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Faculty of Tropical AgriSciences

Evaluation of new means of sugarcane protection against fungal diseases under in vitro conditions

MASTER'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled "Evaluation of new means of sugarcane protection against fungal diseases under in vitro conditions" independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague 19th April, 2024

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Gabriel Laar

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Abstract

Sugarcane (*Saccharum officinarum*) is one of the major commercial crops grown in tropical and subtropical regions of the world. The crop's industrial capacity for producing goods like crystal white sugar, bagasse, power, pressmud, etc. gives it economic significance. Fungal diseases are considered to be one of the main threats to sugarcane productivity among the crop's many production restrictions.

This research aimed to assess essential oils' viability as a novel method for safeguarding sugarcane against fungal infections in a controlled laboratory environment. The study examined the effectiveness of essential oils in impeding the proliferation of fungal pathogens known to afflict sugarcane crops. Eight (8) fungal pathogens strains (*Rhizoctonia solani* 2, *Rhizoctonia solani* 5, *Fusarium spp*, *Setophoma terrestris*, *Schizophyllum commune*, *Alternaria alternata,* *Chalara spp*, and *Fusarium verticillioides*) were obtained from the Czech Collection of Microorganisms in Brno, Czech Republic. The fungal pathogens were sub-cultured and allowed to incubate for 14 days in an incubation chamber to facilitate their growth. The newly grown fungi were used in the experiment. Five already extracted essential oils: *Thymus vulgari*s oil, *Origanumm vulgare* oil, *Saturea montana* oil, *Thymus serpillum* oil, and *Cinnamomum camphora* oil were purchased from Saloos Naturcosmetics and used for the experiment. The study found that *Origanum vulgare* and *Thymus vulgaris* essential oils exhibited exceptional inhibitory effects against all pathogen isolates. In stage two, fungal pathogens were subjected to different concentrations of essential oils for evaluation aimed at establishing the minimum inhibitory concentration (MIC) of the effective essential oils. In the case of OV, the MIC was determined to be between 0.02 % and 0.001 % (EO) with a range of 90.17-100 % inhibition for each pathogen isolate tested. In the case of TV, the MIC was also determined to be in the range of 0.02% and 0.001% (EO) with an average pathogen growth inhibition of 25.9-75.9 % which is a significantly lower efficacy for growth inhibition compared to OV. The results underscore their potential as natural fungicides for safeguarding sugarcane crops against fungal diseases.

Key words: Essential Oils, Sugarcane, Fungal Pathogens, plant protection, *Origanum vulgare*, *Thymus vulgaris.*

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USD = United State Dollars

AA50 = *Alternaria alternata*

SC = *Schizophyllum commune*

FV = *Fusarium verticillioides*

CH = *Chalara sp.*

Rhi2, Rhi5 = *Rhizoctonia sp*.

M3 = *Setophoma terrestris*

M13 = *Fusarium sp*.

EO = Essential Oils

CC = Cinnamomum *camphora*

SM = Satureja *montana*

OV = Origanum *vulgare*

TS = *Thymus serpyllum*

TV = *Thymus vulgaris*

1. Introduction

1.1 Background to the Study

The most common crop produced for sugar production is sugarcane (*Saccharum officinarum L.*) (Sánchez-Elordi et al. 2020). It is a member of the genus *Saccharum* and the family Poaceae. Both sexual and asexual reproductive methods are possible in sugarcane cultivation (Muhammad Fahad Khan et al. 2019). There are two wild species, *S. robustum* and *S. spontaneum*, and four species that were previously cultivated, namely *S. officinarum*, *S. barberi*, *S. sinense*, and *S. edule*, within the Saccharum genus (Wang et al. 2023). The species is native to tropical South Asian regions as well as Southeast Asia. It grows best in tropical and subtropical climates with high organic matter and well-drained soil (pH 7.5–8.5) (Arif et al. 2019).

According to Selman-Housein et al. (2000), the sugarcane sector is significant for tropical and subtropical nations due to the production of sugar, a major export for many emerging economies, and sugarcane's byproducts. For example, sugarcane byproducts are used as raw materials to create paper pulp, plywood boards, alcohols, biofertilizers, and many other beneficial goods. The sugarcane plant is a significant bioenergy crop and is notable for being one of the primary agricultural commodities because its products have high market value (Morais et al. 2022). As a result, sugarcane is grown on almost 28.3 million hectares in 90 different countries, with a global yield of roughly 1.69 billion tonnes (Thibane et al. 2023).

Brazil produces 40% of the world's sugarcane, making it the greatest producer in the world. India, Thailand, China, Pakistan, and Mexico make up the remaining sugar-producing nations. In total, sugarcane is used as the primary raw material for manufacturing 80% of the world's table sugar in more than 100 countries (Aguilar-Rivera 2022). Apart from the conventional uses of sugarcane such as the production of sugar, ethanol, and electricity, it can also serve as a source of eco-friendly carbon for various manufacturing processes (Karp et al. 2022).

1.2 Problem Statement

Like many other crops, sugarcane is susceptible to a range of plant diseases that can lead to significant yield losses, reduced quality of produce, and increased costs for plant protection (Strachan et al. 2022). Among the various pathogens that affect sugarcane, fungal pathogens are of great concern due to their destructive nature and their ability to spread rapidly in the field. Under normal conditions, fungal diseases cause sugarcane losses ranging from 15 to 30%, but under epiphytotic conditions, the yield and sugar losses can reach up to 100% (Tiwari et al. 2017).

Therefore, effective management of fungal diseases in sugarcane is essential for maintaining the quality and productivity of the crop. However, traditional methods of plant protection, such as the use of fungicides, and cultural practices have limitations, including high costs (Gao et al. 2020), environmental concerns (Pang et al. 2023), and the development of resistance among fungal pathogens (Gi et al. 2022). Hence, there is a need for new and innovative means of protecting sugarcane against fungal diseases.

2. Literature Review

2.1 Fungal Diseases Affecting Sugarcane

Fungal diseases pose a substantial threat to the global productivity and quality of sugarcane crops, leading to considerable economic losses for farmers and the industry at large (Tiwari et al. 2017). The spectrum of fungal pathogens affecting sugarcane is extensive, with reports documenting over 100 distinct diseases attributed to fungi (Rajput et al. 2021). Among these, several key fungal diseases significantly impede sugarcane yield in agricultural fields.

Red rot (caused by *Colletotrichum falcatum*), wilt (attributed to *Fusarium sacchari*), smut (caused by *Sporisorium scitamineum*), and pineapple sett rot (linked to *Ceratocystis paradoxa*) stand out as primary adversaries to sugarcane cultivation (Viswanathan & Malathi 2019). These fungal pathogens infect sugarcane at various stages of growth, causing wilting, rotting, and other deleterious effects that diminish both yield and quality. Effective management strategies, including disease-resistant cultivars, cultural practices, and fungicidal treatments, are essential for mitigating the impact of these fungal diseases and safeguarding sugarcane production worldwide

In addition to the primary fungal diseases affecting sugarcane, a range of other significant pathogens threatens the health and yield of sugarcane crops (Wang et al. 2021). These diseases encompass diverse symptoms and causal agents, adding to the complexity of disease management in sugarcane cultivation. Alternaria Leaf Spot (*Alternaria alternata*): This disease manifests as dark spots on sugarcane leaves, ultimately leading to leaf withering and reduced photosynthetic capacity (Zarubova et al. 2015). Arrow Rot (*Fusarium sp.*): Arrow rot affects sugarcane stalks, causing rotting and weakening of the structural integrity, which can result in lodging and reduced crop stand.

Banded Sclerotial Disease (*Rhizoctonia solani*): Characterized by the formation of dark bands or lesions on sugarcane stems, banded sclerotial disease can lead to severe damage and lodging (Viswanathan & Malathi 2019). Pokkah Boeng (*Gibberella moniliformis, Fusarium moniliforme*): This disease primarily affects the internodes of sugarcane, causing swelling and discoloration, which leads to reduced sucrose content and overall yield. Stalk Rot-Rind Disease (*Phaeocytostroma sacchari*): Stalk rot-rind disease causes rotting of sugarcane stalks, compromising structural integrity and reducing yield potential (Viswanathan & Malathi 2019).

Brown Spot (*Cercospora longipes*): Brown spot disease presents as dark, necrotic lesions on sugarcane leaves, impairing photosynthesis and reducing overall plant health Brown Stripe (*Cochliobolus stenophilus, Helminthosporium stenopilum*): Characterized by brown streaks on sugarcane leaves and stems, brown stripe disease can lead to yield losses and compromise sugar content (Tiwari et al. 2017). Seedling Rot (*Damping-off*) (*Pythium, Rhizoctonia*): Seedling rot, also known as damping-off, affects young sugarcane plants, causing rotting of the stem at the soil line and leading to seedling death (Rajput et al. 2021). Managing these fungal diseases requires a comprehensive approach involving disease-resistant cultivars, cultural practices such as crop rotation and proper sanitation, and judicious use of fungicides (Thibane et al. 2023). Additionally, early detection and timely intervention are crucial for minimizing the impact of these diseases on sugarcane production.

2.1.1 Smut (*Sporisorium scitamineum*)

The smut disease of sugarcane, which is caused by *Sporosorium scitamineum*, is regarded as the most severe and pervasive disease known to influence both qualitative and quantitative components and results in significant economic losses (Rajput et al. 2021). Smut costs millions of dollars and causes yield losses of between 50 and 62 percent globally (Strachan et al. 2022). The first known instance of Smut was recorded in 1877 in Natal, South Africa. From there, it spread throughout Africa and neighbouring islands, as well as countries in Southeast Asia such as India, the Philippines, Taiwan, and South America (Tiwari et al. 2017). The disease is currently widespread in all states that grow sugarcane and is one of the main biotic factors impacting sugarcane production (Viswanathan & Rao 2011). Primarily a meristematic tissue disease, whip smut is only spread by young, actively growing plant tissues through either soil-borne teliospores or planting contaminated setts. While secondary infection happens when airborne fungal spores infect a standing healthy crop (Rajput et al. 2021). Whip signs do not usually appear in systemically infected plants, although the pathogen will infect most of their buds. Such symptomless shoots could act as the main source of transmission if they are used as seeds (Viswanathan & Rao 2011).

The most recognizable diagnostic characteristic of plants infected with sugarcane smut is the appearance of a particular structure known as the "smut whip" (Rajput et al.

2021). The length of the whip-like structure that protrudes from the top varies from 6 inches to 1 meter. The whip which is black in color starts off with a shiny coating but eventually breaks open and releases spores into the air, which then go on to infect other plants (Tiwari et al. 2017). When sugarcane plants become infected, they tend to grow less vigorously and their canes become thin and slender with nodes that are spaced further apart than normal (Rajput et al. 2021). The growth inhibition causes them to become stunted, and they tend to produce an unusually high number of tillers - up to 50-100 per plant. Each of these tillers is accompanied by a smut whip (Bhuiyan et al. 2012). Under optimal conditions, such as a temperature range of 25-30 ºC and humidity levels between 65-70 %, the severity of the disease becomes more pronounced (Rajput et al. 2019). The severity of sugarcane smut is directly correlated with the losses in yield and quality parameters - the greater the degree of smut, the more significant the losses will be (Rajput et al. 2021). In Nigeria, cane yield losses ranged from 76 to 97.6 % and 86.3 to 86.8 % on CO 957 and local variety, respectively. In Ethiopia, yield losses reached 43 %. The amount of money lost annually in Ethiopia as a result of the disease exceeded USD 900,000 (Tegene et al. 2021)

Various measures are employed worldwide to prevent and manage sugarcane smut, including techniques like hot water treatment, removing infected plants, planting cultivars that are resistant or tolerant to the disease, and using fungicides (Rajput et al. 2019). The use of resistant varieties is the most effective and cost-efficient method for controlling sugarcane smut (Viswanathan & Rao 2011). Sugarcane breeding programs in all countries where smut is prevalent include efforts to develop resistance to the disease in their cultivars. By developing resistant varieties, the disease can be effectively managed. However, developing resistant varieties of sugarcane is a long-term goal, in the short term, fungicides are used to manage the disease (Tiwari et al. 2017). Fungicides serve a dual purpose of eliminating smut from the planting material and protecting the seed cane from becoming infected by the pathogen inoculum that may be present in the planting soil (Rajput et al. 2019). However, large-scale application of toxic chemicals may cause environmental hazards and residual problems (Pang et al. 2023).

2.1.2 Wilt (*Fusarium sacchari*)

In recent times, researchers working on sugarcane have also focused on the wilt disease as one of the major diseases affecting sugarcane (Tiwari et al. 2017). *Fusarium sacchari* (Butler) W. Gams, a soil-borne pathogen that was formerly known as *Cephalosporium sacchari*, is the cause of the disease (Viswanathan et al. 2022). In India and other South Asian nations, sugarcane wilt is a deadly stalk disease (Viswanathan et al. 2011). The disease was first documented in India over a century ago and has since then resulted in significant harm to sugarcane farming in the nation (Viswanathan et al. 2017). The disease is currently ranked as the second major sugarcane disease in India and is still expanding over many of the world's sugarcane-producing regions (Tiwari et al. 2017). Fusarium-infected setts or soil inoculum are the main origins of the disease, and its transmission to other fields occurs through inocula transported by wind and irrigation water. The pathogen can enter the plant through various means, including cut wounds, root eyes located at the nodes, and infected setts (Viswanathan et al. 2022).

During the monsoon season, symptoms of wilt begin to manifest as the crown turns yellow. As time passes, the cane's weight decreases due to an increase in the pith area. By October, the entire crown wilts, and the cane becomes hollow. When longitudinally split, the nodal area displays light pink to dark purple tissues, along with greyish mycelium present in the hollow internodes. The internodes that have dried out stay attached to the nodes and are not easily detached (Tiwari et al. 2017). Wilt can result in a loss of 2 to 10 tonnes per hectare due to the presence of dead and dried canes, with a yield loss of up to 65 % in ratoon crops. Often, the canes affected by wilt dry out in the field, making them unsuitable for crushing in the mills and resulting in a complete loss of yield. Even partially infected canes can negatively impact juice quality and extractability, as the fungal pathogen causes dehydration (Viswanathan et al. 2022).

Wilt continues to be a significant limitation of sugarcane cultivation. This is due to the fact that the disease-causing pathogen is often present in infected setts. Therefore, the selection of disease-free seed canes is fundamental for managing the disease (Viswanathan 2020). The cultivation of cane varieties that are resistant to diseases is an economically viable option that will ensure the long-term sustainability of sugarcane cultivation (Tiwari et al. 2017). The use of fungicides is not very effective in reducing the severity of sugarcane wilt disease, as it is challenging to apply them in the field and impractical to target soil-borne pathogens with these chemicals. Therefore, biological control presents a viable approach for managing wilt disease in sugarcane (Viswanathan & Malathi 2019).

