina*'NIN IZOLASYONU VE KARAKTERIZASYONU

NURAZ, Momen Adel
Yüksek Lisans Tezi, Biyokimya Bilimi ve Teknolojisi
Danışman: Prof. Dr. Canan CAN
Temmuz 2024
58 sayfa

Sıcak mevsimde her yıl yetiştirilen bir tahıl ürünü olan mısırın verimi iklim değişikliği ve patojenlerden etkilenmektedir. *Macrophomina phaseolina* (MP), konukçu dokularda üretilen ve dokular ayrıştıkça toprağa karışan çok hücreli sklerotlar halinde toprakta yıllarca hayatta kalarak mısır yetiştiriciliğini negatif olarak etkilemektedir. Araştırma, 2022 yılında Türkiye'nin mısır ekimi yapılan illerin toprak örneği üzerinde yürütülmüştür. Her örnek için tarla numarası, etiket numarası (izolat adı), GPS verileri toplanmıştır. Hastalığın şiddetine ilişkin veriler topraktaki mikrosklerot analizleri ile değerlendirilmiştir. Bu çalışmada MP'nin radyal büyümesinin Adana, Osmaniye, Diyarbakır, Gaziantep, Mersin, Mardin ve Kahramanmaraş'ta daha az sayıda ve daha az hastalık şiddetini göstermekte, en yüksek sayı ise Şanlıurfa ve Diyarbakır illerinde saptanmıştır. *M. phaseolina* izolatları arasındaki değişkenliğin belirlenmesi, farklı bölgelere göre hastalık yönetimine yönelik uygun stratejilerin geliştirilmesine rehberlik etmek açısından önem taşımaktadır.

Anahtar Kelimeler: İklim değişikliği, hastalık, *Macrophomina phaseolina*, toprak, mikrosklerot

"Dedicated to my country"

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LIST OF SYMBOLS

α	Alfa
β	Beta
Ω	Ohm
π	Pi

LIST OF ABBREVIATIONS

B-SPM	Bilinear Static Parametric Model
CE	Certainty Equivalence
D/A	Digital to Analog
DC	Direct Current
DIN	Dynamic Inertial System
FD	Fault Detection
FDD	Fault Detection Diagnosis
FDI	Fault Detection and Isolation
FTC	Fault Tolerant Control
g-DIN	Generalized Dynamic Inertial System
ISE	Integral Square Error
ISR	Interrupt Service Routine
LQR	Linear Quadratic Regulator
LTI	Linear Time Invariant
MIMO	Multi Input Multi Output
MRAC	Model Reference Adaptive Control
MSE	Mean Square Error
PDJ	Positive Diagonal Jordan
PEA	Parametric Eigenstructure Assignment
PM	*Macrophomina phaseolina*
PSUPA	Power Supply/Power Amplifier Unit
SISO	Single Input Single Output

SPR Strictly Positive Real

CHAPTER I

INTRODUCTION

The anamorphic fungi *Macrophomina phaseolina* (Tassi) Goid, is accountable for a disease in a range of plants. This particular pathogen impacts over 500 plant species (Elad et al., 1986). It exhibits a widespread presence across various global regions, underscoring its prevalence in diverse soil compositions. Disease symptom severity escalates notably under dry and warm conditions, typically within the range of 28 to 35 ℃ (Dhingra and Sinclair, 1978). The primary source of inoculum stems from the soil itself. This pathogen is pervasive in numerous tropical and sub-tropical countries, with significant repercussions for vital crops worldwide (Indira and Gayatri, 2003). The manifestation of the disease is subject to variables like the development period and the specific plant species involved (Veni et al., 2016).

The other factor is increased temperature caused by global warming leading to a reduction in crop yields and promoting the outbreak and spread of pests and diseases (Ghini et al., 2012). Climate change can affect pathogen viability, spread to a variety of pathologies, alter host susceptibility, and change the effects of disease on crops (Sharma et al., 2019). Previous studies have shown that increased temperatures, CO_2 levels, and droughts due to climate change have led to increased canopy decaying and spotting disease/severity among Australian and South Asian crop production especially, in wheat (Sharma et al., 2007). Due to climate change, reduced rainfall, and water shortages during the harvest season due to global temperature rise, some minor pathogens can reach major pathogen status, and DRR / CR is no exception. This is seriousin legumes worldwide, especially in Pakistan, India, Myanmar (Khan and Muhammad, 2007; Lodha and Mawar, 2020), sub-Saharan Africa (Songa and Hillocks, 1996), and the United States (Wrather and Koenning, 2006).

1.1 Pathogen *Macrophomina phaseolina*

The occurrence of *Macrophomina phaseolina* (MP) infection in sunflowers was initially documented in Sri Lanka in 1927. Subsequently, cases were also recorded in

1996 in Australia, Yugoslavia, and Uruguay, in 1967 in Senegal and Argentina. Similarly; another case was reported in 1970 inin Hungary followed by the USA around 1971. Additionally; India, France, Egypt, and Pakistan reported the same cases in 1973, 1976, 1980, and 1982 respectively (Bhutta, 1997). Finally; Türkiye faced such a crisis in sunflower (Mahmoud and Budak, 2011).

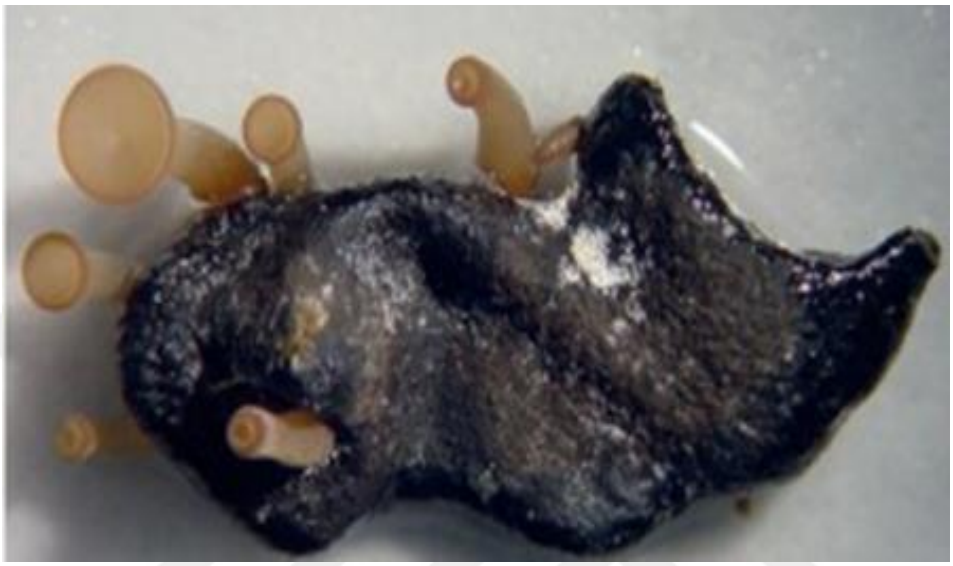

Figure 1.1 Fungus *Macrophomina phaseolina* as a multicellular sclerotia

Table 1.1 Taxonomy of *Macrophomina phaseolina*

Kingdom	Fungi
Division	Ascomycota
Subdivision	Pezizomycotina
Classis	Dothideomycetes
Ordo	Botryosphaeriales
Familia	Botryosphaeriaceae
Genus	*Macrophomina*
Species	*Macrophomina phaseolina* (Tassi) Goidanich

Pathogen *Macrophomina phaseolina* has a wide host range and causes economically important diseases in grains and legumes especially chickpea (*Cicer arietinum* L.), soybean (*Glycine max* L.), mung bean (*Vigna radiata* L. Wilczek), *sunflower* (*Helianthus annuus),* clover *(Trifolium repens*) and maize (*Zea mays*) (Iqbal and Mukhtar, 2014). Following the cropping period, pathogens attack different segments of plants from roots to stems and leaves eventually leading to a loss in the production of seeds (Mayek-Pérez et al., 2002).

A multicellular asexual structure named microsclerotia is produced in the tissues of the host that is disposed of on the soil after the decomposition of tissues. This asexual adult structure helped fungus to live and survive in soil. Having dense and closely packed outer layers with no structural differences; helps to withstand harsh environmental parameters including low nutrition and moisture content with increased dry soil temperatures. However; such conditions are the primary cause of inoculum and septicity (Lodha, 1995). Sclerotia (black sclerotia) shape around the infected tissue, and it could last for a long time (inside the earth for 15 years) (Olaya and Abawi, 1996). The population of *Macrophomina phaseolina* (MP) tends to increase its growth according to years of cultivation of crops in any soil (Lodha *et al*., 1990). Additionally; its population can easily be increased up to 1000 /g soil which is a serious issue for crops and soil (Papavizas and Klag, 1975). The signs and symptoms of charcoal rot are characterized by damping off, plant wilting, and other common bean plant issues including plants with stem blight and untimely defoliation have been recorded. Extreme infections result in death (Islam et al., 2012; Kaur et al., 2012). MP has been found in a different type of soil all over the world, demonstrating that it is ubiquitous. Disease severity is highly dependent on the environmental conditions of MP as inoculum and growth increase during 28 to 35^{o} C (Dhingra and Sinclair, 1974) which is then followed by scarcity or drought (Mayek-Pérez et al., 2002; Pastor-Corrales and Abawi, 1988). This pervasive disease infects valuable crops in the majority of tropical and subtropical regions. The disease's severity is mostly determined by the crop's age and the plant species.

Based on morphological and pathogenic characterization, numerous attempts were made to divide populations of MP into races or Vegetative Compatibility Groups (VCG) (Dhingra and Sinclair, 1973; Cloud and Rupe, 1991). A new era in population studies emerged with the advancement of molecular-based technologies and marker

systems. To reveal the genetic makeup of the causative agent's population, many different marker systems were used. To highlight the population's genetic complexity, various marker systems were used. Several methods are employed for population genetic investigations, including DNA barcoding, RAPD (Random Amplified Polymorphic DNA), SSR (Simple Sequence Repeat), ISSR (Inter Simple Sequence Repeat), and SRAP (Sequence Related Amplified Polymorphism) marker systems, (Tarakanta et al., 2005).

Plant infections that damage the phyllosphere of the plants can be controlled to some extent by fungicidal methods, but soil-borne plant pathogens, like MP, are difficult to eradicate. The best way to combat these pathogens is to use resistant or tolerant plant varieties (Tyler, 2008). For effective breeding programs and the use of resistance resources, it is vital to disclose genetic and pathogenic heterogeneity among pathogen populations.

1.1.1 Fields Effected by the MP and Their Economic Impact

Several crops are affected by the MP. These are given in the Table 1.2.