2.1.3 Pokkah boeng (*Fusarium spp*)

Pokkah Boeng is a fungal disease that affects sugarcane and is caused by *Fusarium spp*. It was initially detected in Java in 1890 and has since spread to various sugarcane-producing countries worldwide. This disease can lead to different levels of economic loss (Li et al. 2022). The pathogen disseminates through rain carried by the wind, contaminated cuttings of sugarcane, and mature sugarcane stem borers in both their pupal and adult stages (Tiwari et al. 2017). The primary symptoms of pokkah boeng ailment are the deformation, yellowing, and drying up of youthful leaves. Plants severely affected by the disease stop growing, and their tops become significantly stunted, ultimately leading to their death due to the rotting of their growing tips (Shan et al. 2021). The progression of disease symptoms occurs in four stages, which are identified as chlorotic phases I and II, top rot, and knife cut phase. Additionally, the apical leaves may exhibit significant wrinkling and twisting, depending on the susceptibility of the plant varieties and prevailing climatic conditions. Furthermore, the disease can cause deformities or harm to the top and stalk of the plant (Tiwari et al. 2017). In certain significant sugarcane-producing nations, including India, South Africa, Malaysia, and China, this disease has recently gained significant relevance and is resulting in significant yield losses. Pokkah boeng can significantly lower the sugar content of infected crops by 40.8 % to 64.5 %, in addition, to yield loss, in high sugar yielding types (Tiwari et al. 2021).

Shan et al. (2021) reported that the prevalence of diseased plants in China was 81.1 % on average and 100.0 % in badly affected fields. The measured yield loss averaged 38.4 % and increased to 48.5 %. Average sugar content decreases were 3.1 %, although they might reach 4.2%. In India, the presence of Pokkah Boeng has been known to result in a significant reduction in crop yield, with estimates suggesting a loss of 40-60 %. In addition, the affected sugarcane crops may experience a reduction of up to 64.5 % in their sugar content. Furthermore, the canes affected by the disease may also exhibit a decrease in weight, internode length, girth, juice content, and total sugar content compared to healthy canes (Viswanathan 2020). The application of various fungicides such as Bavistin (1 g/l of water), Blitox (0.2 %), Copper oxychloride, or 0.3 % Dithane M-45 (3 g/l of water) has been shown to be effective in decreasing the incidence of pokkah boeng disease (Tiwari et al. 2017).

2.1.4 Red rot (*Colletotrichum falcatum*)

In numerous countries, including India, Pakistan, Bangladesh, Thailand, Myanmar, Nepal, Vietnam, and other countries, red rot is considered one of the most severe diseases that affect sugarcane (Viswanathan 2021). For the last 100 years in India, this disease has been a major obstacle to sugarcane productivity and is prevalent throughout the country. It has caused the elimination of several commercial sugarcane varieties during the earlier decades in the country (Viswanathan & Rao 2011). Red rot of sugarcane was initially identified as a sugarcane disease in Java in 1893 by Went. Within a decade of Went's report on the economic harm caused to sugarcane milling in Java, the disease was discovered in other regions across the globe (Viswanathan 2021). *Colletotrichum falcatum*, a type of fungus, is responsible for the occurrence of this disease. It spreads through infected cane stalk setts, diseased debris, and resting spores present in the soil (Nayyar et al. 2017). Under optimal high humidity conditions, a significant amount of inoculum for subsequent infections is provided by crop debris, which consists of a diseased stalk or stubble pieces left behind after harvest. Although the fungus is not a true soil-borne pathogen, there is sufficient evidence to suggest that inoculum borne by debris plays a role in perpetuating the fungal propagules (Tiwari et al. 2017).

The disease leads to a reduction in sugarcane yield by 5-50 %, with sugar recovery rates of only 31 % due to the resulting loss (Hossain et al. 2020). Cane weight and juice extraction in Red rot-affected canes show a decline of 29 % to 83 % and 24 % to 90 %, respectively, as reported by Viswanathan (2021). In addition to reducing the yield attributes, Red rot also has a negative impact on the quality of sugarcane juice, such as reducing the sucrose content, purity, and Brix, which ultimately affects the production of commercial cane sugar (Hossain et al. 2020). The crop losses caused by Red rot in Uttar

Pradesh, India, have escalated from a few thousand hectares during the 2016-2017 season to a significant figure as reported by . The total loss incurred due to the outbreak of this disease during the current season alone ranges from 1.0 to 1.414 billion US dollars. The losses incurred during the previous year may have been at least 40 -50 % of the current loss.

The management of Red rot has been a challenging task for both pathologists and sugarcane breeders. Tiwari et al. (2017) explained that the severity of the disease outbreak depends on various factors such as weather conditions, the presence of virulent pathogens, genotypes, and the time required for disease development. These factors need to be thoroughly studied to achieve effective control of the disease. However, it has been observed that once the disease has established itself in the field, it becomes impossible to control (Tiwari et al. 2017). Bhuiyan et al. (2012) found that traditional methods of managing Red rot disease include cultural practices, the use of resistant varieties, disease-free planting materials, physical, biological, and chemical controls, among others. These methods aim to limit the incidence of Red rot after replanting and improve the productivity of sugarcane plants. However, Hossain et al. (2020) explained that these management strategies have not produced satisfactory results, and no single method has been effective in reducing the incidence of the disease

2.2 Current Methods For Controlling Fungal Diseases in Sugarcane

Due to the complex nature of sugarcane diseases and the multitude of factors contributing to their spread and severity, relying on a singular method for their control is impractical and often ineffective (Shan et al. 2021). Consequently, experts in the field advocate for the implementation of an integrated approach that encompasses various strategies aimed at mitigating the impact of these diseases and ensuring the sustainable management of sugarcane cultivation. The integrated approach proposed by Viswanathan and Rao (2011) emphasizes the need to combine multiple techniques, each addressing different aspects of disease control. These techniques include cultural practices, chemical interventions, host resistance mechanisms, and legislative measures, all working in synergy to combat sugarcane diseases effectively (Aguilar-Rivera 2022).

Cultural practices form a fundamental component of disease management in sugarcane cultivation. These practices encompass a range of agronomic techniques aimed at creating an environment less conducive to disease development. Examples include proper sanitation measures, crop rotation, optimal planting densities, and timely harvesting practices. By implementing these cultural practices, growers can reduce the prevalence and severity of sugarcane diseases within their fields (Arif et al. 2019).

Barney (2020) found that chemical interventions, such as the application of fungicides and pesticides, also play a significant role in disease control. These substances target specific pathogens responsible for sugarcane diseases, helping to suppress their growth and spread. However, it is essential to use these chemicals judiciously, following recommended application rates and adhering to safety guidelines to minimize environmental impact and potential health risks. Host resistance represents another critical aspect of integrated disease management in sugarcane cultivation. Berg et al. (2020) asserted that breeding and selecting varieties with inherent resistance or tolerance to prevalent diseases, growers can reduce their reliance on chemical inputs and minimize yield losses caused by infections. Investing in research and development programs aimed at identifying and breeding resistant cultivars remains paramount in the pursuit of sustainable disease management strategies.

Furthermore, legislative measures and regulatory frameworks can complement on-farm disease management efforts by establishing guidelines and protocols aimed at preventing the introduction and spread of pathogens. These measures may include quarantine protocols, inspection requirements, and certification programs designed to safeguard sugarcane production regions from the incursion of new diseases and pests. In conclusion, the integrated approach advocated by Viswanathan and Rao (2011) underscores the importance of adopting a multifaceted strategy for controlling sugarcane diseases. By combining cultural, chemical, host resistance, and legislative measures, growers can effectively manage disease outbreaks, reduce economic losses, and ensure the long-term sustainability of sugarcane cultivation

2.2.1 Cultural practices which reduce the rate of spread of disease

Cultural practices play a pivotal role in enhancing the growing conditions of plants, primarily by optimizing factors such as nutrition, moisture, light exposure, and minimizing competition from neighboring plants (Castello et al. 2020). Additionally, these practices serve to create an environment that is unfavorable for the proliferation of pathogens, thereby safeguarding the health and productivity of crops. While growers face constraints in manipulating the environment within open fields, they enjoy a higher degree of control in enclosed environments like greenhouses or storage facilities (Clemmensen et al. 2020). Di Somma et al. (2020) explained that in rudimentary greenhouse setups, strategic plant spacing and efficient ventilation systems are implemented to mitigate humidity levels and deter pathogenic infections, such as those caused by *Botrytis*. Conversely, in more sophisticated greenhouse setups, growers employ advanced techniques to regulate relative humidity, particularly during nocturnal periods, with the aim of desiccating plant surfaces and impeding the spread of pathogens like *Botrytis spp.* and *Diplocarpon rosae*, as noted by Zhang et al. (2021).

At the field level, crop management strategies represent a multifaceted approach aimed at optimizing plant health and productivity. Dijkstra and Schröter (2020) found that these strategies entail a range of interventions, each tailored to address specific agricultural challenges and environmental conditions. The study also found among the arsenal of techniques employed, adjustments to planting schedules and cultural practices stand out as fundamental components in fostering favorable growing conditions. The study concluded that one such pivotal practice involves meticulous scheduling of planting activities, which takes into account factors like seasonal variations, climatic conditions, and optimal growth periods for specific crops. The study also asserted that by aligning planting schedules with conducive environmental conditions, growers can capitalize on favorable periods for seed germination, vegetative growth, and reproductive development, thereby maximizing overall crop yield and quality.

In addition to strategic planting schedules, cultural practices play a crucial role in shaping soil health and fertility, thereby influencing plant resilience against diseases and stressors (Hossain et al. 2020). Maintaining low soil pH levels represents a key strategy employed by growers to create an environment conducive to nutrient uptake and microbial balance. Kešnerová et al. (2020) found that adjusting soil acidity levels, growers can optimize nutrient availability and enhance the efficacy of fertilizers, thus fostering robust plant growth while simultaneously inhibiting the proliferation of certain soil-borne pathogens. Furthermore, ensuring adequate concentrations of essential nutrients, such as phosphorus and potassium, is paramount in sustaining plant vitality and bolstering natural defense mechanisms against diseases (Manavalan 2020). Phosphorus, a vital component in energy transfer processes and cellular metabolism, plays a pivotal role in promoting root development, flowering, and fruit set. Similarly, potassium, a micronutrient essential for osmoregulation and enzyme activation, contributes to water and nutrient uptake, stress tolerance, and disease resistance in plants (Merryweather 2020).

The study conducted by Rebitanim et al. (2020) underscores the efficacy of these cultural practices in mitigating the incidence and severity of diseases, particularly in the context of managing sugarcane brown rust. The findings not only validate the importance of maintaining optimal soil conditions but also highlight the broader applicability of these methodologies across diverse crop systems and agricultural landscapes (Kešnerová et al. 2020). In essence, by embracing a holistic approach to crop management that encompasses strategic planting schedules and cultural practices aimed at enhancing soil health and nutrient balance, growers can effectively mitigate the impact of diseases, bolster crop resilience, and optimize agricultural productivity in a sustainable manner (Morais et al. 2022).

Tillage practices represent a fundamental aspect of agricultural management, profoundly influencing soil health, weed populations, and disease dynamics within cropping systems (Failla and Romano 2020). Traditionally, conventional tillage methods involve intensive soil disturbance through plowing, harrowing, and cultivation, aimed at preparing seedbeds, controlling weeds, and managing crop residues. While effective in weed suppression and pathogen control, conventional tillage practices can also lead to soil erosion, compaction, and loss of organic matter, thereby compromising long-term soil health and productivity (Gao et al. 2020). In response to these challenges, modern agricultural systems have increasingly embraced minimum tillage and no-tillage practices as viable alternatives. Minimum tillage involves reduced soil disturbance and minimal disruption of soil structure, while no-tillage entails the complete avoidance of mechanical soil manipulation. These approaches offer several benefits, including the preservation of soil structure, moisture retention, and reduction in energy inputs and greenhouse gas emissions associated with tillage operations (Gavriilidou et al. 2020).

Hossain et al. (2020) found that one of the primary motivations behind adopting minimum tillage and no-tillage practices lies in their potential to mitigate the adverse effects of mechanical soil disturbance on crop plants. Minimizing soil disruption, these practices help alleviate compaction and root damage, thereby promoting healthier root systems and enhancing overall plant vigor and resilience. Furthermore, Karp et al. (2022) found that reduced soil disturbance can limit the exposure of soil-borne pathogens to crop roots, thereby curbing the spread of diseases and mitigating yield losses. However, the transition to minimum tillage and no-tillage practices also introduces new considerations regarding disease management. While these practices can reduce the direct spread of pathogens through soil movement, they may also influence disease dynamics and control strategies in complex ways. For instance, reduced soil disturbance may create microenvironments conducive to certain soil-borne pathogens, leading to shifts in disease prevalence and severity (Kešnerová et al. 2020). Additionally, the accumulation of crop residues on the soil surface under no-tillage systems can provide a substrate for certain pathogens, potentially exacerbating disease pressures in subsequent growing seasons.

The study by Rebitanim et al. (2020) underscores the importance of understanding the nuanced interactions between tillage practices and disease dynamics in agricultural systems. While minimum tillage and no-tillage practices offer compelling benefits in terms of soil conservation and resource efficiency, their impact on disease development and control strategies necessitates careful consideration and proactive management approaches. By integrating agronomic practices, crop rotations, and disease-resistant cultivars, growers can effectively mitigate the risks associated with reduced tillage systems while harnessing their potential for sustainable and resilient agriculture in the long term.

2.2.2 Biocontrol of fungal diseases in Sugarcane

Various microbial antagonists have been developed and commercialized to be used against soil-borne phytopathogenic fungi that cause diseases in the above-ground plant parts. Species of *Trichoderma*, for example, *Trichoderma harzianum*, are one of the most widely used microbial antagonists for biocontrol of plant fungal diseases caused by *Fusarium, Rhizoctonia*, and many soil-borne phytopathogenic fungi (Tiwari et al., 2020). Another microbial fungicide is *Coniothyrium minitans*, which is used for biocontrol of infections of lettuce, oilseed rape, brassicas, beans, and carrots caused by *Sclerotinia sclerotiorum* (Vijayan et al., 2020). The bacterium *Paenibacillus jamilae* HS-26 was reported to have potent antagonistic effects (inhibiting mycelial growth of fungi) on multiple soil-borne fungal pathogens via releasing extracellular antifungal metabolites and the synthesis of hydrolytic enzymes. Formulations of *Streptomyces cellulosae* Actino that produces chitinase were reported to control *Sclerotium rolfsii* causing peanut soil-borne diseases (Manavalan, 2020).

Formulations of microbial fungicides include liquid suspensions, granules, or dust, which are applied in the soil just before cultivation or directly to plant roots. They can also be formulated as conventional sprays and applied on harvested fruits, plant stems, or leaves. Moreover, unique application methods have been developed such as honeybees' delivery during pollination (Merryweather, 2020). Bees usually carry *Monilinia vaccinii-corymbosi* (a phytopathogenic fungus that causes mummy blueberry disease) between the flowers of blueberry during pollination. At the same time, the bees can act as 'flying doctors' and deliver the bacterial fungicide *Bacillus subtilis* to the flowers of blueberry to suppress the disease (Parthasarathy et al., 2020). Additionally, the endophytic bacterium *Bacillus mojavensis* was reported to be fungicidal against various phytopathogenic fungi including *F. oxysporum*, *R. solani*, and *Sclerotinia sclerotiorum* (Dijkstra & Schroter, 2020).

In general, microbial antagonists used for the biocontrol of plant fungal diseases have multiple mechanisms involved in their action (Muhammad et al. 2019). *Trichoderma* species, for example, act against soil-borne phytopathogenic fungi through parasitism, production of antibiotics and enzymes that degrade the fungal cell wall, competition for nitrogen or carbon, and also by production of auxin-like compounds causing plant growth promotion (Zhang et al., 2021).

2.2.3 Use of Resistant Varieties

Plants have evolved an elaborate system of defenses, both inherent and triggered, to withstand pathogen attacks (Ramesh Sundar et al. 2015). A plant's defense against a disease involves the accumulation of various substances such as phytoalexins, which are small antimicrobial compounds that build up at the site of infection, as well as systemic enzymes like chitinases, β-1,3-glucanases, and proteases that break down pathogens (Ramesh Sundar et al. 2015). Additionally, protective biopolymers such as peroxidases and phenoloxidases are generated by systemic enzymes that produce antimicrobial compounds, while others like hydroxyproline-rich glycoproteins, lignin, and callose restrict the spread of pathogens. Finally, regulators such as elicitors of plant and microbial origin, immune signals from primed plants, and compounds that release immune signals can induce or influence the activity of defensive compounds (Tiwari et al. 2017). The primary method for sustainable management of sugarcane diseases is through the utilization of resistant varieties (Viswanathan & Rao 2011).

According to Tiwari et al. (2017), using resistant cultivars has been shown to be an efficient and economical approach to controlling sugarcane smut. Also, the cultivation of disease-resistant cultivars can be a highly effective means of managing Pokkah Boeng (Viswanathan 2020). There are variations in resistance to Pokkah Boeng disease across different sugarcane cultivars, and the most efficient and cost-effective approach to managing the disease is through screening and planting of resistant varieties. Over the past few years, a national collaborative research initiative supported by sugarcane research units in China has resulted in the development of numerous new high-performing sugarcane cultivars with disease resistance. The findings of this study could provide a scientific foundation for the selection and breeding of superior resistant varieties for large-scale cultivation (Shan et al. 2021).

The primary method of effectively controlling red rot has been through the utilization of resistant varieties (Tiwari et al. 2017). The repeated occurrence of red rot in epidemic proportions has prompted breeders to create sugarcane varieties that are resistant to this disease (Hossain et al. 2020). Nevertheless, it should be noted that resistance to red rot in sugarcane varieties is not permanent, and as a result, nearly all the varieties presently grown are vulnerable to the disease (Tiwari et al. 2017). In the past, when severe wilt epidemics had a devastating impact on sugarcane cultivation, the identification and adoption of disease-resistant varieties proved to be a suitable replacement, which saved the sugar industry from threats in India. Therefore, cultivating resistant varieties is not only cost-effective but also ensures the sustained growth of sugarcane in a region (Viswanathan & Rao 2011). Both national and international research studies have affirmed that developing and cultivating resistant cultivars is the most cost-efficient and productive approach for managing brown stripe disease (Wang et al. 2021).

The management of numerous sugarcane diseases relies heavily on the use of cultivars that are resistant to those diseases, and multiple varieties have been created in the past for this purpose (Viswanathan & Rao 2011). However, the recurring occurrence of resistance breakdown in sugarcane varieties is a matter of worry (Viswanathan & Rao 2011).

2.2.4 Chemical Control of fungal diseases in Sugarcane

In agricultural contexts where resistant crop varieties are unavailable and effective non-chemical interventions are limited, the application of chemical fungicides becomes imperative to mitigate the impact of devastating plant diseases, which can precipitate substantial economic losses, as highlighted by Rajput et al. (2019). Among the array of chemical control methods, the immersion of sugarcane setts in a 0.25 % solution of thiophanate methyl and its metabolite carbendazim for 24 hours prior to planting has emerged as a notably effective strategy for managing debris-borne infections, as demonstrated by Tiwari et al. (2017). Chemical fungicides have assumed a central role in the management of Pokkah boeng disease, with Vishwakarma et al. (2013) advocating for the application of fungicidal treatments such as Bavistin, Blitox, Copper oxychloride, or Dithane M-45. These fungicides serve to curtail the proliferation of pathogens responsible for Pokkah boeng disease, thereby safeguarding crop health and productivity.

In the context of smut infection in seedcane, fungicidal treatments have been deployed through various methods aimed at both prevention and remediation. Bhuiyan et al. (2012) elaborate on two prominent approaches: cold water immersion and incorporation of fungicides into hot water treatment tanks. These methods afford dual functionality by concurrently administering preventive measures to mitigate the risk of smut infection and remedial actions to address existing fungal infestations. By integrating fungicidal treatments into seed cane management protocols, growers can effectively mitigate the threat posed by smut disease and sustain crop yields. Rajput et al. (2019) asserted that the utilization of chemical fungicides underscores the significance of integrated pest management strategies, which seek to harmonize chemical interventions with cultural, biological, and physical control measures to achieve comprehensive disease management outcomes.

While chemical fungicides offer indispensable benefits in combating plant diseases, their judicious application must be tempered with considerations of environmental sustainability, resistance management, and human health concerns (Manavalan 2020). As such, ongoing research efforts aimed at developing novel fungicidal formulations, enhancing application techniques, and promoting integrated disease management practices remain pivotal in ensuring the resilience and sustainability of agricultural systems in the face of evolving disease pressures. In vitro research has demonstrated the efficacy of chemical control techniques in effectively suppressing the growth of *C. falcatum*, a notorious pathogen affecting sugarcane. Notably, studies such as those conducted by Hossain et al. (2020) have revealed that chemical fungicides such as Benomyl 50 WP, Folicar, and Radomil 75WP exhibit potent inhibitory effects on fungal growth across a range of concentrations, from 5 to 50 μgmL−1, achieving complete inhibition (100 %). Rahman et al. (2009) reported promising outcomes regarding the application of Topsin M treatment for safeguarding sugarcane against red rot disease. Through the implementation of Topsin M treatment protocols, cane crops experienced enhanced resistance to red rot infection, resulting in notable increases in cane yield. This underscores the pivotal role of chemical fungicides in bolstering crop resilience and productivity in the face of prevalent fungal diseases.

Moreover, the management of Pythium root rot, induced by Pythium arrhonomanes, necessitates proactive measures to protect sugarcane clones or true seedlings from debilitating fungal infestations. Bhuiyan et al. (2012) advocate for the routine application of Metalaxyl fungicides at a concentration of 2.5 mg a.i. L−1 to effectively combat Pythium root rot and mitigate associated yield losses. By integrating Metalaxyl fungicides into disease management protocols, growers can proactively safeguard sugarcane crops against the detrimental effects of Pythium root rot, thereby sustaining crop health and productivity. The findings underscore the critical importance of chemical control techniques in mitigating the impact of fungal pathogens on sugarcane cultivation (Bhuiyan et al. 2012). While chemical fungicides offer effective solutions for disease management, their judicious and responsible use is essential to minimize adverse environmental impacts and mitigate the risk of fungicide resistance. As such, ongoing research endeavors aimed at refining fungicidal formulations, optimizing application techniques, and promoting integrated disease management strategies remain paramount in ensuring the long-term sustainability and resilience of sugarcane cultivation practices (Bhuiyan et al. 2012).

2.3 Limitations of Current Methods for Controlling Fungal Diseases in Sugarcane

Sugarcane, the vital source of our beloved table sugar, faces a formidable foe in the form of fungal diseases (Nguefack et al. 2008). These microscopic adversaries can wreak havoc on plantations, slashing yields, jeopardizing livelihoods, and impacting global sugar production. While the arsenal against these fungal enemies is not empty, current methods face significant limitations, leaving researchers, farmers, and the entire sugar industry grappling for more effective solutions (Parthasarathy et al. 2020). This section delves into the critical challenge of controlling fungal diseases in sugarcane. The section discusses the shortcomings of existing strategies, from fungicides and resistant cultivars to cultural practices (Khan et al. 2019). By examining these limitations, this section aims to illuminate the pressing need for novel approaches, paving the way for a future where sugarcane thrives, unburdened by the ever-present threat of fungal blight.

2.3.1 Phytopathogenic Fungi

Fungi play a vital role in ecosystems as decomposers, breaking down dead organic matter and recycling nutrients back into the environment (Niem et al. 2020). However, Parthasarathy et al. (2020) and Moons et al. (2022) found that only a small fraction of fungal species, comprising less than 10 % of all known fungi, have the ability to colonize living plants. Within this subset, an even smaller proportion consists of phytopathogenic fungi, which have the capacity to infect and cause diseases in crop plants (Moons et al., 2022). Despite their limited representation among fungal species, phytopathogenic fungi wield significant influence due to their ability to trigger catastrophic epidemics in cultivated crops. These fungal pathogens pose a persistent threat to global food security by causing substantial and often irreparable damage to agricultural yields each year (Moons et al., 2022).

Li et al. (2022) found that the economic ramifications of fungal-induced crop diseases are profound, prompting concerted efforts from scientists, plant breeders, and farmers to develop strategies for combatting these pathogens. Manavalan (2020) found that by leveraging interdisciplinary approaches encompassing genetics, genomics, plant pathology, and agronomy, researchers seek to unravel the molecular mechanisms underpinning fungal pathogenicity and host susceptibility. This knowledge serves as the foundation for devising innovative disease management strategies aimed at mitigating the impact of fungal epidemics on crop production (Manavalan 2020). Plant breeders endeavor to develop crop varieties endowed with genetic resistance to fungal diseases, thereby reducing reliance on chemical interventions and promoting sustainable agricultural practices. Through meticulous selection and breeding programs, resistant crop cultivars can be developed with enhanced tolerance to specific fungal pathogens, thereby bolstering crop resilience and minimizing yield losses.

Furthermore, farmers employ integrated disease management practices that integrate cultural, biological, and chemical control measures to mitigate the spread of fungal diseases and preserve crop health (Manavalan 2020). These strategies encompass diverse interventions, including crop rotation, sanitation practices, biological control agents, and judicious use of fungicides, tailored to the specific epidemiological characteristics of fungal pathogens. In conclusion, the threat posed by phytopathogenic fungi underscores the imperative for collaborative efforts aimed at understanding, preventing, and managing fungal diseases in agricultural systems By fostering synergies between research, breeding, and farming practices, stakeholders can forge a path towards sustainable disease management strategies that safeguard crop yields, enhance food security, and promote resilience in the face of evolving fungal threats (Li et al. 2022).

2.3.2 Excessive Utilization of Chemical Fungicides

In commercial agriculture, chemical fungicides represent the primary line of defense against fungal pathogens, acting to disrupt and impede the growth and reproduction of these detrimental organisms (Failla & Romano 2020). Their widespread adoption is attributed to their efficacy, affordability, and ease of application, making them indispensable tools in disease management strategies (Failla & Romano 2020). However, the accessibility and convenience of chemical fungicides have led to their often indiscriminate or excessive use in agricultural practices. Farmers may apply these chemicals preemptively or repeatedly, sometimes as a precautionary measure, contributing to the accumulation of fungicide residues in soil, water, and agricultural produce. Such practices have raised concerns about the potential adverse effects on beneficial organisms, human and animal health, and the broader environment.

Clemmensen et al. (2020) found that the unintended consequences of fungicide overuse extend beyond ecological considerations. Excessive fungicide applications can disrupt natural ecosystems by indiscriminately targeting non-target organisms, including beneficial insects, soil microbes, and aquatic fauna. Moreover, the buildup of fungicide residues in food crops can pose risks to human health, potentially leading to toxicity and long-term health complications. Furthermore, the overreliance on chemical fungicides has fueled the emergence of resistant strains among fungal pathogens, rendering conventional fungicidal treatments increasingly ineffective (Clemmensen et al. 2020). Merryweather (2020) found that fungal populations possess inherent genetic variability, enabling them to adapt and evolve in response to selective pressures exerted by fungicidal compounds. Consequently, the widespread use of fungicides can inadvertently promote the proliferation of resistant fungal strains, exacerbating the challenge of disease management and necessitating the development of alternative control strategies.

To address these challenges, sustainable approaches to disease management are imperative. Nayyar et al. (2017) asserted that Integrated Pest Management (IPM) strategies integrate cultural, biological, and chemical control measures, offer a holistic framework for mitigating fungal diseases while minimizing reliance on chemical inputs. Promoting practices such as crop rotation, sanitation, biological control agents, and targeted fungicide applications. IPM strategies seek to optimize disease control outcomes while minimizing environmental impacts and reducing the risk of fungicide resistance. Failla and Romano (2020) found that while chemical fungicides remain indispensable tools in the arsenal of agricultural pest management, their judicious and responsible use is essential to mitigate adverse ecological, human health, and resistance-related consequences. Embracing sustainable agricultural practices and adopting integrated pest management approaches can help strike a balance between effective disease control and environmental stewardship, fostering resilient and sustainable agricultural systems for future generations.

Considering these detrimental effects of fungicides on both the environment and human health in both the short and long term, it is necessary to investigate eco-friendly alternatives for managing fungal infections (Tiwari et al. 2021). Alternative and effective methods for controlling plant fungal diseases include biological control of phytopathogenic fungi, microbial and botanical fungicides, agronanotechnology, as well as fungal cell deactivation and evacuation via ghost techniques, all of which are safe (Barney, 2020).

2.4 Essential Oils as Potential Plant Protectants

Essential oils represent intricate blends of volatile compounds derived from diverse plant parts such as leaves, flowers, fruits, and seeds (Pavela and Benelli 2016). Through intricate chemical processes, plants synthesize these compounds, which serve multifaceted purposes within their natural environments. Among their myriad functions, essential oils act as formidable shields, fortifying plants against a plethora of threats including insects, bacteria, fungi, and other adversities that could compromise their vitality (Pavela and Benelli 2016). The composition of essential oils is rich and varied, comprising a complex amalgamation of organic molecules (Zarubova et al. 2015). Within these compositions lie a spectrum of aromatic compounds, terpenes, phenolics, and other bioactive constituents, each contributing distinct characteristics to the oil's aroma, flavor, and therapeutic properties. It is this diversity that renders essential oils potent allies in organic and sustainable agricultural practices.

Raveau et al. (2020) found that in the realm of agriculture, essential oils have emerged as promising alternatives to synthetic pesticides and antimicrobials, aligning harmoniously with the principles of eco-friendly cultivation. By harnessing the natural defenses of plants, essential oils offer a sustainable means of safeguarding crops against pests and pathogens, mitigating the ecological footprint associated with conventional agricultural practices. Furthermore, the application of essential oils in agriculture extends beyond mere pest control (Isman 2020). Their inherent properties exhibit multifunctional roles, including soil enhancement, plant growth promotion, and stress alleviation. Through targeted formulations and strategic deployment, essential oils can foster resilient agroecosystems that thrive in harmony with nature's rhythms. Bhavaniramya et al. (2019 asserted that the integration of essential oils into agricultural systems represents a symbiotic relationship between science and nature, where the innate wisdom of plants converges with human ingenuity to cultivate sustainable solutions for global food security. As the momentum for eco-conscious practices continues to gain traction, essential oils stand poised at the forefront of a burgeoning movement towards regenerative agriculture and stewardship of the earth's precious resources (Nayyar et al. 2017).

Plants possess a remarkable inherent capability to synthesize a diverse array of molecules, among which secondary metabolites are prominent (Pritts 2020). These secondary metabolites play crucial roles in defending plants against pathogens and environmental stresses. As highlighted by Pritts (2020), many of these molecules exhibit biological properties that contribute to the plant's resilience and survival. The medical benefits of essential oils (EOs), which have been known for centuries, make them one important class of secondary metabolites. Various plant parts, including leaves, flowers, stems, roots, and seeds, are the source of complex combinations of volatile molecules that make up essential oils. Their antibacterial, antioxidant, and anaesthetic qualities are only a few of their numerous advantages (Shan et al. 2021).