Table 1.2 Crops that are affected by the MP

Crops	**Reference**
Ginger	(Nasser and Bhai, 2022)
Peanut	(Gupta et al., 2002)
Sorghum	(Spagnoletti et al., 2020; Cloud and Rupe, 1994)
Soybean	(Spagnoletti et al., 2020)
Maize and Strawberry	(Yildiz et al., 2010)

Sunflower	(Mahmoud and Budak, 2011)
Maiz and Cotton	(Degani et al., 2020)

MP is a pathogen that can be found in seeds as well as in soil. It targets the seeds of various pulse crops, leading to significant declines in both germination rates and seed viability (Bhattacharya et al., 1994; Sarita et al., 2014). MP further infects stored seeds, causing their quality to deteriorate and resulting in substantial losses. The deterioration of seeds has been noted in legumes named urban, chickpea, mungbean, soybean, and peas of pigeon, causing a loss of approximately 40% in seed yield across countries of South Asia (Haider and Ahmed, 2014; Ashwini and Giri, 2014; Patil et al., 2012; Ali et al. 2010; Singh and Kumar, 2002; Kumar and Singh, 2000; Rahman et al., 1999). For instance, mungbean seeds experienced reduced germination rates and protein content (12.3%) due to the pathogen's impact (Kaushik et al., 1987; Patil et al., 1990). The interaction between humidity and temperature of the atmosphere leads to the corrosion of seeds belonging to the legume family by this pathogen. Higher levels of former parameters correlate with increased contamination, leading to varying seed infection percentages across different countries (Mbofung et al., 2013).

The pre and post-emergence mortality of crops belonging to the legume family is another devastating effect of the pathogen. The size of the pod, production of seeds, and yielding are further reduced due to the infection of foliar. Approximately 30% of mungbean reduced yield is reported in India due to MP (Kaushik et al., 1987; Raghuchander et al., 1993), while in Pakistan, losses of 44% have been reported (Bashir and Malik, 1988). Similarly, urdbean and chickpea in India have experienced around 40% disease incidence due to the pathogen (Indira and Gayatri, 2003; Lakhran et al., 2018). In 1994, soybean crops faced an estimated yield loss of 1.2 million metric tons due to charcoal rot in the countries that play a vital part in soybean production (Wrather et al., 2001). Subsequently, Iowa State of the USA faced a devastating soybean epidemic due to charcoal rotting in 2003 (Wrather and Koenning, 2006).

Moreover, 30-50% of annual soybean yield was decreased in Missouri (USA) (Wyllie, 1988) while an 80% reduction in India (Gupta and Chauhan, 2005). Additionally, chickpeas (Lakhran et al., 2018), sunflowers (Khan, 2007), and soybeans (ElAraby et al., 2003) are highly infected by MP colonies due to their warm and dry atmospheric conditions; leading to a substantial loss in their yields. While the pathogen has been found to infect Kenya pea crops including common, cow and pigeon peas (Songa and Hillocks, 1996) however; the exact percentages of infection, loss of yield, and their incidence can never be quantified. Nevertheless, West Africa's cowpea production experienced a 10% yield loss due to charcoal rot, with an estimated value of about USD 146 million. This reduction was additionally observed in Niger with similar reporting of cases in Senegal (Ndiaye et al., 2010). Additionally, in European countries, the production of mungbean sprouts has also been adversely affected by MP (Fuhlbohm et al., 2013).

1.1.2 Pathogen's Development in the System of Host

The MP's development within the host system is never consistently organized. Early infections are influenced by soil moisture levels, favoring occurrences under drought conditions and elevated temperatures in the course of infection of roots (Burton et al., 1987; Raut, 1985). After infecting roots; MP progressed upwards form to stem and leaves leading to development (Bhutta et al., 1995). Within 48 hours; fungus starts to develop within the seedling from where it enters into the tissues of hosts. As soon as sowing; in the stage of cotyledon, infection starts to spread to seeds within 3-7 days. Variations in the severity of disease among hosts are attributed to differences in genes, geographical origin, or isolate source. Approximately 20 to 30% of leaves of plants harbored the pathogen with no noticeable necrotic spots within 7 days. Infections on stems at the 1st node were noticeable after 60 days of germination. Severe infections displayed substantial sclerotia on the stem's bark. Notably, the pathogen tends to becomeinseparable from the leaves, stems, and flowers, especially at the 3rd node, and 5th internode respectively (Hodges, 1962; Dhingra and Sinclair, 1978). Subterranean signs initiated the development of the lesions on the taproot and stem of basal, leading to wilting in susceptible plants. Initial above-ground infections occurred at the stem base, progressing to rapid leaf desiccation. Significantly; the yielding of plants started to decrease during the stage of flowering to maturity phase due to wilting (Prioletta and Bazzalo, 1998).

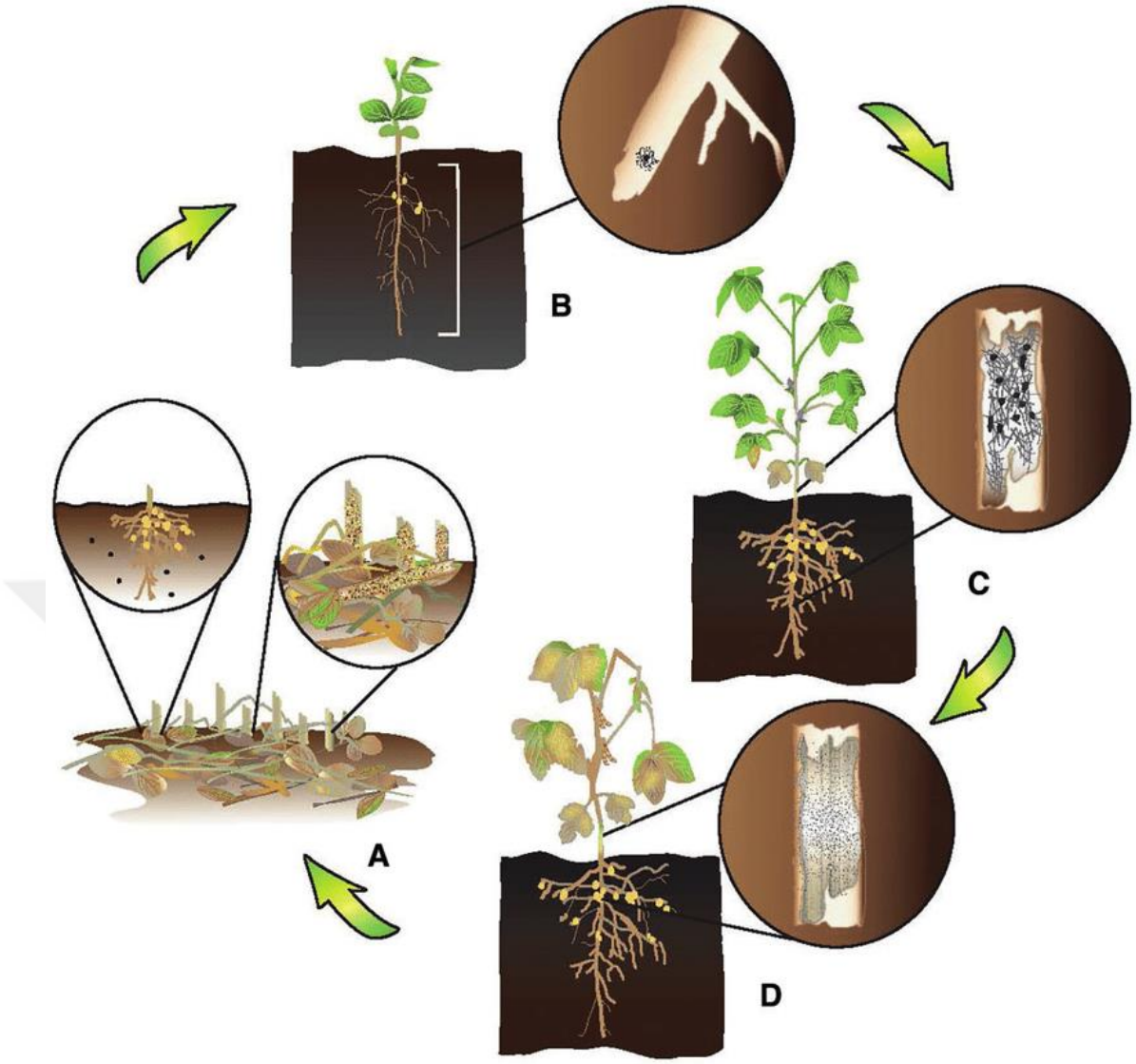

Figure 1.2 The stages of infection and transmittance in plant parts.

1.1.3 Factors Affecting the Growth of MP

1.1.3.1 Perpetuation and Over - Wintering

There has been a noted positive connection between the amount of MP present in the seedbed and the severity of the resulting disease. The fungus persists through the winter, transitioning from one season to the next, and can be found either as independent sclerotia in the soil or within leftover plant material. Factors such as mechanical harm, dense plant populations, and insect infestations are seen as contributing elements to the transmission of the disease (Ilyas et al., 1975; Shiekh and Ghaffar, 1984; Dodd, 1980; Ahmed et al., 1991; Cook et al., 1973).

1.1.3.2 Host-Pathogen Interaction

The host and pathogen binding and the pathogen's ability to infect the host are highly dependent on the amount of interaction between host and pathogen. The concepts of resistance and susceptibility are inherent traits (Yang et al., 1999). Fresh insights into the pathogenic relationship between MP and sunflowers have recently emerged. Consequently, it is imperative to comprehend the genetic aspects and the behaviors of both the host and the pathogen throughout the disease development process and their interconnected relationship. This understanding is essential for the development of robust breeding programs aimed at disease resistance. Numerous endeavors have been undertaken to explore diverse facets of the interaction between host and pathogen, particularly in the context of the infection pathway.

1.1.3.3 Disease Cycle

MP, a warm climate pathogen, primarily targets hosts during periods of low rainfall and elevated temperatures reaching 35 to 40 °C (Sarr et al., 2014). The disease cycle of the pathogen is further divided and completed into four phases germinated phase, penetrating phase, parasitic phase, and saprophyte phase (Dhingra and Sinclair, 1978). This pathogen can persist in soil and crop residue as microsclerotia, a type of aggregated hyphal cells, for 3 years, acting as a major cause of infection during favorable conditions (Su et al. 2001; Tonin et al. 2013). These microsclerotia, which range in shape from spherical to oblong and in color from black to chocolate brown, germinate under optimal conditions (28–35 °C) to produce hyphae. Under conditions of water stress in the host, these microsclerotia germinate on the surfaces of roots, generating appressoria that breaches the cellular walls of the epidermal layer of cells by normal openings (Olaya and Abawi, 1996). Additionally, MP can infect plants through cuts to the surface of roots. After infection; hyphae invade roots proceeding through the intercellular matrix by deep penetration into the cortical layer of tissue. Finally, the vascular structure of the plant is infiltrated by the hyphae that spread deeply in the entire system of roots leading to the generating mycelia and sclerotia within the vascular tissue. This whole procedure causes the blockage of vessels of the xylem (Babu et al., 2007) hindering the transportation of nutrients and water from the roots to the different and upwards plant structures. Consequently, the host wilts and can succumb to systemic infection. Throughout the process of infection, multiple

toxins including botryodiplodin/phaseolinone, are released as a result of reactions of enzymes and the mechanical pressure leading to the progression of disease (Bhatt and Vadhera, 1997; Abbas et al. 2019). Furthermore; the whole progression cause desiccation of the plant with decaying roots giving a shredded look. Notable disease symptoms such as lesions on stems and roots in charcoal brown color, dark mycelia, and black microsclerotia are observed. As soon as the decomposition of tissues initiated; colonization of mycelial and formation of microsclerotia started to show within the host tissue. Additionally; these colonies started to be distributed in the soil causing the degradation of debris of infected plants, perpetuating the cycle of infection. In natural settings, microsclerotia can endure severe environmental parameters, underscoring their crucial part in the pathogen's cycle of life, epidemiology, and the initiation and progression of the disease. Multiple factors of the environment including such as moisture, temperature, and relative humidity have an important part in influencing the pathogen's survival and spread.

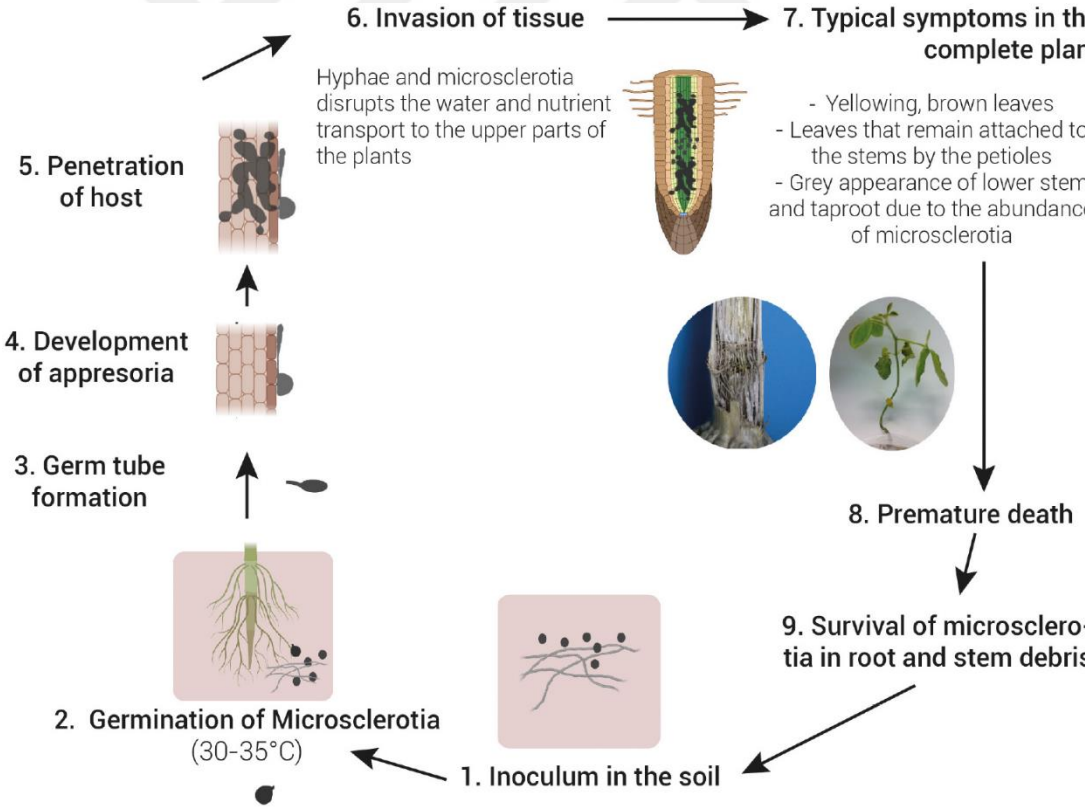

Figure 1.2 Life cycle of microsclerotia

1.2 Aim and Objectives

Knowing the inoculum amounts is especially important in determining effective control methods against soil-borne plant pathogenic fungi. In this study, studies were carried out to determine the amounts of *M. phaseolina* sclerotia in soil samples taken from 20 districts of 7 provinces where corn is grown in Türkiye. It is thought that the data obtained will support studies on the control of *M. phaseolina*.

CHAPTER II

LITERATURE REVIEW

Due to *Macrophomina phaseolin's* significance as the cause of the charcoal rot disease on a broad list of crops that are grown for various purposes, including food, oil, and biofuel industries, numerous research was undertaken worldwide to define the variety of MP populations. Phenotypic characterization and the use of data in breeding and disease management programs were the main goals of study in the latter part of the XX century. (Smith, 1969: Mirza, 1984; Kaiser and Das, 1988) enlightened the global issue caused by fungal infections and an abnormal response to changes in climate conditions towards the legumes. The diseases caused by fungus lead to restriction of the production of legumes through dry and charcoal rooting of roots. MP is an important disease of legumes. The fungus has the potential to spread from the soil to roots, stems, and seeds. According to the data, this fungus belongs to the root inhabitant fungi group developing black sclerotia in a tube or cushion form with a diameter of 1-8 mm. These sclerotia are a vital part of the organism's life. (Ghaffar and Erwin, 1969) stated that decreased moisture in soil or water in plants is considered to be more susceptible to disease caused by MP than elevated temperature. More severe infections due to *Macrophomina* sp occur if the soil dries out during the stage of seedling or at the ripening of seed. Significantly; 15 to 28 days observed an increase in temperature and water level. However, in Arizona, sorghum faced severe drought conditions during the flowering stage. Except for flowers, the rate of charcoal rotting is further affected by the water level in the sorghum grain during the stage of grain filling in regions having semi-arid conditions (Pande et al., 1989). The survival rate of pathogens is further observed through soils of loam as sandy and silty clay, having various gradients related to moisture. However, having raised moisture levels with an extended period of incubation helps to reduce the growth of these pathogens, especially in such kinds of soils and loams. According to reports; fungus population rapidly increased within 50 to 60% soil moisture content but the number of bacteria increased by 5-6 times during the incubation period of 7 weeks. Additionally; the rate of rotting in plants is also observed during rain session that further confirmed the effect

of increased moisture level of soil on fungal population rate and sclerotia reduction (Ghaffar and Erwin, 1969). The results of Dhingra and Sinclair (1974) concluded tant the bacteria presents in unimproved soil can attack MP during high moisture level similarly like in germ tube mycelium attacked by bacteria leading to decomposition and digestion of cellular wall of fungus. However, fields of cotton, soybean, maize, wheat, and alfalfa showed decreased sclerotia numbers on contamination (Meyer et al., 1973). The density of inoculum is highly associated with severity of disease while infection is mainly regulated by soil moisture (Keim & Webster, 1974). When a field is flooded for irrigation, the sclerotia float freely on the surface and form the principal inoculum for sprouting seedlings (Keim, 1974; Webster et al., 1976). The dislodged sclerotia started to float on the surface of water with the bed of flooded seed, or any other force disrupting the surface layer throughout the growth season, such as water wave motion caused by wind. Under dry soil, the fungus may persist for more than ten months.

The disease severity is proportional to the number of sustainable sclerotia present in the soil. The mycelium of MP in the soil, on the other hand, does not considered as the only cause of inoculum (Meyer et al., 1974). Up to 25 sclerotia of MP have been found in 1g soil in Pakistan, and data suggests a single cell from sclerotia have the capability to kill the whole plant. Additionally, MP strain can raise the pre-emergence rate of mortality by infecting the whole embryo (Burney et al., 1984; Dhingra and Sinclair, 1978). These strains can easily penetrate within 5 to 10 mm thickened membrane that is clear out of damage while those sections of MP are free of pre-emergence capability move towards the upper and other parts of plants. The amount of nutritional absorption of such strains with the tissues of affected plant is connected to their pathogenicity (Khan et al., 2000).

Having a larger variety and range of host plants, MP can cause deaths of more than 500 plants that can either be cultivated or belonging to any wild species (Kunwar et al., 1986). So far, 67 economic hosts of MP have been identified in Pakistan, including cotton, rice, maize, cucurbits, okra, and wheat (Shahzad et al., 1988). MP has a broad host range, indicating that it is a non-host specific fungus. The fungus' physiological specialty has yet to be shown. Even when isolated from various regions of the same plant, there is a lot of heterogeneity in morphology, physiology, and pathogenicity stated that variations observed in level of water and temperature affecting the

metabolism, growth, and regeneration capacity of the fungus present in the soil (Olaya and Abawi, 1996).

Tan et al. (2008) stated born Canadian man originally belongs to Sri Lankan became infected with the fungal plant disease MP as he was having renal transplants surgery in India. *Scytalidium dimidiatum* infection led to the patient's death. The identification was confirmed by molecular sequence analysis as they belonged to the family named as Botryosphaeriaceae-Ascomycete. The reclassification of *S. dimidiatum* as *Neoscytalidium dimidiatum* and *Nattrassia mangiferae* was highly discussed in this study. Pervious cases including this study show how plant pathogenic fungus can induce invasive human diseases that are resistant to antifungal treatment.

According to researches and best management, related to crop specific, techniques concluded nitrogen leaching shoulder be decreased in improving the cultivation of sweet corn crops especially *Zea mays* L. var. *saccharata* (He et al., 2012). The irrigation of sweet corn on sandy Florida land along with BMPs of nitrogen was studied in the current research through the usage of Agrotechnology transfers model named as CERES-maize. In single factor simulations, 24 irrigation schedules, 21 N, 30 N, and 20 N were carefully investigated. Then, in a multifactor study, 54N fertilizer and total six irrigation application techniques focused on timing and amount was used for investigation of single factor.

Sweet corn output was strongly influenced by irrigation frequency. Similarly; when soil water was depleted more than 60%, the growth of corn yield is highly reduced. This reduction is described as the result of irrigation associated to MAD (max. allowed depletion).

Above 168 kg N ha1, the increase in yield was close to nil. If N fertilizer was applied through small or large leaf stage, it had no effect on yield; however, when zero N applied in stage of small leaf, the least amount of N leaching occurred. The yield simulation is significantly depended on the application of N ha 1/ fertigation as it increased with <100-70 kg N ha 1 and modestly raised at <70 kg N ha 1/ fertigation with lesser leaching changes of N on plants. Total 6 viable of BMPs were chosen as optimizing yield while limiting N leaching from 324 management scenarios. These whole six plans included two schedules of irrigation, two N levels and split plans respectively along with two application techniques of N/ fertigation. The irrigation

should be performed at the depth of 5 mm with 20% MAD value with 7.5mm having 30% value. The N level and split plans are adjusted with 196 Kg N ha1 of 0-1/4-3/4 and 224 with 0-1/3-2/3. These splits would be applied on all small and large leaf's during their stages of development. The whole plan was completed with the application of N at the rate of 30 and 40. It should be noted that these conclusions are references based on modelling hypothesis, and their practical and economic viability should be evaluated in actual field operations.