Essential oils have long been widely used in traditional medical systems throughout various nations and civilizations (Puentes-Téllez and Salles 2020). Their therapeutic potential has been explored and documented in various contexts, ranging from treating common ailments to managing more complex health conditions. The diverse chemical compositions of essential oils contribute to their wide-ranging biological activities and applications in healthcare (Rahman et al. 2009). The utilization of essential oils in traditional medicine reflects the accumulated wisdom of generations, where their efficacy and safety have been observed and validated over time. As our understanding of plant chemistry and pharmacology continues to advance, essential oils remain a subject of ongoing research and exploration in modern medicine and complementary therapies (Sánchez-Elordi et al. 2020). Their potential for enhancing human health and well-being continues to inspire scientific inquiry and innovation in the field of natural products and herbal medicine

Essential oils (EOs) represent a fundamental wellspring of biologically active compounds renowned for their diverse range of properties, as elucidated by Di Somma et al. (2020). These compounds exhibit antibacterial, insecticidal, fungicidal, nematicidal, herbicidal, antioxidant, and anti-inflammatory characteristics, making them valuable assets in various applications. Based on Kešnerová et al. (2020), essential oils have gained widespread use in the food industry, cosmetics, and flavorings, with about 300 being sold commercially. Essential oils are obtained from aromatic plants and are extracted using techniques like steam distillation and hydrodistillation to preserve the organic content of the plant sources.

Different plant parts, such as flowers, buds, leaves, seeds, fruits, roots, rhizomes, wood, and bark, can produce essential oils, though in small amounts. Due to compositional differences, essential oils are normally colorless, though they can have shades ranging from pale yellow to brown. They are stored in specialized structures such as glandular hairs, secretory cells, cavities, canals, and epidermal cells. Their physical states vary from liquid to resinous or solid, with densities that reflect their chemical makeup and molecular structure (Castello et al. 2020).

Essential oils demonstrate poor solubility in water but possess high solubility in organic solvents, a feature that enhances their versatility in formulation and application. Notably, they are categorized as fat-soluble compounds, underscoring their affinity for lipid-based mediums, as detailed by Gavriilidou et al. (2020). In essence, essential oils represent nature's reservoir of potent bioactive compounds, offering a myriad of practical and therapeutic benefits across industries and disciplines. Their intricate chemistry and multifaceted properties continue to inspire exploration and utilization in diverse fields, shaping the landscape of modern science and innovation.

According to Gavriilidou et al. (2020), the essential oil's chemical makeup is subject to variation, contingent on the specific organ from which the oil is derived. The essential oil's quantity and chemical composition can vary considerably, even for the same plant species, as a result of several factors such as the growth and developmental conditions of the plant, climatic conditions like temperature, rainfall, humidity, and light intensity, the soil composition, acidity, pollution, and mineral nutrition availability at the cultivation site, and the time of harvesting (Berg et al., 2020) and the root colonisation by symbiotic microorganisms, in particular, arbuscular mycorrhizal fungi (Gavrilidou et al., 2020). Differences in terms of chemical composition also appear between plant species of the same genus and more precisely between varieties of the same plant species, especially regarding the main compounds' proportions (Berg et al., 2020).

Puentes et al. (2020) showed in their study that the main aroma constituents of peppermint and sweet basil, menthol in the case of peppermint essential oil and linalool/eugenol in the case of basil oil, have antifungal properties against certain pathogenic fungi like *Rhizopus stolonifer* and *Sclerotinia sclerotiorum*. According to

Niem et al. (2020), *Calocedrus macrolepis* essential oil and its constituents have been shown to have an antifungal effect on *Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia solani*, *Pestalotiopsis funerea*, and *Colletotrichum gloeosporioides*. According to the study, essential oils from 25 different species of medicinal plants can inhibit the growth of six important mycelial-stage fungi that are toxic and pathogenic. These fungi include *Penicillium brevicompactum*, *Fusarium verticillioides*, *Fusarium oxysporum*, and *Penicillium expansum*. According to a different study by Berg et al. (2020), essential oils from *Nigella sativa L.*, *Eucalyptus globulus Labill*, and *Allium cepa L.* have antifungal action against *Fusarium oxysporum*, *Fusarium verticillioides*, *Fusarium solani*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum*. An investigation on the fungicidal qualities of essential oil obtained from *Solidago canadensis L.* was recently carried out by Zhang et al. (2021). *Aspergillus niger*, *Botrytis cinerea*, *Monilinia fructicola*, and *Penicillium expansu*m are among the postharvest phytopathogenic fungi against which the study validated its efficacy.

2.5 Sources Of Essential Oils

Several botanical sources harbor essential oils with notable potential as plant protectants, offering a diverse array of compounds that confer protective benefits against pests and pathogens. The various sources are discussed below.

2.5.1 Herbs and Spices

Herbs and spices have long been treasured not only for their culinary delights but also for their multifaceted contributions to agriculture and medicine (Prakash et al. 2015). Among these botanical treasures, certain herbs and spices stand out for their rich reservoirs of essential oils, which harbor potent pesticidal and fungicidal properties (Prakash et al. 2015). Some notable examples:

Thyme (*Thymus vulgaris*): Thyme, with its aromatic foliage adorned with tiny leaves, contains high concentrations of thymol, a powerful phenolic compound known for its broad-spectrum antimicrobial activity (Pandey et al. 2017). Thymol is particularly effective against bacterial and fungal pathogens, making thyme essential oil a valuable asset in the fight against plant diseases.

Figure 1: ***Thymus vulgaris*** **(**Pandey *et al*. 2017)

Oregano (*Origanum vulgare*): Oregano is celebrated for its robust flavor and aroma, which stem from its abundance of essential oils, including carvacrol and thymol (Pandey et al. 2017). These compounds exhibit potent antimicrobial properties, effectively combating both bacterial and fungal infections in plants. Oregano essential oil is commonly employed in organic agriculture to bolster plant health and resilience (Pandey et al. 2017).

Figure 2: ***Origanum Vulgare*** **(**Pandey *et al*. 2017)

Rosemary (*Rosmarinus officinalis*): Rosemary's distinctive fragrance and flavor are attributed to its rich essential oil content, primarily comprising cineole, camphor, and pinene. These compounds possess notable insect-repelling properties, deterring pests like aphids, whiteflies, and beetles. Additionally, rosemary oil exhibits fungicidal activity, offering protection against various plant pathogens (Blowman et al. 2018).

Figure 3: *Rosmarinus officinalis* (Blowman *et al*. 2018)

Mint (*Mentha spp*.): The refreshing aroma and flavor of mint are owed to its high levels of menthol and menthone, two compounds renowned for their insecticidal properties. Mint essential oil serves as a natural deterrent against pests such as ants, aphids, and moths, making it a valuable ally in integrated pest management strategies (Zarubova et al. 2015).

Figure 4 : ***Mentha*** **spp (**Zarubova *et al*. 2015)

Basil (*Ocimum basilicum*): Basil essential oil, with its rich blend of aromatic compounds including linalool and eugenol, possesses potent antimicrobial and insecticidal properties. It is effective against a wide range of pathogens and pests, making it a versatile tool in organic pest and disease management (Isman 2020).

Figure 5: ***Ocimum basilicum*** (Isman, 2020).

These culinary herbs and spices represent a treasure trove of natural remedies for the challenges faced by farmers and gardeners alike. By harnessing the power of their essential oils, growers can cultivate healthier crops while minimizing reliance on synthetic pesticides and fungicides, thereby promoting sustainable and environmentally conscious agricultural practices.

2.5.2 Citrus Fruits

Citrus fruits, esteemed for their vibrant flavors and refreshing aromas, offer more than just culinary delights; their rinds harbor a wealth of essential oils teeming with insecticidal and antimicrobial prowess (Blowman et al. 2018). Oranges, lemons, grapefruits, and other citrus varieties yield oils rich in compounds that serve as formidable defenses against pests and pathogens (Blowman et al. 2018). The essential oils extracted from citrus fruit rinds not only serve as natural solutions for pest and disease management in agriculture but also find applications in various industries, including cosmetics, aromatherapy, and household products (Blowman et al. 2018). Through sustainable extraction methods, these oils can be harnessed to promote eco-friendly practices in agriculture while minimizing the environmental impact associated with conventional pesticides (Blowman et al. 2018). By leveraging the innate defenses of citrus fruits, growers can cultivate resilient crops while reducing reliance on synthetic chemicals, thereby fostering healthier ecosystems and promoting sustainable agricultural practices that resonate with the rhythms of nature.

Oranges (*Citrus sinensis*)

The peels of oranges yield an essential oil abundant in limonene, a monoterpene compound renowned for its potent insect-repelling properties (Park et al. 2023). Limonene disrupts the sensory receptors of insects, deterring pests such as ants, flies, and mosquitoes. Additionally, orange essential oil exhibits antimicrobial activity, inhibiting the growth of bacteria and fungi that threaten plant health (Park et al. 2023).

Figure 6: Orange (Park *et al*. 2023)

Lemons (*Citrus limon*)

Lemon essential oil, derived from the zesty rinds of lemons, is prized for its citrusy fragrance and diverse therapeutic properties. It contains compounds like citral and citronellal, which possess strong insecticidal properties, making lemon oil an effective natural deterrent against pests like aphids, mites, and fruit flies (Ferrer et al. 2023). Furthermore, lemon oil's antimicrobial activity helps safeguard plants from bacterial and fungal infections (Ferrer et al. 2023).

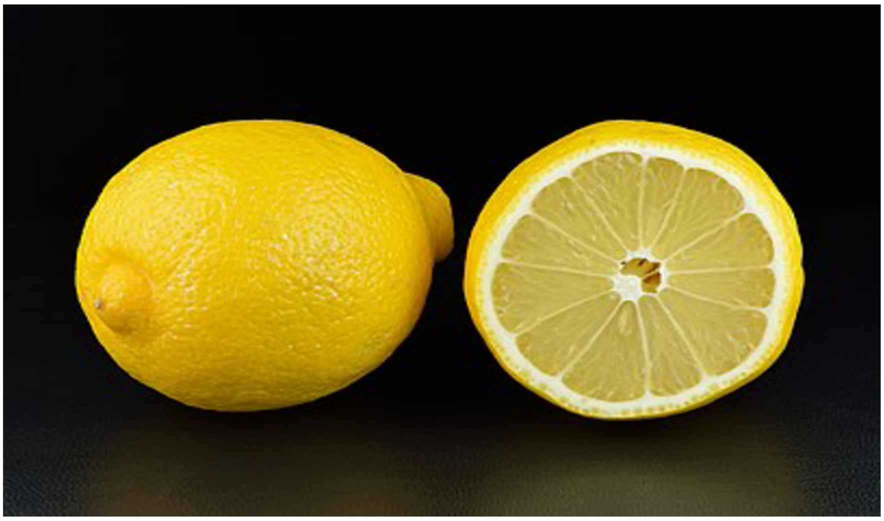

Figure 7: Lemon external surface and cross-section (Ferrer *et al*. 2023)

Grapefruits (*Citrus paradisi*)

Grapefruit essential oil, extracted from the peels of grapefruits, contains high levels of limonene and other terpene compounds known for their insecticidal and antifungal properties (Bustamante et al. 2016). Grapefruit oil serves as a potent insect repellent, warding off pests such as thrips, whiteflies, and caterpillars. Moreover, its antimicrobial activity contributes to the protection of plants against microbial threats in the soil and on foliage (Bustamante et al. 2016).

Figure 8: Grapefruits (Bustamante *et al.* 2016)

2.5.3 Trees

Trees, with their towering presence and majestic stature, harbor within them a wealth of natural remedies that extend far beyond their leafy canopies (Teigiserova et al. 2021). From the rugged bark to the resinous sap, trees yield essential oils brimming with potent compounds that serve as formidable defenses against insects and fungal diseases, offering a glimpse into nature's pharmacopoeia (Park et al. 2023). Among the arboreal treasures, species such as neem, cedarwood, and pine stand out for their remarkable efficacy in safeguarding plant health. Okla et al. (2019) found that harnessing the natural defenses of trees and extracting their precious essential oils, growers can cultivate healthier crops while minimizing reliance on synthetic pesticides and fungicides, thus promoting sustainable and eco-conscious agricultural practices. Park et al. (2023) found that the intricate connections between nature and agriculture, trees emerge as steadfast allies, offering potent remedies that resonate with the rhythms of the earth. Through harmonious stewardship of these arboreal treasures, the vitality of plants and the integrity of ecosystems converge in harmonious abundance. Some of the trees for essential oils are discussed below.

Neem (*Azadirachta indica*)

Revered for its myriad medicinal properties, the neem tree yields an essential oil renowned for its potent insecticidal and antifungal properties. Azadirachtin, the primary active compound in neem oil, disrupts the life cycles of insects, inhibiting feeding, reproduction, and metamorphosis. Neem oil also contains other bioactive constituents like limonoids and terpenoids, which confer broad-spectrum antimicrobial activity, making it an invaluable tool in organic pest and disease management (Okla et al. 2019).

Figure 9: Neem Tree (Okla *et al.* 2019)

Cedarwood (*Cedrus spp.*)

The majestic cedar trees bestow upon us an essential oil imbued with woody aromas and potent insect-repelling properties (Ferrer *et al*. 2023). Cedarwood oil contains compounds such as cedrol and cedrene, which act as natural insecticides, deterring pests like moths, ants, and termites. Additionally, cedarwood oil's antifungal properties make it effective in preventing and controlling fungal diseases that afflict plants, offering a sustainable alternative to synthetic fungicides (Ferrer *et al*. 2023).

Figure 10: Cedarwood Tree (Ferrer *et al.* 2023)

Pine (*Pinus spp.*)

Pine trees, with their towering stature and resinous sap, yield an essential oil prized for its invigorating scent and versatile applications. Pine oil contains compounds like pinene and limonene, which exhibit potent insecticidal properties, repelling pests such as beetles, aphids, and caterpillars (Bustamante *et al*. 2016). Moreover, pine oil's antifungal activity helps protect plants from fungal pathogens that thrive in humid environments, making it a valuable asset in plant disease management

2.5.4 Flowers

Flowers, with their delicate petals and captivating fragrances, offer more than just visual and olfactory pleasures; within their blooms lie a treasure trove of essential oils brimming with potent bioactive compounds (Zarubova *et al*. 2015). Among the floral wonders, lavender, geranium, and clove emerge as botanical powerhouses, harnessing nature's arsenal to safeguard plants against pests and fungal diseases (Zarubova et al. 2015). Harnessing the natural defenses of flowers and extracting their precious essential oils, growers can cultivate healthier crops while minimizing reliance on synthetic pesticides and fungicides, thus promoting sustainable and eco-conscious agricultural practices. Blowman et al. (2018) found that flowers are steadfast allies, offering potent remedies that resonate with the rhythms of the earth. Through harmonious stewardship of these floral treasures.

Lavender (*Lavandula spp.*)

Lavender, with its serene hues and soothing aroma, yields an essential oil revered for its diverse therapeutic properties. Lavender essential oil contains compounds such as linalool and linalyl acetate, which exhibit potent insect-repelling properties, deterring pests like mosquitoes, moths, and fleas (Isman 2020). Moreover, lavender oil's antifungal activity helps protect plants from fungal pathogens that threaten their health and vitality, making it a versatile ally in organic pest and disease management (Isman 2020).

Figure 11: Lavender Flower (Isman 2020)

Geranium (*Pelargonium spp.*)

Geraniums grace gardens with their vibrant blooms and aromatic foliage, harbouring within them an essential oil prized for its insecticidal and fungicidal properties. Geranium oil contains compounds like citronellol and geraniol, which act as natural insect repellents, warding off pests such as aphids, whiteflies, and caterpillars (Raveau *et al*. 2020). Additionally, geranium oil's antifungal activity helps combat fungal diseases that afflict plants, offering a sustainable alternative to synthetic fungicides (Raveau *et al*. 2020).

Figure 12: Geranium Flower (Raveau *et al*. 2020).

Clove (*Syzygium aromaticum*)

Clove, with its rich aroma and culinary allure, yields an essential oil renowned for its potent insecticidal and antimicrobial properties. Clove oil contains eugenol, a powerful compound that disrupts the nervous system of insects, effectively repelling pests like ants, cockroaches, and beetles (Pandey et al. 2017). Furthermore, clove oil's antifungal activity helps protect plants from fungal pathogens that thrive in warm and humid conditions, making it a valuable asset in plant disease management (Pandey et al. 2017).

Figure 13: Clove Flower (Pandey et al. 2017)

Figure 14: Dried Clove Flower (Pandey et al. (2017)

2.6 Effectiveness of Essential Oils for Controlling Fungal Diseases in Other Related Crops

In recent years, there has been a surge in research dedicated to exploring essential oils (EO) as a promising source for new biopesticides. The emphasis has been placed on their relative effectiveness against pathogens, their multiple mechanisms of action, and their relatively low toxicity to mammals and humans (Failla & Romano, 2020). In vitro experiments conducted by Rashad et al. (2022) have notably highlighted lavender essential oil (EO) for its potent antifungal properties against *Fusarium solani*, a causative agent of damping-off in sorghum. The study reported complete inhibition of fungal growth at a concentration of 1.6 % EO, suggesting its potential as a biopesticide.

Additionally, another study conducted by Sreenivasa et al. (2011) suggests that essential oils, particularly citronella oil, could be practically applied to control the growth of *Fusarium* species in stored maize and sorghum grains. This further underscores the versatility and potential of essential oils as biopesticides in agricultural settings

Cymbopogon citratus, *Ocimum gratissimum*, and *Thymus vulgaris* essential oils have shown exceptional efficacy in preventing seed-borne infections and the spread of pathogens from seed to seedling in rice. According to Nguefack et al. (2008), these essential oils successfully prevented *Alternaria padwickii*, *Bipolaris oryzae*, and *Fusarium moniliforme* from infecting naturally occurring rice seeds. This highlights the potential of essential oils as natural agents for managing seed-borne diseases in agricultural crops. Furthermore, Ravali et al. (2020) discovered that Cedarwood oil and Lemongrass oil exhibit potent antifungal properties against *Rhizoctonia solani*, a common pathogen affecting various crops. Their study revealed that Cedarwood oil treatment, particularly under field conditions, resulted in a substantial 57.38 % disease control rate and a remarkable 48.24 % increase in yield compared to the control group. These findings underscore the practical application of essential oils as effective and eco-friendly alternatives for disease management in agricultural settings, offering potential benefits for crop productivity and sustainability

3. Aim of The Thesis

The primary objective of this research is to assess the viability of essential oils as a novel method for safeguarding sugarcane against fungal infections in controlled laboratory environments. The investigation will focus on examining the effectiveness of different essential oils in impeding the proliferation of fungal pathogens known to afflict sugarcane crops. Furthermore, the study aims to substantiate the degree of effectiveness exhibited by these natural plant compounds in combating fungal diseases that commonly affect sugarcane plants. Through rigorous experimentation and analysis, the research endeavors to elucidate the potential role of essential oils as a sustainable and environmentally friendly solution for mitigating fungal infections in sugarcane cultivation.

3.1 General objective

The primary goal of the research is to assess and affirm the effectiveness of different essential oils in suppressing the proliferation of fungal pathogens that impact sugarcane crops.

3.2 Hypotheses of the Study

(H_1): There is a statistically significant difference between the *Alternaria alternata* growth inhibition values on different essential oils and the control.

(H_2): There is a statistically significant difference between the *Schizophyllum commune* growth inhibition values on different essential oils and the control.

(H_3): There is a statistically significant difference between the *Fusarium verticillioides* growth inhibition values on different essential oils and the control.

(H_4): There is a statistically significant difference between the *Chalara sp*. growth inhibition values on different essential oils and the control.

(H_5): There is a statistically significant difference between the *Rhizoctonia sp*. growth inhibition values on different essential oils and the control.

(H_6): There is a statistically significant difference between the *Setophoma terrestris* growth inhibition values on different essential oils and the control.

(H_7): There is a statistically significant difference between the *Fusarium* sp. growth inhibition values on different essential oils and the control.

4 Materials and Methods

The experiment was conducted at the Laboratory of Molecular Diagnostics of Pathogens and Plants Pests at the Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague.

4.1 Materials

4.1.1 Fungal Pathogens Material

Eight (8) fungal pathogens strains (*Rhizoctonia solani 2, Rhizoctonia solani 5, Fusarium spp, Setophoma terrestris, Schizophyllum commune, Alternaria alternata, Chalara spp,* and *Fusarium verticillioides*) were obtained from the Czech Collection of Microorganisms in Brno, Czech Republic. The fungal pathogens were sub-cultured and allowed to incubate for 14 days in an incubation chamber to facilitate their growth. The newly grown fungi were used in the experiment.

4.1.2 Essential Oils

Five already extracted essential oils: *Thymus vulgari*s oil, *Origanumm vulgare* oil, *Saturea montana* oil, *Thymus serpillum* oil, and *Cinnamomum camphora* oil were purchased from Saloos Naturcosmetics and used for the experiment.

4.2 Methods

4.2.1 Preparation of Poison Petri Dish

Thirty-nine grams (39 g) of already formulated PDA (Potato Dextrose Agar) was suspended in 1 litre of distilled water in a conical flask. The mixture was stirred to fully dissolve all components. The media was covered with aluminium foil and sterilized in an autoclave at a temperature of 121 °C for 15 minutes. After sterilization, the PDA was allowed cool to approximately 45 °C. 250 µL of essential oil and 13 ml of TWEEN in a water mixture were pipetted into 250 ml of PDA. The mixture was swirled to mix it thoroughly. Approximately 17 ml of the mixture was poured into sterile petri dishes and left to solidify under sterile conditions.

4.2.2 Fungal Pathogen Inoculation

Two different techniques were used to transfer the fungal pathogens onto the poison petri dish.

1. Inoculation by hyphal transfer: A small piece of mycelium from the fungal culture was transferred onto the surface of the growth medium in the petri dish using a sterile needle under sterile conditions. The growth of the fungal culture was monitored over time, looking for signs of contamination or abnormal growth patterns.

2. Inoculation by cork borer: Sterile cork borer, forceps, and scalpel blade were used for the inoculation. The tools were sterilized by heating them in a flame. Using the sterile cork borer, small agar plugs were cut out from a culture of the fungal pathogen. Using sterile forceps or a scalpel blade, the agar plugs were placed onto the surface of the growth medium in the Petri dish (poison petri dish). The plugs were placed in the centre of the growth medium. The Petri dishes thus inoculated were sealed with parafilm paper. The inoculated Petri dishes were kept in an incubation chamber at the appropriate temperature and humidity for the fungal pathogen being cultured.

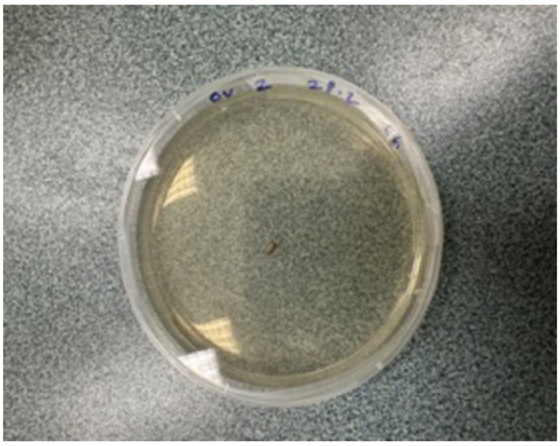

Figure 15: Inoculation by Hyphal transfer

Figure 16: Inoculation by Cork borer

4.2.3 Measuring the fungal growth

The growth of fungi was monitored, and control plates that had reached full growth were removed along with the treatment plates to measure their dimensions using a digital caliper. In addition, plates that had not reached full growth within two weeks were also measured. Before taking measurements, it was confirmed that the fungal colony had a well-defined edge. The Petri dishes were carefully placed on a flat surface. The digital caliper was placed at the edge of the fungal colony and the measurement was recorded in an Excel spreadsheet. Multiple measurements were taken from different sides of the colony to ensure accuracy.

4.3 Experimental Design and Statistical Analysis

The experiment consisted of two stages. During the initial stage, fungal pathogens were subjected to 0.1 % concentration of essential oils for evaluation. The study utilized a completely randomized block design with five replications. Each treatment was tested on five plates per experimental unit and one control. Following incubation and measurement, it was observed that *Origanumm vulgare* and *Thymus vulgaris* were able to entirely suppress the growth of all fungal species.

The subsequent stage aimed to establish the minimum inhibitory concentration of the effective essential oils. At this stage, fungal pathogens were subjected to different concentrations of essential oils.

Table 1: Concentration levels of EO used

Percentage (%) of Essential Oil	Concentration Level
0.02	200
0.04	400
0.06	600
0.08	800

4.4 Statistical Analysis

The mycelial growth measured on plates treated with poison was compared to the control for a specific variant, and the percentage of growth inhibition was determined by I = (C-T)/C × 100, where C is the growth of mycelium in the control plate, T is the test species growth of mycelium in the inserted plate, and I is the mycelial growth inhibition. The data was processed using the arcsine function to transform the percentage data for analysis.

$$y = \arcsin\sqrt{\frac{x}{100}}$$

=ARCSIN(ODMOCNINA(B3/100)

Since negative numbers cannot undergo transformation, the study focuses on the positive effects of EO rather than negative ones. Therefore, any negative cases will be treated as having 0 % inhibition.

The experimental data underwent statistical analysis. Initially, a normality test was conducted on the distribution of the data. The findings revealed that the data did not follow a normal distribution, hence necessitating the application of a non-parametric test similar to ANOVA. Consequently, the Kruskal-Wallis test was selected, regarding the utilization of essential oils and their impact on phytopathogenic fungi.

Table 2: Fungi and Acronym

Fungi	Acronym
Alternaria alternata	AA50
Schizophyllum commune	SC
Fusarium verticillioides	FV
Chalara sp.	CH
Rhizoctonia sp.	Rhi2, Rhi5
Setophoma terrestris	M3
Fusarium sp.	M13

Table 2 lists the various fungi and their corresponding acronyms used in this study

Table 3: Essential Oils and Acronym

Essential Oils	Acronym
Cinnamomum camphora	CC
Satureja montana	SM
Origanum vulgare	OV
Thymus serpyllum	TS
Thymus vulgaris	TV

Table 3 shows the various essential oils and their respective acronyms used in the experimental set-up.

5. Results

The results show whether there exist statistically significant variations in the suppression of mycelial growth caused by specific fungal pathogens when exposed to different essential oils, as evidenced by the Kruskal-Wallis test performed. The fungal pathogens examined include *Alternaria alternata* (AA50), *Schizophyllum commune* (SC), *Fusarium verticillioides* (FV), *Chalara sp.* (CH), *Rhizoctonia sp.* (Rhi2, Rhi5), *Setophoma terrestris* (M3), and *Fusarium sp.* (M13). The essential oils considered are derived from *Cinnamomum camphora* (CC), *Satureja montana* (SM), *Origanum vulgare* (OV), *Thymus serpyllum* (TS), and *Thymus vulgaris* (TV).

The results are presented in two different formats, the first is using boxplot to summarise the distribution of the set of fungal pathogen growth values for different essential oils. This is followed by a Table presentation of each fungal pathogen growth value with their respective p-values to show which ones are statistically significant among them.

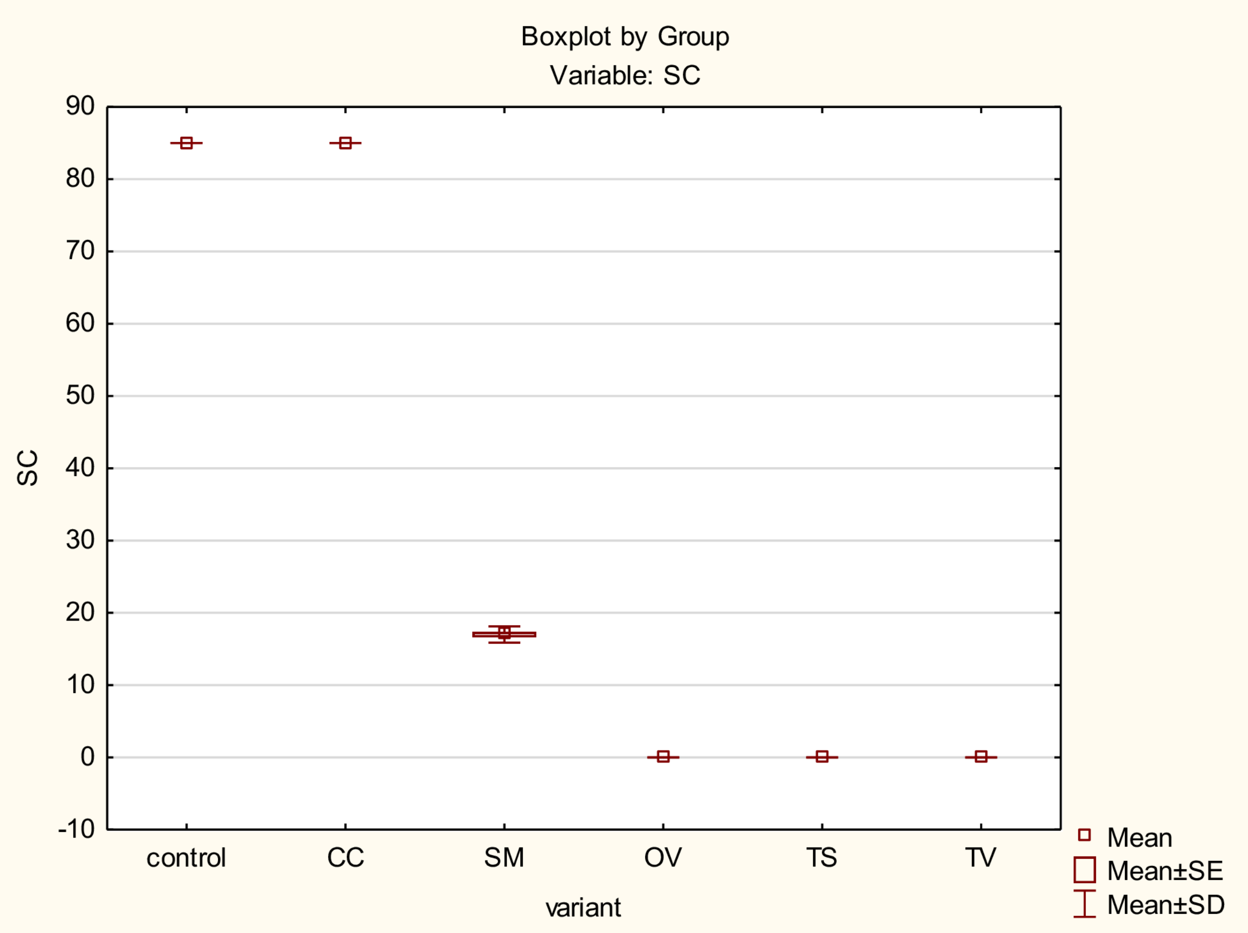

Figure 17: *Schizophyllum commune* (SC) Treatment with Essential Oils

In the boxplot representation, the horizontal axis defines the various essential oils utilized in the experiment (CC, SM, OV, TS, and TV), while the vertical axis denotes the fungal pathogen growth values. Each box in the plot illustrates the interquartile range (IQR), summarizing the middle 50 % of the data, with the bottom and top of the box indicating the first quartile (Q1) and third quartile (Q3) respectively. The median, dividing the data evenly, is depicted by the line within the box. Whiskers extend to the farthest non-outlier data points, while outliers beyond the whiskers are represented individually as circles. The notches in the box signify the 95 % confidence interval for the median, offering insights into its variability across different samples.