In the cut-stem method Othello was only infected once, whereas the other genotypes received a 2nd with 3rd inoculation at the lateral side branches of plants, showing resistance, 7 days following the first inoculation (even till harvest) in the greenhouse is a rigorous test (Twizeyimana et al., 2012). This was done to check the genotypes showing higher level of resistance during their reproductive and vegetative phases towards ASB. It might be accompanied with screening of ASB in the testing field. The tested bean genotypes including 195PC 50, I 9365-31 along with 92 BG -7 showed fractional resistant towards the MP. To improve ASB resistance levels, it is therefore required for the proper identification of the genetic pool of Phaseolus that described as resistance genetic parent. Secondly; the resistance in seeds was also increased by the combination of resistant gene/ QTL with germplasm. Finally; MP played a pivotal role in the production of bacterial nanoparticles (Ag/AgCl) in the crops providing extra protection to yields.

Lakhran et al. (2018) stated that the roots and internodes at basal area have the fibrovascular system played an important role in the transportation of water and minerals to entire plant's parts. This system was attacked by MP causing progressive wilting, early death, loss of vigour, and lower output along with damping effect, rotting of stem and roots, blighting of seeds and fast maturity of sunflower crops are all caused by the pathogen. Moreover; the common symptoms that emerge after flowering includes grey dark staining, shredding and hollowing of stem and tip of the taproot. MP can be distinguished by using either light or dark brown septal hyphae or thin-walled hyaline hyphae. On parent hyphae, branches from the main hyphae are usually generated from the constricted origin portion angled at right angle. Micro-sclerotia is a spherical and oval shaped light brown mycelium, hardened in structure that become brownish black in color on its mature phase. On the other hand; a larger and dark brownish colored, rough and irregular shaped structure rarely seen in nature is named

as Pycnidia; highly different from former mycelium. These kind of difference and heterogeneity is extremely detected on the synthetic medium. This difference includes distribution of microsclerotia, color, and production of pycnidia with phenotypes of chlorate.

Nonetheless, magnification of the internal transcribed spacers (ITS) revealed that the isolates were all from the same species (Almomani, et al., 2013). The morphological polymorphism could be related to the fungi's responses to environmental variables or variety in the species of hosts, according to certain theories. He also has shown a strong link between virulence and phenotype (morphological changes). According to the reports described that because of the economic prospects and prospective applications in several disciplines, a greater attention is diverted towards the development of metallic nanoparticles by using living things and naturally occurring materials. We employed the MP's exudate to create firm nanoparticles of silver & silver chloride (Ag/AgCl-NPs) using a low-cost green synthesis approach. The synthesis of NPs was further adjusted using reaction parameters such as medium and AgNO3 concentration. Silver NPs has the surface plasmon resonance with a peak around 420 nm, according to spectral analyses. SEM analysis revealed NPs with a spherical shape and a size range of 5–30 nm. X-ray diffraction (XRD) analysis was used to check the crystalline nature of the produced NPs. The green NPs have antibacterial action against both gram-positive and negative bacteria. Fungi and yeast cells showed little effect, whereas bacteria growth kinetics showed a significant inhibitory effect. Moreover; disruption of cellular membrane and raised oxidative stress is observed in SEM through the binding of bacteria and Ag/AgCl-NPs that defined the significant bactericidal activity. The Ag/AgCl-NPs and its dosage was further investigated to analyses its seed protection ability on soybean (*Glycine max L.*) seed during its germination phase. The introduction of these biogenic particles and its dosage had no noteworthy effect on germination, length of seed incubation along with zero oxidative damage.

Main variables affecting sunflower plant growth are rotting of roots and charcoal rotting triggered through MP (Nafady et al., 2019). The goal of study was to analyze the effect of combined introduction of *Brettanomyces naardensis* and arbuscular mycorrhizal fungi in the soil along with farming with crops. Being a growth enhancing yeast and fungus, it is quite powerful in controlling the colonization of MP causing

deformity and harm. This prevention is due to production of indole acetic acid, NH 3 and good solubilization properties for phosphate. Farming the crops yeast and AMF considerably improved height of stem, weight of dry plants, and number of leaves in affected and unaffected crops. The results validated the marked reduction of malondialdehyde content (MDA) with boosting of availability of different nutrients in the sunflower's immune system by the injected combination of yeast and AMF that finally upgraded growth with decreased symptoms. The mixture of bio-fungicide with bio-fertilizer in single formula, a marked protection of crops is gained through this technique of bio-controlling. As a combined in one bioformulation, the double bioagents present a promising biocontrol technique in crop protection.

Khan and Javaid, (2020) described that Quinoa (*Chenopodium quinoa* Willd) is a South American grain that has lately made its way to Pakistan. Previous research has shown that wild Chenopodium species from Pakistan have antifungal properties against the plant disease MP, which is spread by seeds and soil. Quinoa stem was tested for antifungal activity against this damaging plant disease in this study. Methanolic stem extracts (1-5%) of four kinds of quinoa were tested in vitro for their antifungal effects on diseased mung bean plants. Various extracts exhibited strong antifungal efficacy and reduced fungus biomass by 48–89%. Quinoa variant V7 performed best against the pathogen, reducing its development by 80–89%. The polarity of four organic solvents were used to fractionate the methanolic extract of this cultivar. For each fraction, concentrations of 1.562 to 200 mg mL-1 divided into eight sets were finally used in the bioassays especially of antifungal. The pathogen's growth was completely stopped at every concentration of n-hexane and chloroform fraction. Ethyl acetate and n-butanol fractions were shown to be less antifungal from 66 to 100% and 52 to 100% declines in pathogen biomass, respectively. The existence of significant antifungal chemicals in n-hexane and chloroform fractions was indicated by GC-MS analysis of 9,12-octadecadien-1-ol, (Z, Z)- (22.23%), followed by 8,11-octadecadienoic acid, methyl ester (16.68%), 1,2-benzedicarboxylic acid, disooctyl ester (12.48%), and hexade to summarize, the n-hexane and chloroform fractions of the stem extract of the V7 type of quinoa include potent antifungal components against the pathogen.

Sridharan et al. (2020) evaluated that *Trichoderma longibrachiatum* EF5 is a fungal endophyte that attacks rice. *Sclerotium rolfsii* and MP are two soil-borne fungal

diseases that are treated with it. *T. longibrachiatum EF5* suppresses the progression of these parasites both directly and through the formation of microbial volatile organic chemicals (mVOCs). By changing the mycelial structure, the mVOCs slowed mycelial growth and stopped the generation of sclerotia. When T. longibrachiatum EF5 came into contact with the two pathogens, we measured 138 mVOCs. Several molecules, including longifolene, caryophyllene, 1-Butanol 2-methyl, cedrene, and cuprenene, are up- or downregulated by *T. longibrachiatum* EF5 during these interactions. These chemicals are involved in the sesquiterpenoid and alkane biosynthesis processes and the degradation pathway of trimethylamine. The light was thrown on the numerous areas in which *T. longibrachiatum* EF5 exerts antagonistic activities, containing hyperparasitism, competition, and antibiosis via mVOCs. These metabolites, in addition to their antibacterial capabilities, may also encourage plant development**.**

Upadhyay et al. (2020) stated that soybean is a widely grown oilseed. Soybean yields are lost by charcoal rotting due to MP borne in soil. Fortified MP and cultural medium of MS was used to generate a toxic culture containing a purified filtrate. This filtrate was used to treat callus and cellular suspension creating from embryonic axis of both immature and mature nature and cotyledon explants from soybean suspected as diseased. The toxic medium was selected through four cycles of continuous method while a non-toxic medium was selected during 2^{nd} and 3^{rd} cycle of discontinuous method of silence. This method of discontinuation played an important role in the regeneration of calli. Constant contact to hazardous culture filtrate resulted in mortality rates of 65-75 percent. During S1 generation, four JS335 lines and nine JS95-60 lines were discovered resistant among a variety of putative resistant/tolerant lines.

Banaras et al. (2020) stated that the goal of this research was to find antifungal chemicals in *Sonchus oleraceous L.* (family Asteraceae) that could be used to combat *M. phaseolina* that is an exceedingly damaging fungoid infection. Initially, 10 to 50 mg mL-1 extract of methanolic of this Asteraceous weed's stem, leaf, inflorescence, and root were tested against *M. phaseolina* that decreased fungoid biomass respectively from 54–84% to 7–73%. This reduction further observed as 51–87%, and 49–82%, respectively uncontrolled. Four solvents having different polarities were used for the separation of the extract from the stem. Tests in laboratories with concentrations ranging 3.125- 200 mg mL^{-1} found that the fraction of chloroform and n-hexane fraction had the significant antifungal properties helps in managing and controlling

biomass to 60–90% and 15–66% respectively. The chloroform fraction was subjected to GC-MS analysis, which yielded the identification of 14 chemicals. Hexadecanoic acid, 13.26% methyl ester, 13.12 % of 11-octadecanoic acid, methyl ester, 12.96% of 9, 12-octadecadienoyl chloride, (Z, Z), 8.62% of 1-docosanol (8.62%), and 8.28% of 1, 2-benzenedicarboxylic acid, disooctyl ester were the predominating chemicals in this portion.

It is stated that *M. phaseolina* is the causative agent of rotting disease development in melons, which reduce its production globally (de Sousa Linhares et al., 2020). Although using resistant cultivars to treat this disease is a good idea, still very little knowledge is available about the temperature effects on the resistance activities of identifying the accessions related to melon. The goal of this research was to see how temperature affected the reactions of six accessions of melons that had earlier been chosen for their *M. phaseolina* resistance. In a repeatable experiment, *M. phaseolina* isolated form CMM-1531 was used for the inoculation of the accessions that further cultivated in extremely managed and controlled conditions having varied temperature values as 25 to 28°C and 31 to 34°C. This variation in temperature showed exacerbation of signs in the most of species however 'Piel de Sapo,' and ‘agrestis Ag-15591 Ghana’ as wild plant of African showed resistance towards these temperature changes. Being resistant and sensitivity control accession; this behavior can easily be explained. This temperature variation helps in the grouping and classification of QMPAfg, Con-Pat81Ko, Ag-C38Nig and Can-NYIsr in one group by showing similar resistance behavior at 25 °C with severe damage at raised temperature. However; last two plants and Dud-QMPAfg had shown moderate resistance at 28°C to 31°C while Con-Pat81Ko showed significant resistance till 31°C. However, all the formers’ types were vulnerable with increase in temperature especially at 34 °C showing that inability to survive at hot weather conditions. Among all, Ag-15591 Ghana shown significant resistance at the temperature range above 34 °C while growing in the greenhouse that still needed to be studied under the natural growing conditions and fields with infestation, the controlled screening procedures described here are critical for characterizing new resistance sources and conducting genetic investigations where a greater amount of plants needed to be managed in a controlled setting.

It was stated that charcoal rotting, caused by *M. phaseolina* is major disease of green and black gramme in Pakistan, causing production losses of up to 40%. As no options

is available for the long-term management and controlling of *M. phaseolina* and affecting seeds and soil, seven indigenous *Trichoderma* species were examined *in vitro* and in vivo for their efficiency against MP with the goal of identifying alternatives to pernicious fungicides in the current study (Iqbal and Mukhtar, 2020). In vitro, all seven *Trichoderma* species considerably slowed *M. phaseolina* development. *T. harzianum* caused the greatest reduction (79.63 percent), followed by *T. hamatum* (76.3 percent), and *T. pseudokoningii* caused the smallest reduction (58.14 percent). *Trichoderma* species also had an impact on the quantity and size of sclerotia.