The fungal pathogen, *Schizophyllum commune* (SC), demonstrates the most significant growth inhibition when exposed to the essential oil TV, OV and TS, evident from the lowest median value and the smallest box in the boxplot representation. Additionally, the essential oils CC and SM also exhibit inhibitory effects on fungal pathogen growth compared to the control, albeit to a lesser degree than TV, OV and TS. Variability in growth values for each essential oil is apparent, as depicted by the varying sizes of the boxes and whiskers, with no outliers present in the dataset. In summary, the boxplot indicates that all five essential oils exhibit some degree of inhibition on fungal pathogen growth, with TV, OV and TS emerging as the most effective among them with respect to *Schizophyllum commune* (SC).

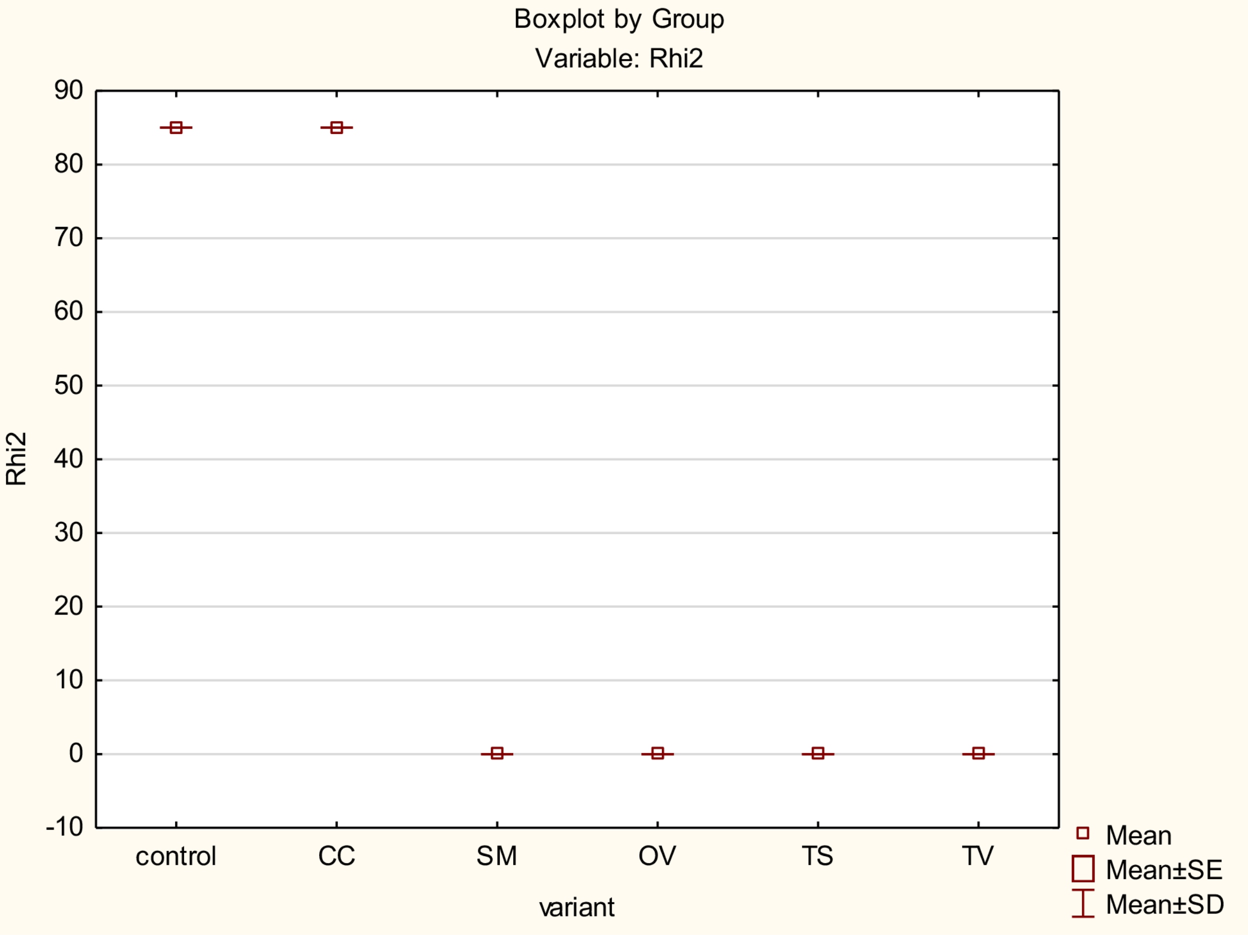

Figure 18: *Rhizoctonia sp*. (Rhi2) Treatment with Essential Oils

The fungal pathogen, *Rhizoctonia sp.* (Rhi2), when subjected to essential oils, displays a general trend of growth escalation over time, although the growth rate varies among pathogens. In the control group, which includes no essential oil, most pathogens exhibit the highest growth. *Rhizoctonia sp*. (Rhi2) also exhibit the highest growth in the essential oils CC. This shows that the essential oils CC do not suppress the fungal growth of *Rhizoctonia sp*. (Rhi2). Essential oils such as SM, TS, OV and TV demonstrate a more pronounced effect of impeding fungal growth compared to the control.

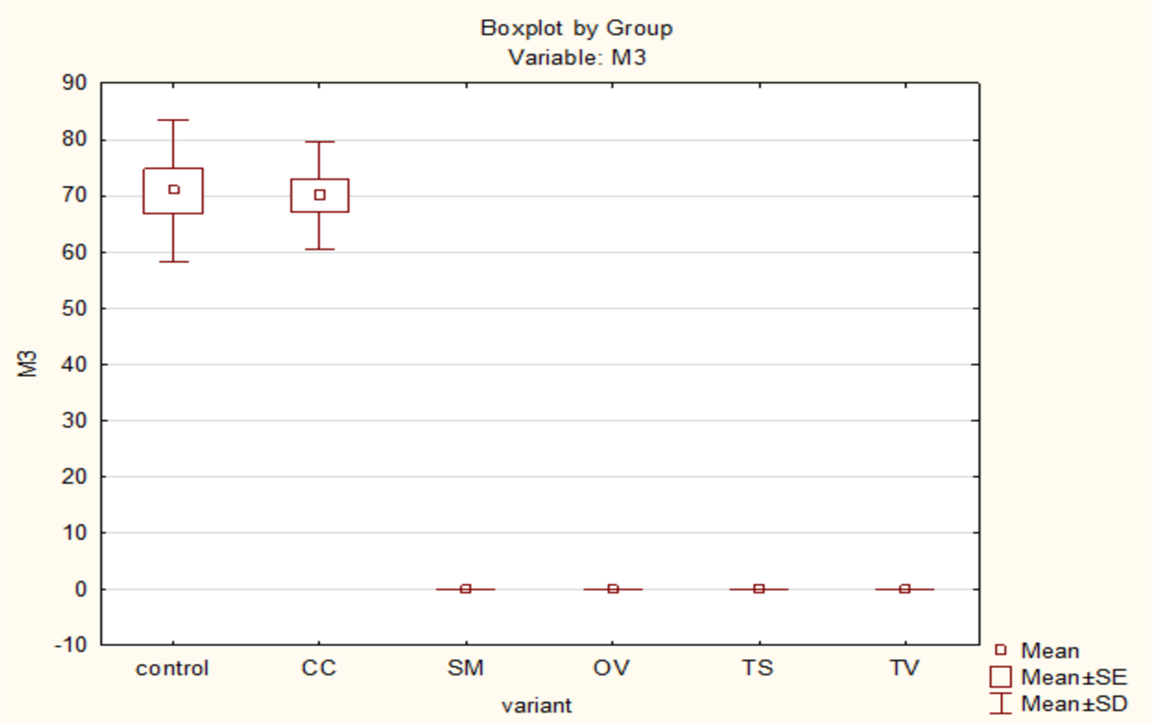

Figure 19: *Setophoma terrestris* (M3) Treatment with Essential oils

The essential oils CC, SM, TS, TV, and OV demonstrated inhibition of *Setophoma terrestris* (M3) growth to varying degrees compared to the control group, as evidenced by lower median growth values for each essential oil. Among them, *Origanum vulgare* (OV) emerges as the most effective in suppressing the growth of *Setophoma terrestris* (M3), with its median growth value being the lowest among all essential oils. While there is some variability in growth inhibition observed for each essential oil, as indicated by the sizes of the boxes and whiskers in the boxplot, no outliers were detected in the dataset.

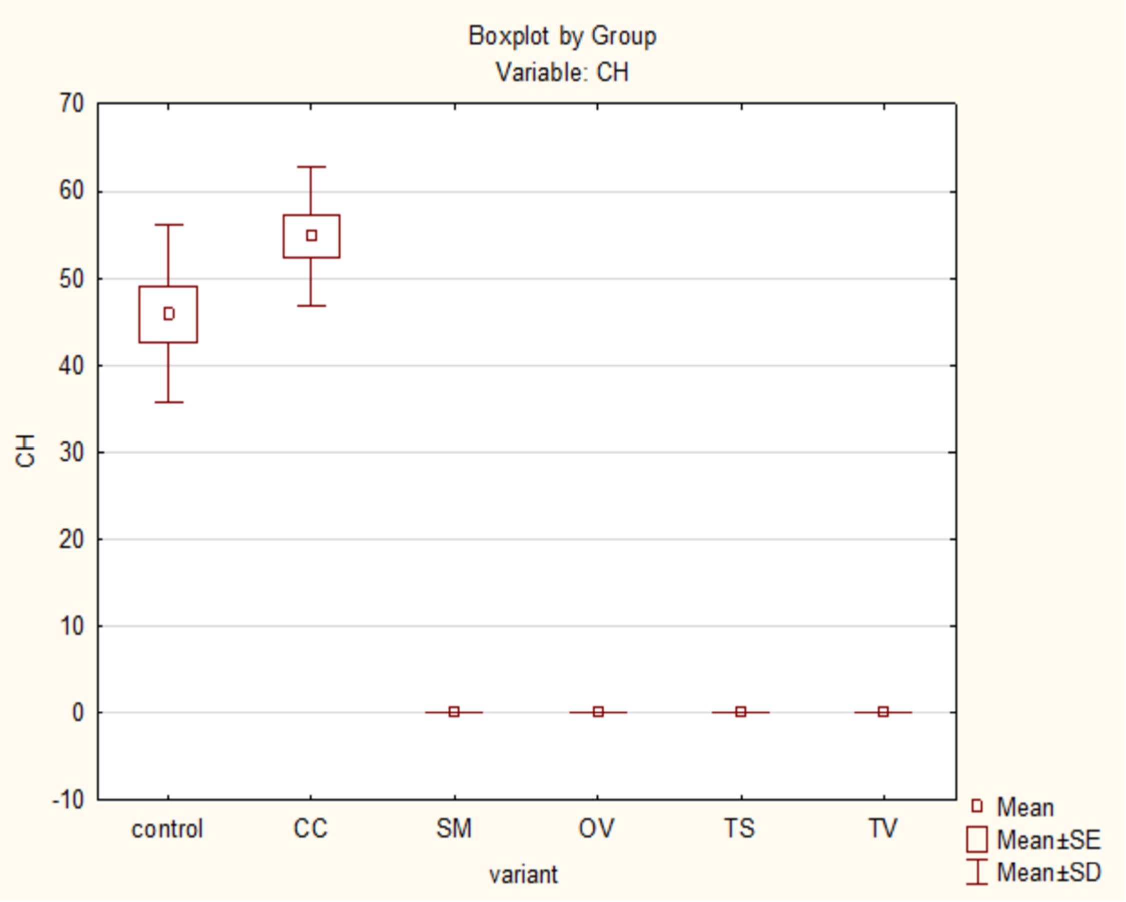

Figure 20: ***Chalara sp.*** **(CH) with Essential Oils**

The results of the treatment on *Chalara sp.* (CH) with essential oils show that SM, OV, TS, and TV demonstrated inhibition of *Chalara sp.* (CH) growth to varying degrees compared to the control group, as evidenced by lower median growth values for each these essential oil. All of these essential oils SM, OV, TS, and TV have very similar effects in suppressing the growth of *Chalara sp.* (CH), with its median growth value being the lowest among the essential oils.

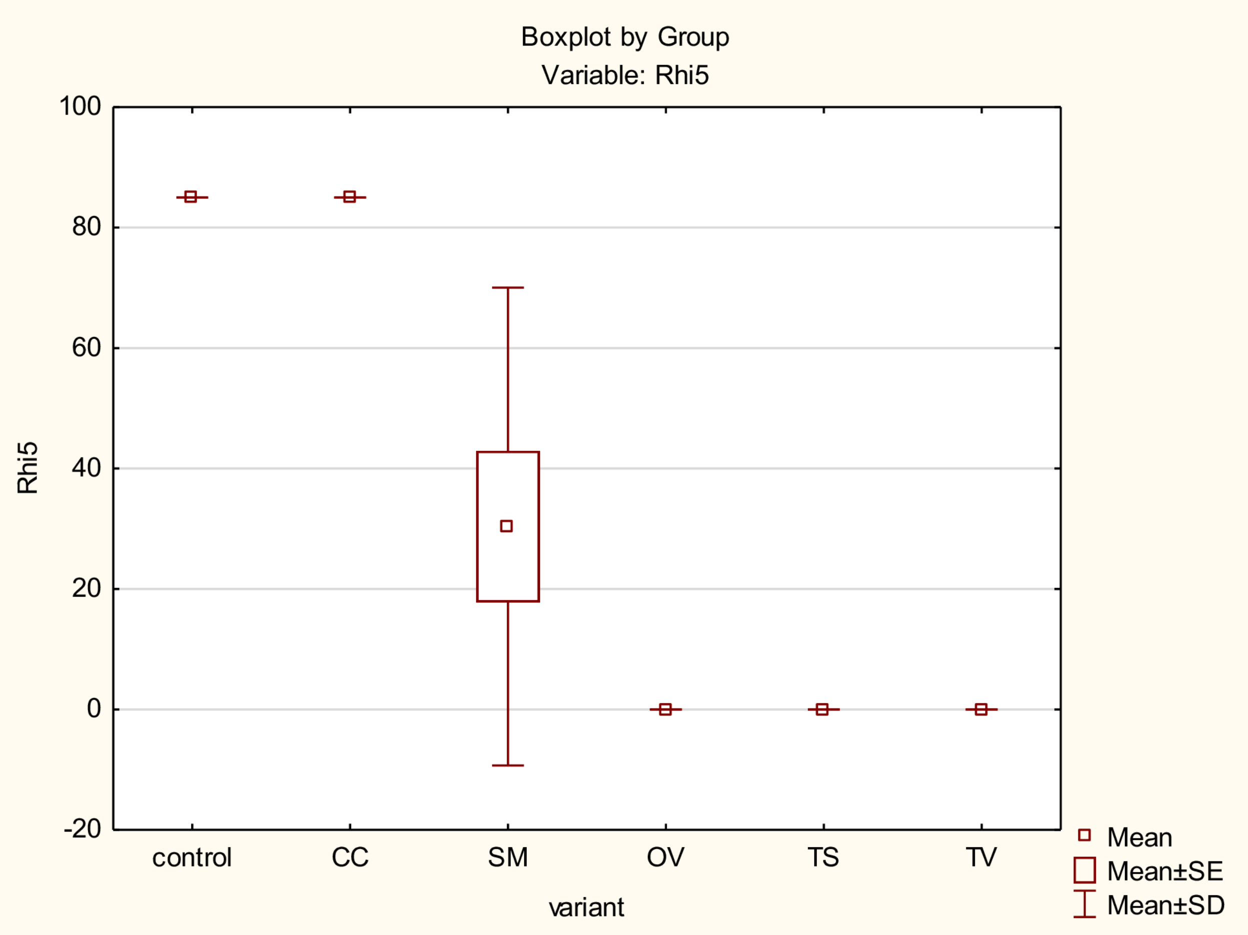

Figure 21: *Rhizoctonia sp.* (Rhi5) Treatment with Essential Oils

The *Origanum vulgare* (OV) group exhibits the highest median inhibition of *Rhizoctonia sp.* (Rhi5). This suggests that *Origanum vulgare* (OV) essential oil was the most successful treatment in reducing the growth of *Rhizoctonia sp.* (Rhi5) in this experiment. Similarly, the *Thymus vulgaris* (TV) group shows a notably high median inhibition of *Rhizoctonia sp.* (Rhi5). The results also indicate that *Thymus vulgaris* (TV) essential oil might also serve as an effective treatment for this pathogen. Conversely, the control group, as well as the *Cinnamomum camphora* (CC) group and the *Satureja montana* (SM) essential oils, exhibit significantly lower median inhibition rates for *Rhizoctonia sp.* (Rhi5).