In the presence of *T. hamatum, M. phaseolina* produced the fewest sclerotia, followed by *T. harzianum*, which reduced the number of sclerotia by 69.5 and 66.84 percent, respectively, above control. *T. harzianum* caused the greatest reduction in sclerotia size. *T. harzianum* had the best green and black gramme plant survival, followed by *T. hamatum* and *T. viride*. At a concentration of 2108 (propagules/ml), *T. harzianum* produced the highest individual germination (86.67 %), whereas *T. pseudokoningii* produced the lowest (33.33 %). Plant survival was likewise affected by *Trichoderma* concentrations, with the maximum concentration having the greatest effect. The survival rate of plants was reduced as antagonist concentrations fell, indicating a direct link between plant survival and antagonist concentrations.

Ghosh et al. (2018) stated that *M. phaseolina* is a significant necrotrophic phytopathogenic fungus that causes significant harm to several oilseed crops. Twelve isolates of *M. phaseolina* with different biological characteristics were chosen for the investigation of the bio-informatics along with sequence based meta-transcriptomic to find viruses that infect *M. phaseolina*. The study discovered 40 incomplete or almost full viral genome segments, with 31 of them being unique viruses. 43% of the viral genomes in this study were found to be the majority of viral genomes were double-stranded RNA (dsRNA), whereas the remaining 47% were positive single-stranded RNA (ssRNA). Negative sense-stranded RNA (ssRNA) accounted for ten percent of the total. Two viruses belong to the Potyviridae and Bromoviridae families, which previously had no mycovirus species.

In addition, nine new Totiviridae, Endornaviridae, and Partitiviridae viruses were discovered to be linked with *M. phaseolina*. Multiple viral co-infection is common in MP, with each isolate containing anything from 3-18 viruses. In addition, the impacts

of the viruses on the hosts of fungus were investigated based on the biological parameters of each isolate. MP hypovirus 2, MP fusagravirus virus 1-5 (MpFV1-5), MP endornavirus 1-2 (MpEV1-2), MP ourmia-like viral 1-3 (MpOLV1-3), MP mitovirus 4 (MpMV4), and MP mycobunyavirus 1-4 (MpMBV1-4) were all found solely in hypovirulent isolate. Hypovirulent viruses could be utilized as biological control agents instead of chemical fungicides, which would be more environmentally friendly. These findings add to our knowledge of mycoviruses in MP and show that there is a significant difference between viruses isolated from various hosts in different geographic regions. The current research adds to our understanding of viral evolution and fungus-virus coevolution.

According to Javed et al. (2021), having an antifungal property, the extract of *Monotheca buxifolia* was used in different fractions for the testing of MP as it is a specie having more than 500 types that are highly opportunistic and rare. Fungal biomass was decreased by 39–45 percent at different doses of methanolic extract (3.125 to 200 mg mL1). Fungal biomass was inhibited by 32–52, 29, and 29–35%, respectively, in isolated n-hexane, chloroform, and ethyl acetate fractions. The triterpenes lupeol and lupeol acetate (1, 2) were recovered from n-hexane, while the chloroform fraction yielded betulin, -sitosterol, -amyrin, and oleanolic acid (3–6). Vanillic acid, protocatechuic acid, kaempferol, and quercetin (7–10) were extracted from the ethyl acetate fraction and identified by mass spectroscopy and nuclear magnetic resonance (NMR).

The isolated compounds at various doses (0.0312 to 2 mg mL1) was assessed and compared to that of the broad spectrum fungicide mancozeb. Mencozeb was found to suppress fungal biomass by 83–85 percent at various doses. Lupeol acetate (2) was found to have the strongest antifungal activity against MP, followed by betulin (3), vanillic acid (7), protocatechuic acid (8), -amyrin (5), and oleanolic acid (6), with antifungal activity of 79–81 percent, 77–79 percent, 74–79 percent, 67–72 percent, 68–71 percent, and 68–71 percent, respectively. The remaining compounds were also antifungal, reducing MP biomass by 41–64 percent.

CHAPTER III

MATERIALS AND METHODS

3.1 List of Chemicals and Instruments

Table 3.1 A list of chemicals and instruments used during this study.

Instrument and chemical name	Company name
Agar	Sigma-Aldrich
Potato dextrose agar	Merck KGaA
Tween 20	Sigma-Aldrich
Hydrochloric acid	Sigma-Aldrich
Sodium hypochlorite solution	Domestos
Weighing balance	Dikomsan
Magnetic stirrer with hotplate	Heidolph
Burner	
Tripod stand	
Spatula	
Sieve 177 mm, 44mm diameter	Elegi
Beaker	Ertick
Measuring cylinder	Eisco
Petridis's glass	
Aluminium foil	Commercial use

3.2 Methodology

3.2.1 Soil Sample Assay

3.2.1.1 Preparation of Soil Sample

The study was carried out in the mycology laboratory of the biology department in Gaziantep, Türkiye between 2021 and 2022 with little modification (Altınok and Can, 2010). Soil samples were collected from twenty districts of Türkiye. The field number, tag number (isolate name), and GPS data were collected. Each sample was more divide was more divided into sub-samples. Approximately up to 10 grams of soil

sample was taken, to isolate several MP from soils in contact with the through the sterile sieve of mesh 177 mm then 44 mm in diameter. Soil samples contained large particles were removed.

Figure 3.1 Steps in soil sampling of microsclerotia A) Sieve of mesh size 177 mm and 44 mm diameter, B) 177 mm diameter sieve screening the large particles, C) 44 mm in diameter sieve with required particle size, D) weighing of sample

3.2.1.2 Post Elimination Process Soil

A 50 % sugar solution was made and 0.25 grams of sample was transferred to it. Using the difference in intensity from the decomposition of microsclerotia in the soil particles it is expected that the parsing process will take 5 minutes. After this, the remaining

microsclerotia will be found on the surface. Before this step, sterile preparation had been undertaken, and areas of 1 cm^2 divided filter papers were used for sampling.

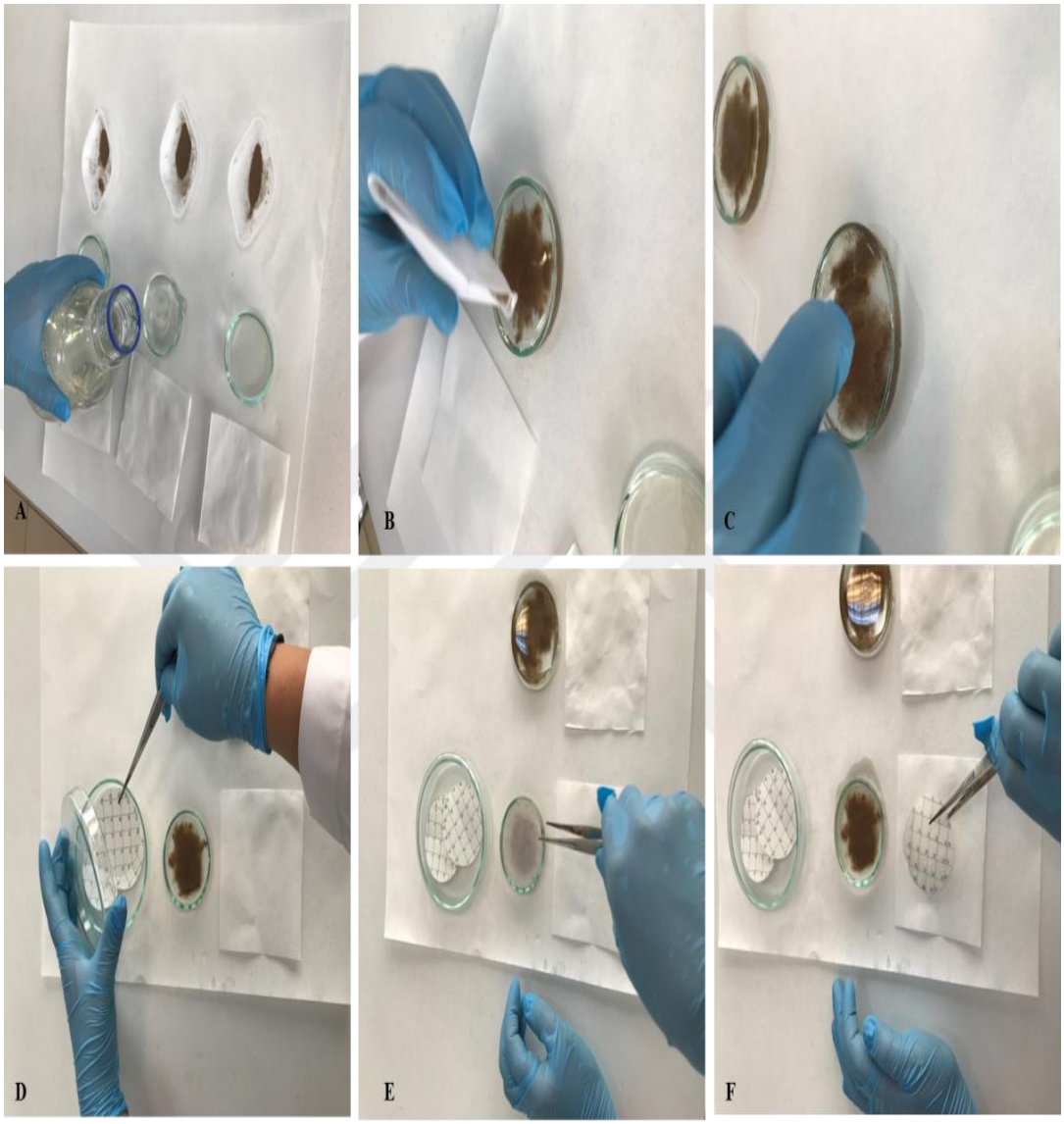

Figure 3.2 Screening process post- microsclerotia parsing and sterile filter papers withdraw. A), B), C) microsclerotia parse; D), E), F), G), H), I) microsclerotia in areas of 1 cm 2 divided sterile filter papers were withdrawn

3.2.1.3 Analysis of Sample Using Microscope

The filter paper was analyzed under a stereo microscope total average number of microsclerotia was counted from 1 gram of soil. Repetitive attempts were made. Data of count was made from soil survey information of samples and sweetcorn also.

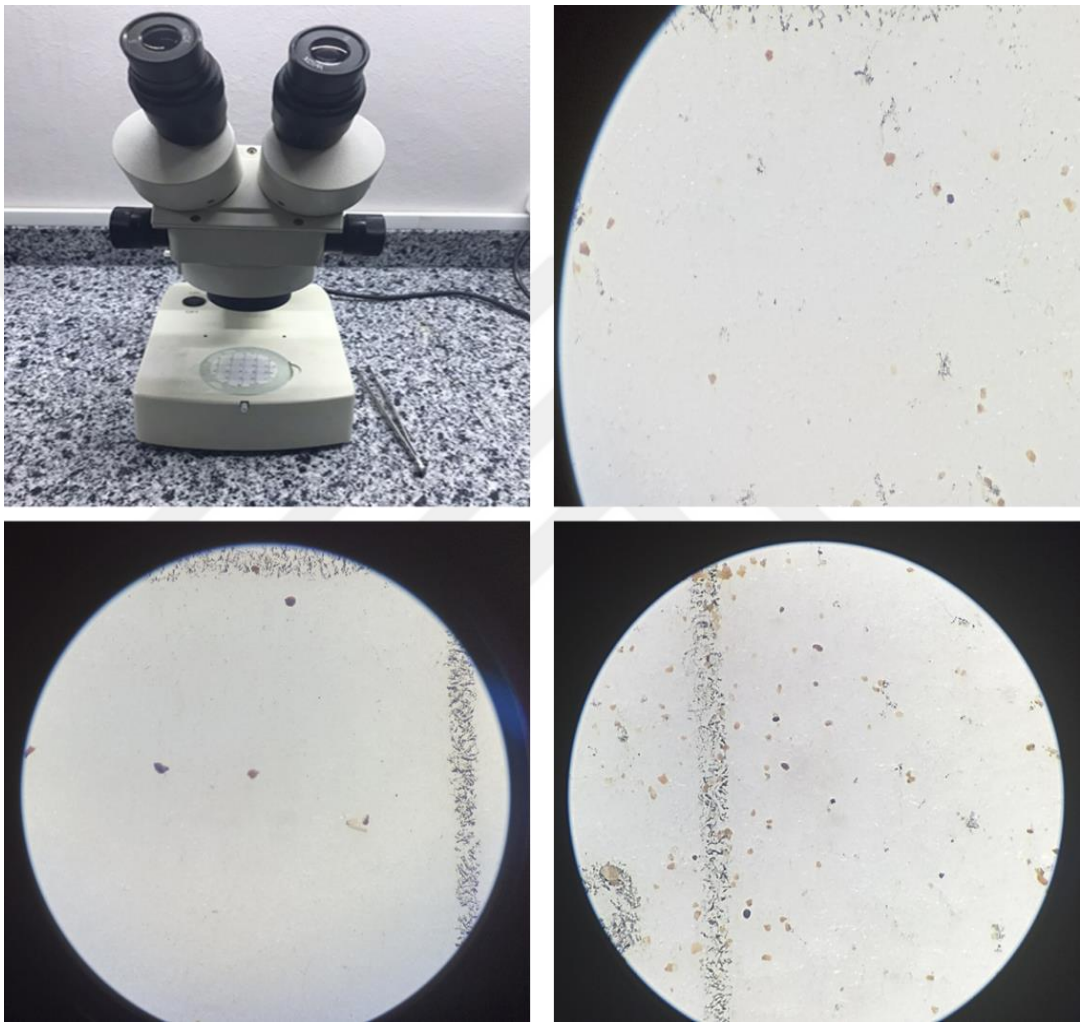

Figure 3.3 Microsclerotia binocular stereo microscope image of microsclerotia

3.2.1.4 Preparation of different medias for incubation

After the completion of the counting process, the filter papers bearing microsclerotia were densely packed and subjected to surface sterilization using a 1.5% NaOCl solution with the addition of 0.5ml/L Tween 20 for 5 minutes. Subsequently, the papers

were rinsed with sterile distilled water and subjected to surface sterilization. To facilitate the process, the filter papers were smoothly opened on water agar and ½ PDA (Potato Dextrose Agar) medium at a temperature of 27-28°C. After 24 hours of incubation, the hyphal developments were closely monitored, and the formation of microsclerotia was observed. Cultures with microsclerotia were subcultured in ½ PDA medium and left to incubate at 27-28°C. After a further 3 days of incubation, a technique involving the reduction of spores from the cultures was used to obtain pure

cultures, as shown in Figure. These pure cultures were prepared into glycerol stocks and stored at -20°C for future studies.

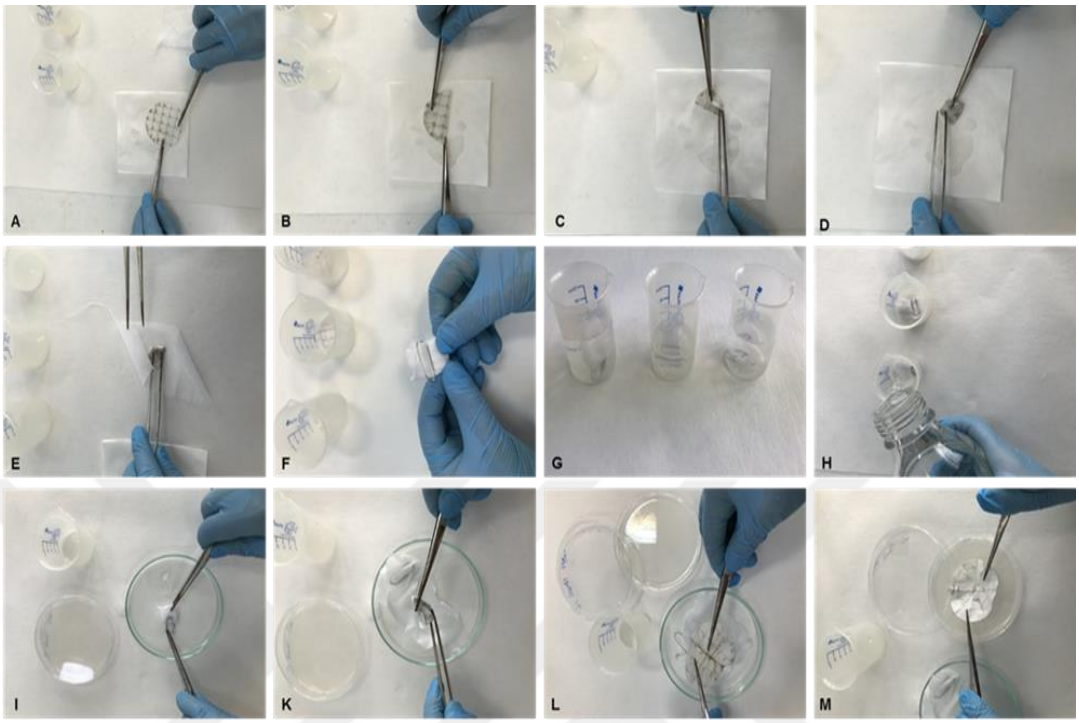

Figure 3.4 The insulation stages A), B), C), D), E) and F); Packing of microsclerote-bearing filter papers, G), Surface sterilization with 1.5 % NaOCl solution for 5 minutes, H); Rinsing with distilled water, I), K) and L); Opening the packaged filter paper after rinsing , M); Placing the filter papers containing microsclerotia in ½ PDA medium.

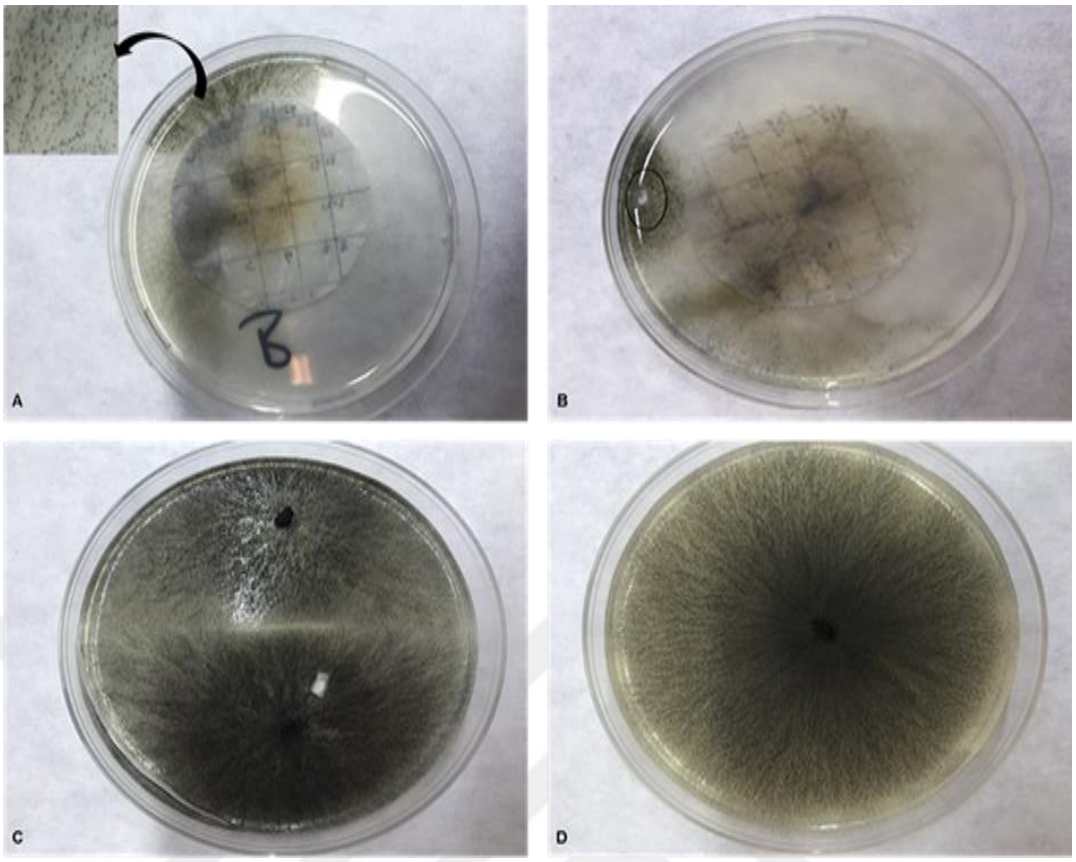

Figure 3.5 *Macrophomina phaseolina* culture A) insulation, B) Cultures with microsclerotia C) Subculture in ½ PDA medium D) Pure culture

CHAPTER IV

RESULTS

The field number, isolate first name, and Global Positioning System of districts from where exact soil samples were taken are given in Table 4.1 and Figure 4.1.