This implies that *Cinnamomum camphora* (CC) group and the *Satureja montana* (SM) essential oils treatments were not particularly effective in curtailing the growth of *Rhizoctonia sp.* (Rhi5). Variability in the data within each group, as depicted by the width of the boxes, suggests that treatment effectiveness may vary depending on the specific experimental conditions. Notably, there are no outliers detected in the dataset.

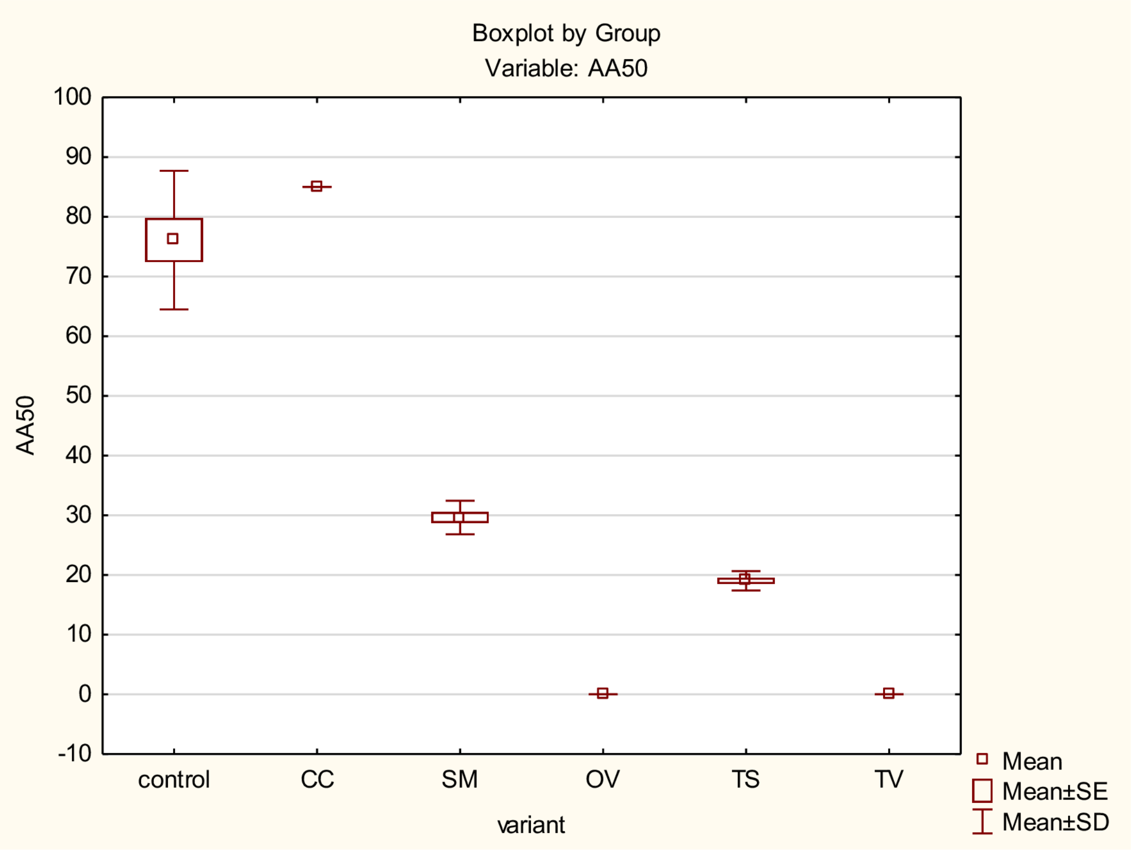

Figure 22: *Alternaria alternata* (AA50) with Essential Oils

The *Thymus vulgaris* (TV) and *Origanum vulgare* (OV) group exhibit the highest median inhibition of *Alternaria alternata* (AA50). This reveals that *Thymus vulgaris* (TV) and *Origanum vulgare* (OV) essential oil were the most successful treatments in reducing the growth of *Alternaria alternata* (AA50) in this experiment. Similarly, the *Thymus serpyllum* (TS) essential oil shows a promising median inhibition of *Alternaria alternata* (AA50). However, the results also indicate that *Cinnamomum camphora* (CC) and *Satureja montana* (SM) were not mostly effective in restraining the growth of *Alternaria alternata* (AA50).

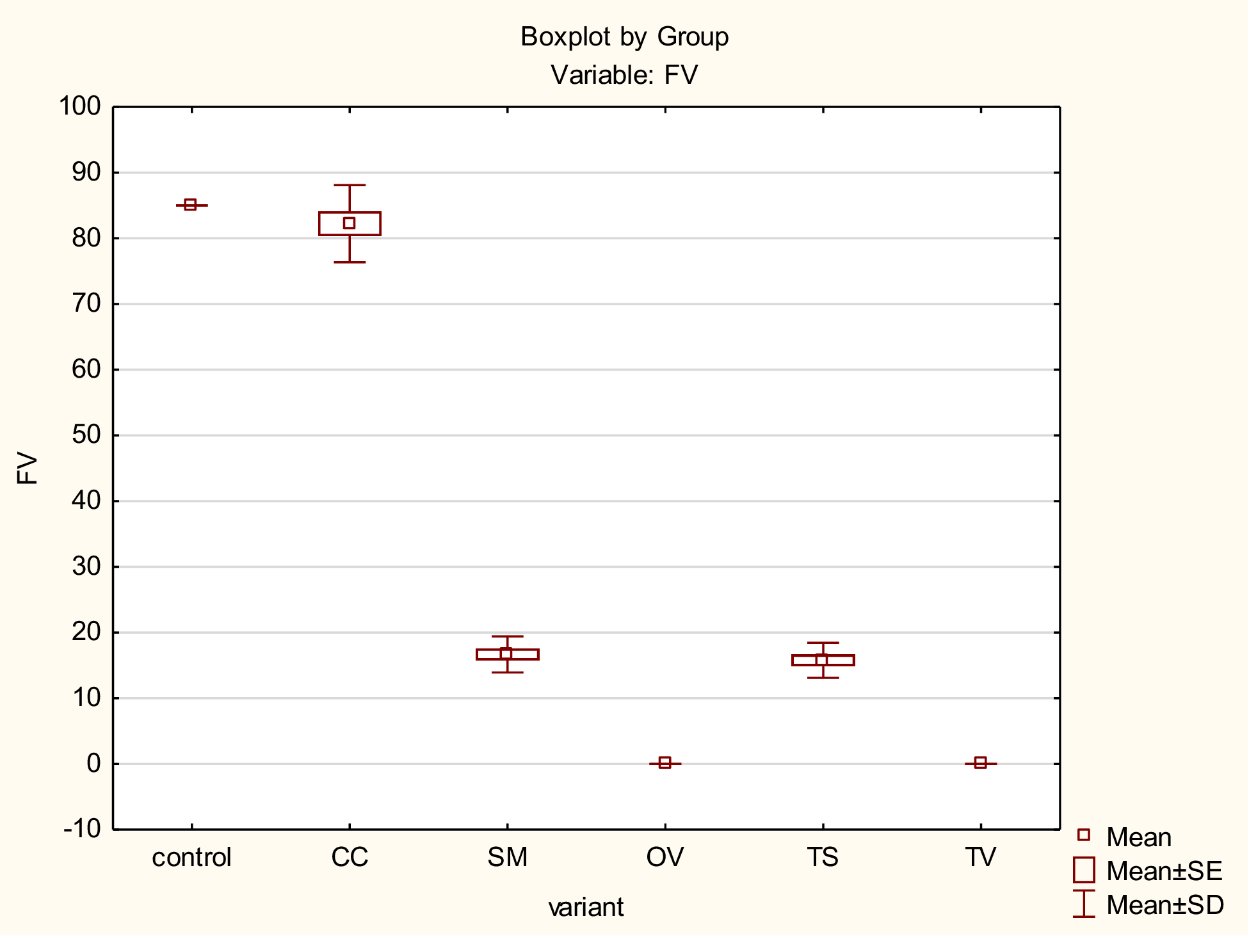

Figure 23: *Fusarium verticillioides* (FV) with Essential Oils

The *Origanum vulgare* (OV) and *Thymus vulgaris* (TV) essential oil group exhibit the highest median inhibition of *Fusarium verticillioides* (FV). This demonstrates that *Origanum vulgare* (OV) and *Thymus vulgaris* (TV) are the most effective essential oils in the inhibition of *Fusarium verticillioides* (FV). *Thymus serpyllum* (TS) and *Satureja montana* (SM) essential oils group have shown promising median inhibition of *Fusarium verticillioides*. Nevertheless, the results indicate that *Cinnamomum camphora* (CC) is not effective in inhibiting the growth of *Fusarium verticillioides* (FV).

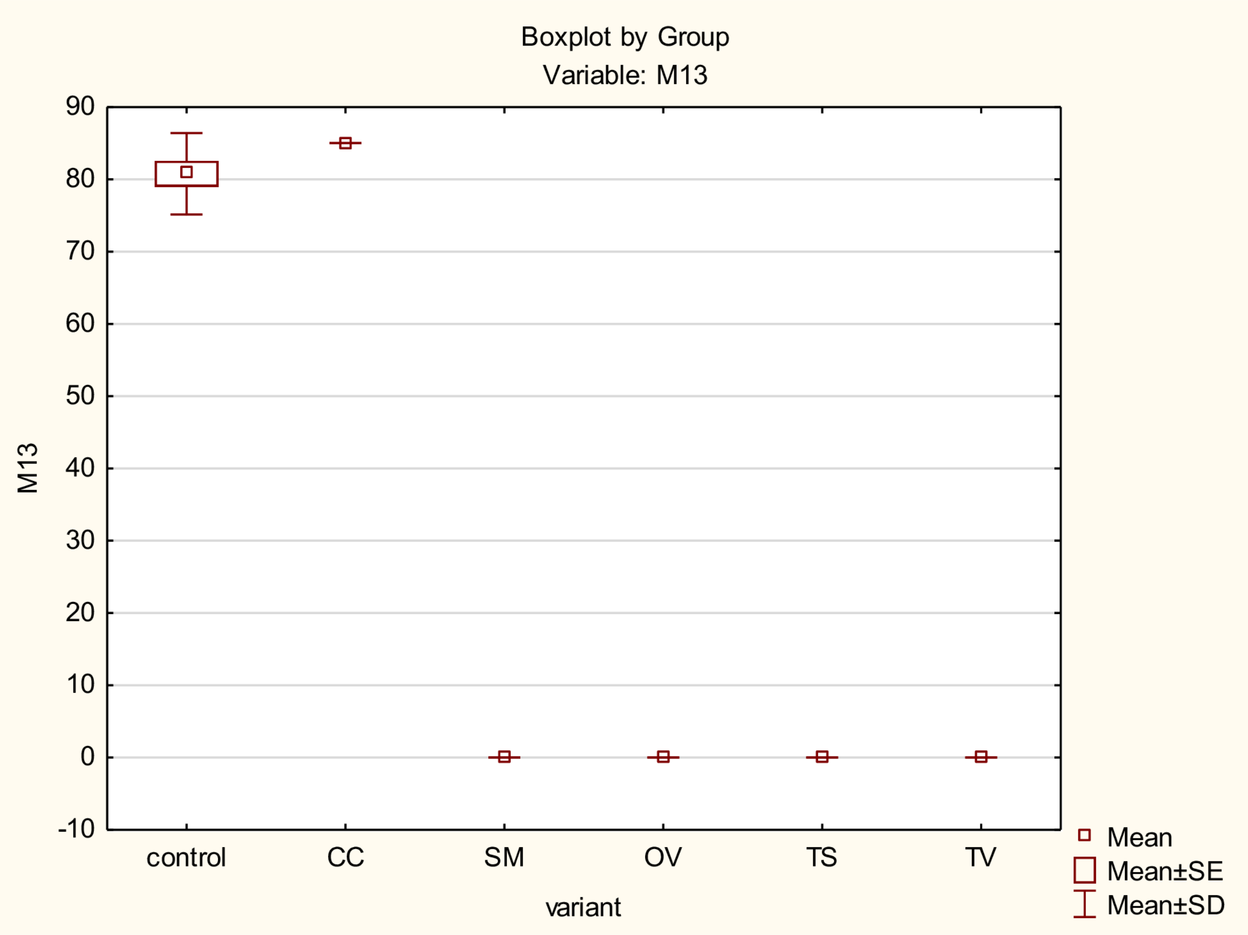

Figure 24: *Fusarium sp.* (M13) with Essential Oils

The results indicate that *Satureja montana* (SM), *Origanum vulgare* (OV), *Thymus serpyllum* (TS) and *Thymus vulgaris* (TV) essential oil group exhibit the highest median inhibition of *Fusarium sp.* (M13). This shows that *Satureja montana* (SM), *Origanum vulgare* (OV), *Thymus serpyllum* (TS) and *Thymus vulgaris* (TV) are the most effective essential oils in the inhibition of *Fusarium sp.* (M13). However, the *Cinnamomum camphora* (CC) essential oil has shown that it is not effective in inhibiting the growth of *Fusarium sp.* (M13).

Effect of Essential Oils on Fungal Pathogen Growth

The table presents the results of an experiment examining the effect of different essential oils on the growth of the fungal pathogen. The growth values indicate the degree of inhibition or promotion of fungal growth caused by each essential oil. Lower growth values suggest greater inhibition of fungal growth, while higher values indicate less inhibition or even promotion of growth.