Table 4.1 Field number, isolate name and GPS data of soil samples

Field No	Isolate Name	GPS Data (N/S)
1	Adana/Seyhan-3	36.981663/35,120838
2	Adana/Yumurtalık-3	36,86459/35,839062
3	Adana/Yregir-8	36,920918/35,380848
4	21 Bismil /Celtikli-1	37,862928/40,878107
5	21 Bismil /Coltepe-1	37.846398/40,801951
6	21 Bismil /Gultepe-2	37,865106/40.816748
7	21 Sur/Tanoglu-1	37.931046/40.259247
8	Gaziantep-3	37.23/37.1852
9	Hatay-13	36.377888/36.415657
10	Hatay-15	36.347241/36.404121
11	Hatay-17	36,367775/36,450237
12	Hatay-18	36,324127/36.416363
13	Hatay-26	36.372784/36.226391
14	Kahramanmaraş-1	37.317211/36.808567
15	Kahramanmaraş-4	37.370518/36,84964
16	Mersin/Silifke/Arkum-4	36,350216/34,027802
17	Mersin/ Silifke/Çeltikçi-1	36.345779/33,982803
18	Mersin/Tarsus-3	36,89397/34.857777
19	Mersin/Tarsus/Akarsu-6	36.876766/34,965076
20	Osmaniye-1	37.341309/35.925613

Figure 4.1 Sampling hotspots and Global Positioning System of districts of Türkiye

Table 4.2 Province, GPS data, field area, total microsclerotia and disease severity in the field

No.	Province	District	Neighborhood	GPS Data (N/S)	Field Area (Da)	Number of microsclerotia (1 gr soil)	Disease severity in the field %
1	Adana	Yüregir	İncirlik	36.97154/35.44292	160	290.67	88.89
2	Adana	Yüreğir	İncirlik	36.96141/35.44439	140	254.67	0
3	Adana	Yüreğir	Ismailiye/Köprüce	36.94583/35.43208	50	73.33	48.61
4	Adana	Yüreğir	Camili	36.93087/35.43566	930	193.33	88.89
5	Adana	Yüreğir	Köklüce	36.92356/35.4223	250	332,00	88.89
6	Adana	Yüreğir	Yukarıçiçekli	36.92092/35.38085	30	466.67	40.28
7	Adana	Seyhan	Gökçeler	36.98166/35.12084	90	534.67	88.89
8	Adana	Seyhan	Gökçeler	36.96842/35.11316	50	280	0
9	Adana	Yumurtalık	Gölovasi	36..88911/35.86871	30	458.67	83.33
10	Adana	Yumurtalık	Narlıören	36.86459/35..83906	400	301.33	61.11
11	Adana	Ceyhan	Merkez	37.04455/3577816	100	290.67	30.56
12	Osmaniye	Kadirli	Çukurköprü	37.34131/35.92561	100	406.67	55.56
13	Osmaniye	Kadirli	Kiremitli	37.35209/35.95121	100	342.67	27.78

No.	Province	District	Neighborhood	GPS Data (N/S)	Field Area (Da)	Number of microsclerotia (1 gr soil)	Disease severity in the field %
14	Osmaniye	Kadirli	Göçmenler	37.361374/36.01588	800	373.33	27.78
15	Osmaniye	Bahçe	Taşoluk	37.1731/36.50616	30	52	0
16	Osmaniye	Bahçe	Taşoluk	37.17939/36.49546	200	36	0
17	Osmaniye	Düziçi	Akçakoyunlu	37.18887/36.42515	12	282.67	29.17
18	Osmaniye	Düziçi	Yarbaşı	37.21016/36.41638	21	450.67	32
19	Diyarbakır	Sur	Tanoğlu	37.93105/40.25925	1000	634.67	48.61
20	Diyarbakır	Çınar	Şükürlü	37.80585/40.37037	80	878.67	34.72
21	Diyarbakır	Bismil	Obalı	37.50625/40.38365	450	145.33	0
22	Diyarbakır	Bismil	Korukçu	37.84898/40.74336	75	828,00	13.89
23	Diyarbakır	Bismil	Çöltepe	37.8464/40.80195	570	1281.33	38.89
24	Diyarbakır	Bismil	İsalı	37.84898/40.83216	1400	396	88.89
25	Diyarbakır	Bismil	Çeltikli	37.86293/40.87811	65	428,00	69.44
26	Diyarbakır	Bismil	Koyunlu	37.8624/40.95523	300	442.67	83.33
27	Diyarbakır	Bismil	Gültepe	37.85433/40.80828	100	384	36.11

No	**Province**	**District**	**Neighborhood**	**GPS Data (N/S)**	**Field Area (Da)**	**Number of microsclerotia (1 gr soil)**	**Disease severity in the field %**
28	Diyarbakır	Bismil	Gültepe	37.86511/40.81675	360	410.67	52.78
29	Diyarbakır	Bismil	Aralık	37.81545/40.70288	470	561.33	37.5
30	Diyarbakır	Bismil	Bozçalı	37.80749/40.73188	200	769.33	77.78
31	Diyarbakır	Bismil	Top output	37.79606/40.78539	160	393.33	36.11
32	Diyarbakır	Bismil	Kurudere	37.79605/40.83422	45	1174.67	13.89
33	Diyarbakır	Bismil	Kenberli	37.77391/40.80156	100	98.67	83.33
34	Diyarbakır	Sur	Yukarıkılıçtaşı	37.99379/40.25543	100	718.67	0
35	Gaziantep	Şahinbey	Bostancik 2	37.23/37.1852	15	532,00	56.25
36	Gaziantep	Şahinbey	Bostancik 3	37.23/37.1852	25	597.33	60
37	Gaziantep	Şahinbey	Çapalı 1	37.1011/37.2040	20	457.33	0
38	Gaziantep	Şahinbey	Çapalı 5	37.612/37.1944	20	118.67	0
39	Gaziantep	Şahinbey	Sarıt	37.1011/37.2111	13	709.33	2.78
40	Gaziantep	Oğuzeli	Gündogan	36.5232/37.3344	24	388	2.78
41	Gaziantep	Oğuzeli	Belören 2	36.5149/37.3420	50	97.33	0

No	Province	District	Neighborhood	GPS Data (N/S)	Field Area (Da)	Number of microsclerotia (1 gr soil)	Disease severity in the field %
42	Gaziantep	Oğuzeli	Çaybeyi 1	36.476/373520	25	424	8.33
43	Gaziantep	Oğuzeli	Büyükkaracaoren 3	36.4615/37.333	60	354.67	2.78
44	Hatay	Arsus	Arpaderesi	36.54684/36.08281	20	292,00	45.83
45	Hatay	Arsus	Arpaderesi	36.54337/36.08978	350	364	58.33
46	Hatay	Kırıkhan	Topboğazı	36.43906/36.30071	170	66.67	0
47	Hatay	Kırıkhan	Karadırmışlı	36.46483/36.38532	20	226.67	23.61
48	Hatay	Kumlu	Akkerpiç	36.37789/36.41566	140	408	23.61
49	Hatay	Kumlu	Karasüleymanlı	36.34724/36.40412	170	382.67	44.44
50	Hatay	Kumlu	Sökmen	36.36778/36.45024	130	565.33	44.44
51	Hatay	Reyhanlı	Suluköy	36.32413/36.41636	400	265.33	16.67
52	Hatay	Reyhanlı	Suluköy	36.27392/36.40316	700	245.33	27.78
53	Hatay	Antioch	Mustafa Kemal	36.33365/36.20615	60	318.67	11.11
54	Hatay	Antioch	Zülüflühan	36.37278/36.22639	200	266.67	80.56
55	Hatay	Arsus	Arpaderesi	36.54684/40.72125	20	292,00	45.83

No	Province	District	Neighborhood	GPS Data (N/S)	Field Area (Da)	Number of microsclerotia (1 gr soil)	Disease severity in the field %
56	Kahramanmaraş	Türkoglu	Akçalı	37.31721/36.80857	15	48	0
57	Kahramanmaraş	Türkoğlu	Geceli	37.3609736.84356	60	53.33	0
58	Kahramanmaraş	Türkoğlu	Geceli	37.37311/36.85576	90	996	88.89
59	Kahramanmaraş	Türkoğlu	Geceli	37.37052/36.84964	20	302.67	88.89
60	Kahramanmaraş	Pazarcık	Bölükcam	37.44622/37.20866	300	402.67	27.78
61	Kahramanmaraş	Pazarcık	Damlataş	37.45716/37.26327	250	233.33	88.89
62	Kahramanmaraş	Narlı	Merkez	37.38285/37.14376	90	556	88.89
63	Mersin	Silifke	Arkum	36.36878/34.04021	10	381.33	5.56
64	Mersin	Silifke	Arkum	36.35088/34.0308	10	57.33	0
65	Mersin	Silifke	Arkum	36.35022/34.0278	3	364	50
66	Mersin	Silifke	Kurtuluş	36.34307/33.98911	20	306.67	44.44
67	Mersin	Silifke	Çeltikçi	36.34578/33.9828	40	205.33	48.61
68	Mersin	Tarsus	Cezaevi	36.90508/34.9506	250	48	0
69	Mersin	Tarsus	Merkez	36.88718/34.96503	360	409.33	48.61

No	Province	District	Neighborhood	GPS Data (N/S)	Field Area (Da)	Number of microsclerotia (1 gr soil)	Disease severity in the field %
70	Mersin	Tarsus	Akarsu	36.87677/34.96508	530	252	88.89
71	Mersin	Tarsus	Akarsu	36.86255/34.9651	260	308	88.89
72	Şanlıurfa	Haliliye	Boydere	37.11205/38.87425	50	142.67	0
73	Şanlıurfa	Suruc	Yazıkoy	36.92671/38.535	75	1105.33	0
74	Şanlıurfa	Akcakale	Tatlıca	36.71998/38.91068	100	1176	0
75	Şanlıurfa	Akcakale	Şanlı	36.7605/38.75528	50	836	0
76	Şanlıurfa	Haliliye	Boydere	37.11205/38.87425	50	142.67	0
77	Şanlıurfa	Eyyübiye	Akçamescit	37.10108/38.84003	45	66.67	0
78	Mardin	Kızıltepe	Akça	37.19294/40.45346	220	118.67	0
79	Mardin	Artuklu	Çiftlikköy	37.28624/40.721245	220	116	0

The results of Table 4.2 showed that the number of microsclerotia in 1 g of soil was highest in Diyarbakır-Bismil (Çöltepe, Kurudere) and Şanlıurfa-Suruç (Yazıköy). The maize fields in Adana and Osmaniye provinces had the lowest microsclerotia. The severity of in disease in the field was highest in Kahramanmaraş (Pazarcık, Narlı, and Türkoğlu), Mersin (Tarsus), Diyarbakır (Bismil), and Adana (Seyhan, Yüreğir, İncirlik) provinces. The provinces of Mersin, Gaziantep, Mardin, and Kahramanmaraş had less damage due to MP. The temperature ranges of Gaziantep, Türkiye whose maize crop has the lowest PM is given in Figure 4.2 showing the lowest and highest temperature range.

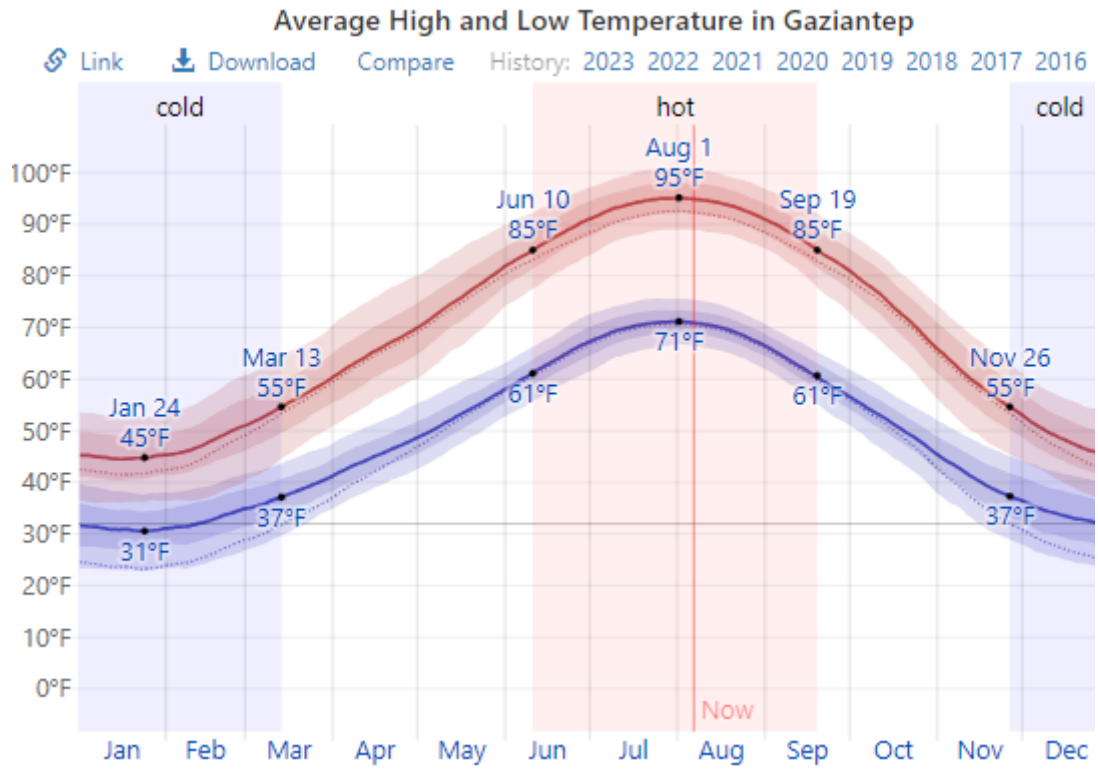

Figure 4.2 Diversity of temperature at Gaziantep, Türkiye.

CHAPTER V

DISCUSSION

M. phaseolina, a fungus present in both soil and seeds, causes charcoal rot in various crops like maize. In this research, 20 samples of soil collected from different districts of Türkiye exhibited differences in several morphological characteristics such as the number of microsclerotia in 1 g of soil and severity of illness. These variations in numbers could be attributed to the diverse environmental conditions across districts, including climate change, differences in temperature, moisture levels, soil composition, and other local factors. Türkiye, a vast peninsula, serves as a geographical bridge connecting the Asian and European regions. The country is bordered through by the Black Sea, the Mediterranean Sea, and the Aegean Sea from three sides. Istanbul is the largest city of Türkiye located within the seaway of Bosporus. Türkiye possesses a diverse climate, with its various regions differing from one another owing to the wide-ranging landscape and the presence of mountain ranges that are completely parallel to coasts. The areas of coastal lands experience more temperate climates, while the Anatolia plateau, an inland, endures summers and winters, with scarce rain. As the study was carried out at Gaziantep, Türkiye, its temperature varied from 31 to 23 ℃ during 2015-2023 (https://weatherspark.com/). The optimum temperature required for growing was 30 ℃ (Manici et al., 1995). During this study, we observed that the ideal temperature for the growth of MP is 26-30 ℃, and temperature below and above this specified limit is not suitable for MP growth.

The severity of illness in the field was highest in Kahramanmaraş Pazarcık/Damlataş, Kahramanmaraş Narlı / Center, and Kahramanmaraş Türkoğlu / Geceli, Mersin Tarsus / Akarsu, Diyarbakır Bismil/İsalı, Adana Seyhan / Gökçeler, Adana Yüregir / Camili, Adana Yüregir/Köklüce, and Adana Yüreğir / İncirlik (88.89%) among all fields may be due to ideal temperature in these areas for the growth of MP.

There is a correlation with cropping history, as crops are extensively cultivated in any area due to limited irrigation, facilitating the establishment of the fungus in these regions (Wagan et al., 2019). The temperatures in these areas such as Myrtle, Gaziantep, Mardin, and K. Maras are not suitable for fungal growth while irrigation and water potential are also the factors for lower growth. The presence of propagules of MP in various soils having warm settings and the severity of in illness the field is highly reduced by the moisture stress present in the topsoil.

The stimulatory and inhibitory biochemicalinteractions are highly occurring between plants and organisms known as "allelopathy." Specifically, higher plants utilize root exudates to boost phytopathogens from contaminating harvests, as demonstrated by (Ushiki et al., 1996). Natural phytosanitary products, such as the extracts of plants with oils having a volatile nature, have also been explored as alternatives to conventional fungicides, aiming to either replace or reduce their usage. Within the defense mechanisms of plants, secondary metabolites can be categorized into several distinct chemical groups, namely terpenes, phenolics, and nitrogen and sulfur-containing compounds. A significant proportion of these secondary metabolites possess antifungal properties, as highlighted by (Zaynab et al., 2018).

As global temperatures continue to rise, the geographic distribution and virulence of MP are expected to expand, potentially affecting a broader range of crops and altering host-pathogen interactions. This shift highlights the need for adaptive agricultural practices to manage the increased risk of these fungal diseases.there are certain factors that reduce the growth of MP are such as otating field, cropping practices, use of chemicals, bio-fungicides and development of resistant varieties through breeding could be developed, evaluated and pooled to partially cope with the impact of *M. phaseolina* their detail is given as. First factor is implementing cultural practices like crop rotation with non-host annual crops such as wheat and rice can serve as a cost-effective strategy for managing dry root rot (DRR) and charcoal rot (CR) (Singh et al., 1990). However, crop rotation might lead to the development of new pathogen strains by altering the genetics *of M. phaseolina*, enabling it to infect additional hosts due to climate change adaptation (Almeida et al., 2008). Consequently, in addition to crop rotation, soil solarization and soil amendments with zinc sulfate, neem cake, or Brassica-mixed farmyard manure residues can be recommended to eliminate microsclerotia/MP populations from the soil (Ansari, 2010).

Second factor in order of decreasing efficacy is fungicides such as benomyl, thiophanate-methyl, thiram, thiobendazole, triforine, and captan reduced the viability of microsclerotia in soil and in jute stem pieces during laboratory tests (Ilyas et al., 1976). Field studies with inoculated soil showed that while fungicides did not impact seed emergence, benomyl significantly reduced microsclerotia numbers, with other fungicides having a lesser effect. The effectiveness of fungicides might be affected by climate change (Juroszek and von Tiedemann, 2011). Therefore, it is essential to study how climate change impacts the efficacy of these chemicals, their residues in the soil and plants, and the potential development of resistance in microbial pathogen populations. Additionally, with the evolving climate, pathogens may become more virulent, necessitating management strategies that include identifying and targeting more aggressive antagonists.

Third factor to reduce the MP is soil treatment with benomyl and thiobendazole were most effective at controlling seedling infection, although both exhibited some phytotoxicity (Ilyas et al., 1975). Soil fumigation with sodium methyldithiocarbamate lowered pathogen populations on jute residue and in the roots of field-grown plants (Kittle and Gray, 1982). Additionally, fumigation with methyl bromide significantly increased jute yields and decreased the number of viable microsclerotia and the prevalence of *M. phaseolina* (Watanabe et al., 1970).

The most important thing for the growth of MP has been inhibited by the evaluation of complete plants or extracts of leaves belonging to medicinal plants. The examination of these extracts revealed various compounds, including alkaloids, saponins, tannins, flavonoids, anthraquinones, octadecadienoic acid, pentadecanoic acid, 1,2,3,4, butaneteterol, octadecanoic acid, and linoleic acid are present. Multiple literature, reported by (Aftab et al., 2019; and Elaigwu et al., 2018) have confirmed the antifungal activity of these extracts.

Moreover, certain extracts have demonstrated the ability to activate defense enzymes in plants that were exposed to MP and inhibited it, as observed in the research conducted by (Lorenzetti et al., 2018). Additionally, the oil extract of plants displayed a significant inhibitory effect on the mycelial growth of MP, making it a promising alternative for controlling this fungus. The application of sodium methyldithiocarbamate for soil fumigation resulted in a decrease in the pathogen's

populations present in both jute residue and the roots of plants cultivated in field plots, as observed in the study (Kitttle and Gray,1982). Moreover, the utilization of methyl bromide for fumigation had a notable effect on jute yields by leading to a significant increase, alongside a reduction in the count of viable microsclerotia and the occurrence of *M. phaseolina*, as reported by (Watanabe et al., 1970).

The use of fungicides for seed treatment has shown a certain level of effectiveness in minimizing the damages inflicted by *M. phaseolina* on crops, especially during their vulnerable seedling stage. Applying thiophanate-methyl and furcarbanil at a concentration of 1000 ppm each resulted in the most significant control over *M. phaseolina* in jute (Vir et al., 1972). Additionally, seed treatment using captafol and mancozeb demonstrated effectiveness in managing root rot and enhancing the emergence of jute seedlings, as documented by (Singh et al., 1990).

CHAPTER VI

CONCLUSION

The diverse range of across districts, including climate change, differences in temperature, moisture levels, soil composition, *M. phaseolina* and other local factors. The factors that can be controlled are the growth of *M. phaseolina* in areas such as Kahramanmaraş Pazarcık/Damlataş, Kahramanmaraş Narlı/Center, and Kahramanmaraş Türkoğlu/Geceli, Mersin Tarsus/Akarsu, Diyarbakır Bismil/İsalı, Adana Seyhan/Gökçeler, Adana Yüregir/Camili, Adana Yüregir/Köklüce, and Adana Yüregir/İncirlik with the great MP should be given the treatment like using sandy and loam, silty clay soils having diverse gradients of moisture effect the survival and growth of pathogens. Such soil parameters and incubation period help to rapidly reduce in *M. phaseolina* population. The other methods are soil and seeds treatment, fertilizers, different other available methods should be practiced to kill the *M. phaseolina* otherwise the pathogens and climate change together may destroy the economy of the world.

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CURRICULUM VITEA

MOMEN ADEL NURAZ

PROFESSIONAL SUMMARY

Dedicated Educational Instruction Worker offering training and support solutions for learning program development. Enjoys collecting information on various learning systems to compare effectiveness and implement next-level approaches. Actively assesses expressed needs of individuals and community to guide program research and creation.

WORK HISTORY

2016 – Teacher Trainer
Among the teaching staff in schools that worked in refugee camps 2016 in Iraq

EDUCATION

Bachelor of Agricultural Engineering - 2012 – 2015
Field of Study: Soil and Water Resources

ADDITIONAL INFORMATION

Participant In several cultural and poetic festivals at the Iraq and international levels.
Writing a book of poetic texts entitled (Lam al-Huruf).

SKILLS

Communication	Written Communication
Multitasking ability	Planning & Coordination
Problem solving	MS Office