Table 4: Effect of Essential Oils on Fungal Pathogen Growth

Fungal Pathogen	Essential Oil	Growth Value	p-value
***Alternaria alternata* (AA50)**	*Cinnamomum camphora (CC)*	87	0.785
	Satureja montana (SM)	28	0.071
	Origanum vulgare (OV)	0	0.002
	Thymus serpyllum (TS)	18	0.008
	Thymus vulgaris (TV)	0	0.012
***Schizophyllum commune* (SC)**	*Cinnamomum camphora (CC)*	88	0.654
	Satureja montana (SM)	27	0.070
	Origanum vulgare (OV)	0	0.005
	Thymus serpyllum (TS)	15	0.027
	Thymus vulgaris (TV)	0	0.039
***Fusarium verticillioides* (FV)**	*Cinnamomum camphora (CC)*	80	0.831
	Satureja montana (SM)	18	0.071
	Origanum vulgare (OV)	0	0.000
	Thymus serpyllum (TS)	25	0.058
	Thymus vulgaris (TV)	0	0.001
***Chalara sp.* (CH)**	*Cinnamomum camphora (CC)*	57	0.771
	Satureja montana (SM)	0	0.009
	Origanum vulgare (OV)	0	0.001
	Thymus serpyllum (TS)	0	0.002
	Thymus vulgaris (TV)	0	0.008
***Rhizoctonia sp.* (Rhi2, Rhi5)**	*Cinnamomum camphora (CC)*	70	0.642
	Satureja montana (SM)	24	0.031
	Origanum vulgare (OV)	0	0.005
	Thymus serpyllum (TS)	0	0.019
	Thymus vulgaris (TV)	0	0.006
***Setophoma terrestris* (M3)**	*Cinnamomum camphora (CC)*	70	0.614
	Satureja montana (SM)	0	0.011
	Origanum vulgare (OV)	0	0.00
	Thymus serpyllum (TS)	0	0.001
	Thymus vulgaris (TV)	0	0.001
***Fusarium sp.* (M13)**	*Cinnamomum camphora (CC)*	84	0.621
	Satureja montana (SM)	0	0.021
	Origanum vulgare (OV)	0	0.008
	Thymus serpyllum (TS)	0	0.000
	Thymus vulgaris (TV)	0	0.015

$P < 0.05$

In terms of growth values and p-values for *Alternaria alternata* (AA50), *Origanum vulgare* (OV) and *Thymus vulgaris* (TV) are the essential oils that significantly limit growth. They both have growth values of 0, which means that fungal growth is completely inhibited. The corresponding p-values, which are below the significance threshold of 0.05 (0.002 for OV and 0.012 for TV), also show this. *Thymus serpyllum* (TS) also has a growth value of 18 with a p-value of 0.008. Therefore, these results suggest that *Origanum vulgare* (OV), *Thymus serpyllum* (TS) and *Thymus vulgaris* (TV) are statistically significant in their ability to inhibit the growth of *Alternaria alternata* (AA50). However, *Cinnamomum camphora* (CC) essential oil has a fungal growth growth value of 87 and *Satureja montana* (SM) has a growth value of 28 with associated p-values of 0.785 and 0.071 which shows that (CC) cannot inhibit fungal growth in *Alternaria alternata* (AA50).

Regards to *Schizophyllum commune* (SC), growth values and p-values, it's notable that *Origanum vulgare* (OV), and *Thymus vulgaris* (TV) all display a growth value of 0 indicating complete inhibition of fungal growth for OV and TV. Moreover, the associated p-values for these oils (0.005 for OV, and 0.039 for TV) are all below the significance threshold of 0.05, suggesting statistical significance in their ability to inhibit the growth of *Schizophyllum commune* (SC). *Thymus serpyllum* (TS) exhibits a lower growth value (27) with a corresponding p-value (0.027) indicating statistical significance in their ability to inhibit the growth of *Schizophyllum commune*.

Satureja montana (SM) exhibits a growth value (20), and its corresponding p-value (0.070) is above 0.05, indicating no significant inhibition just like *Cinnamomum camphora* (CC) (growth value of (88) with a higher p-value of 0.654 indicating it's ability to inhibit fungal growth.

In summary, *Origanum vulgare*, *Thymus serpyllum* and *Thymus vulgaris* appear to be statistically significant in inhibiting the growth of *Schizophyllum commune* when compared to *Satureja montana* and *Cinnamomum camphora* which cannot inhibit fungal growth.

When it comes to *Fusarium verticillioides*, analyzing both the growth values and p-values, it's evident that *Origanum vulgare*, and *Thymus vulgaris* all demonstrate a growth value of 0, indicating complete inhibition of fungal growth. Moreover, the associated p-values for these essential oils (0.000 for OV and 0.001 for TV) are all below the significance threshold of $P < 0.05$, suggesting statistical significance in their ability to inhibit the growth of *Fusarium verticillioides*.

In contrast, *Cinnamomum camphora* (CC), *Satureja montana* (SM) and *Thymus serpyllum* (TS) display a higher growth value (80), 18 and 25 respectively with corresponding p-values of 0.831, 0.071 and 0.058 indicating an inability to inhibit fungal growth compared to the other essential oils tested. Therefore, *Cinnamomum camphora* (CC), *Satureja montana* (SM) and *Thymus serpyllum* (TS) are not statistically significant in their ability to inhibit the growth of *Fusarium verticillioides.*

Origanum vulgare (OV), and *Thymus vulgaris* (TV) are statistically significant in their efficacy in inhibiting the growth of *Fusarium verticillioides* (FV) when compared to the control (CC).

For *Chalara sp.* (CH), examination of both the growth values and p-values, it's apparent that *Satureja montana* (SM), *Origanum vulgare* (OV), *Thymus vulgaris* (TV) and *Thymus serpyllum* all display a growth value of 0, indicating complete inhibition of fungal growth. Moreover, the associated p-values for these essential oils (0.009 for SM, 0.001 for OV, 0.002 for TS, 0.008 for TV and 0.002 for TS) are all below the significance of $P < 0.05$. This suggests statistical significance in their ability to inhibit the growth of *Chalara sp.* (CH). However, *Cinnamomum camphora* (CC) exhibits a higher growth value (57) along with a relatively high p-value of 0.7. This implies *Cinnamomum camphora* (CC) is not statistically significant in its ability to inhibit the growth of *Chalara sp.* (CH). *Satureja montana* (SM), *Origanum vulgare* (OV), *Thymus serpyllum* (TS), and *Thymus vulgaris* (TV) appear also to be statistically significant in their efficacy in inhibiting the growth of *Chalara sp.* (CH) when compared to *Cinnamomum camphora* (CC).

Regards to *Rhizoctonia sp.* (Rhi2, Rhi5), the results reveal that both the growth values and p-values, *Satureja montana* (SM), *Origanum vulgare* (OV), *Thymus serpyllum* (TS), and *Thymus vulgaris* (TV) all display a growth value of 0, indicating complete inhibition of fungal growth. Moreover, the associated p-values for these essential oils (0.031 for SM, 0.005 for OV, 0.019 for TS, and 0.006 for TV) are all below the significance $p< 0.05$. This suggests statistical significance in their ability to inhibit the growth of *Rhizoctonia sp.* (Rhi2, Rhi5). While *Cinnamomum camphora* (CC) exhibits a higher growth value (70), its associated p-value of 0.614 is not statistically significant in its ability to inhibit the growth of *Rhizoctonia sp*. Therefore *Satureja montana* (SM), *Origanum vulgare* (OV), *Thymus serpyllum* (TS), and *Thymus vulgaris* (TV) appear to be statistically significant in their efficacy in inhibiting the growth of *Rhizoctonia sp*. (Rhi2, Rhi5) except *Cinnamomum camphora* (CC).

Results on *Setophoma terrestris* (M3) show that the growth values and p-values are evident that *Satureja montana* (SM), *Origanum vulgare* (OV), *Thymus serpyllum* (TS), and *Thymus vulgaris* (TV) all display a growth value of 0, indicating complete inhibition of fungal growth (Setophoma terrestris). Furthermore, the associated p-values for these essential oils (0.011 for SM, 0.00 for OV, and 0.001 for both TS and TV) are all below the significance $p< 0.05$. This indicates statistical significance in their ability to inhibit the growth of Setophoma terrestris (M3). While *Cinnamomum camphora* (CC) exhibits a higher growth value (70), its associated p-value of 0.614 exceeds the significance $p < 0.05$. This suggests that *Cinnamomum camphora* is not statistically significant in its ability to inhibit the growth of *Setophoma terrestris* compared to the other essential oils tested. Consequently, *Satureja montana* (SM), *Origanum vulgare* (OV), *Thymus serpyllum* (TS), and *Thymus vulgaris* (TV) appear to be statistically significant in their efficacy in inhibiting the growth of *Setophoma terrestris* (M3) compared to *Cinnamomum camphora* (CC).

When it comes to *Fusarium sp*. (M13) the growth values and p-values show that *Satureja montana* (SM), *Origanum vulgare* (OV), *Thymus serpyllum* (TS), and *Thymus vulgaris* (TV) all indicate a growth value of 0, demonstrating a complete inhibition of fungal growth (*Fusarium sp.*). Furthermore, the associated p-values for these essential oils (0.021 for SM, 0.008 for OV, 0.035 for TS, and 0.015 for TV) are all below the significance $p< 0.05$. This suggests statistical significance in their ability to inhibit the growth of *Fusarium sp*. (M13). *Cinnamomum camphora* (CC), on the other hand, exhibits a higher growth value (84), and its associated p-value of 0.621 is above the significance threshold of 0.05. This indicates a less pronounced growth inhibition compared to the other essential oils tested. Consequently, *Satureja montana* (SM), *Origanum vulgare* (OV), *Thymus serpyllum* (TS), and *Thymus vulgaris* (TV) appear to be statistically significant in their efficacy in inhibiting the growth of *Fusarium sp*. (M13) except *Cinnamomum camphora* (CC) which is not statistical significance in its ability to inhibit the growth of *Fusarium sp.* (M13)

Table 5: Hypotheses Testing

Hypotheses	Decision				
	Thymus vulgaris	**Origanumm vulgare**	**Saturea montana**	**Thymus serpillum**	**Cinnamomum camphora**
H1: There is statistically significant difference between the *Alternaria alternata* growth inhibition values on different essential oils and the control.	Accepted	Accepted	Rejected	Accepted	Rejected
H2 There is statistically significant difference between the *Schizophyllum commune* growth inhibition values on different essential oils and the control.	Accepted	Accepted	Rejected	Accepted	Rejected
H3 There is statistically significant difference between the *Fusarium verticillioides* growth inhibition values on different essential oils and the control.	Accepted	Accepted	Rejected	Rejected	Rejected
H4: There is statistically significant difference between the *Chalara sp.* growth inhibition values on different essential oils and the control.	Accepted	Accepted	Accepted	Accepted	Rejected
H5 There is statistically significant	Accepted	Accepted	Rejected	Accepted	Rejected

difference between the *Rhizoctonia sp.* growth inhibition values on different essential oils and the control.					
H$_6$: There is statistically significant difference between the *Setophoma terrestris* growth inhibition values on different essential oils and the control.	Accepted	Accepted	Accepted	Accepted	Rejected
H$_7$: There is statistically significant difference between the *Fusarium sp.* growth inhibition values on different essential oils and the control.	Accepted	Accepted	Accepted	Accepted	Rejected

5.1 Determination of Minimum Inhibitory Concentrations (MIC)

The results show that OV and TV are the most effective against all fungi, even in the case of FV and AA50. Therefore, the minimum inhibitory concentrations were chosen for testing. In the case of OV, the MIC was determined to be between 0.1 and 200 with a range of 90.17-100 % for each pathogen isolate tested. Lower concentrations were not tested.

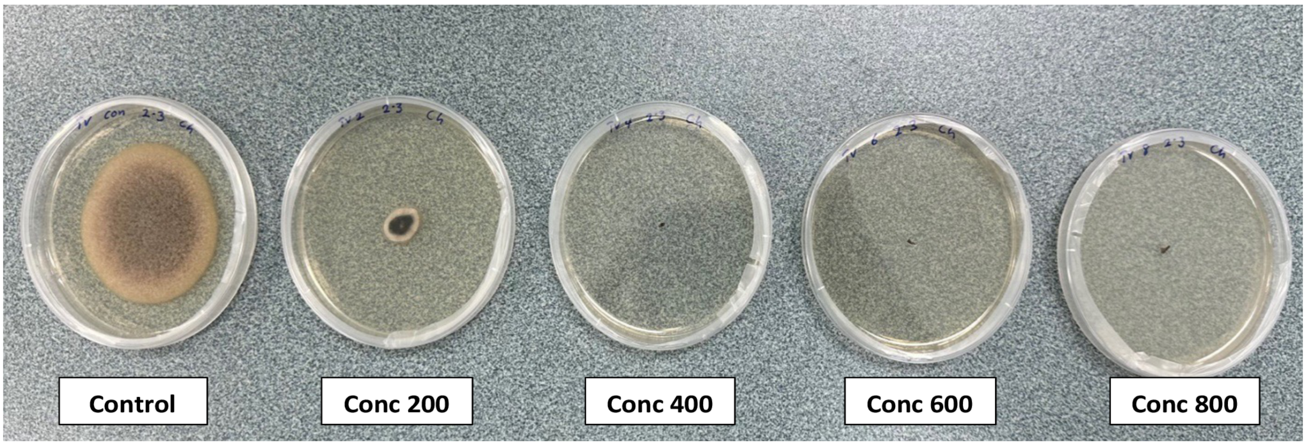

Figure: 25: Poison food technique showing antifungal Activity of *Thymus vulgaris* on Fungal growth (*Chalara sp.*) at different concentrations.

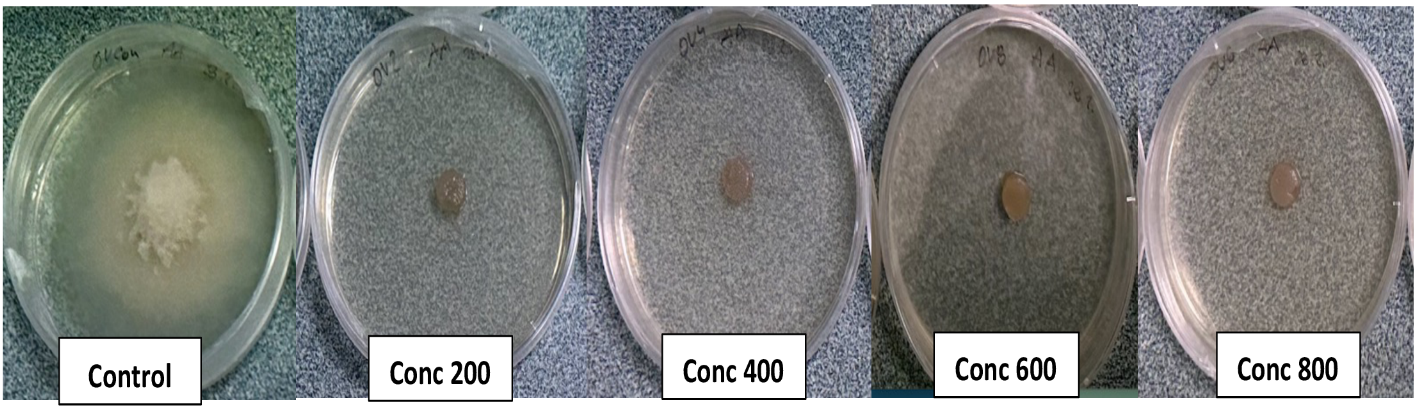

Figure 26: Poison food technique showing antifungal activity of *Origanum vulgare* on fungal growth (*Alternaria alternata*) at different concentrations.

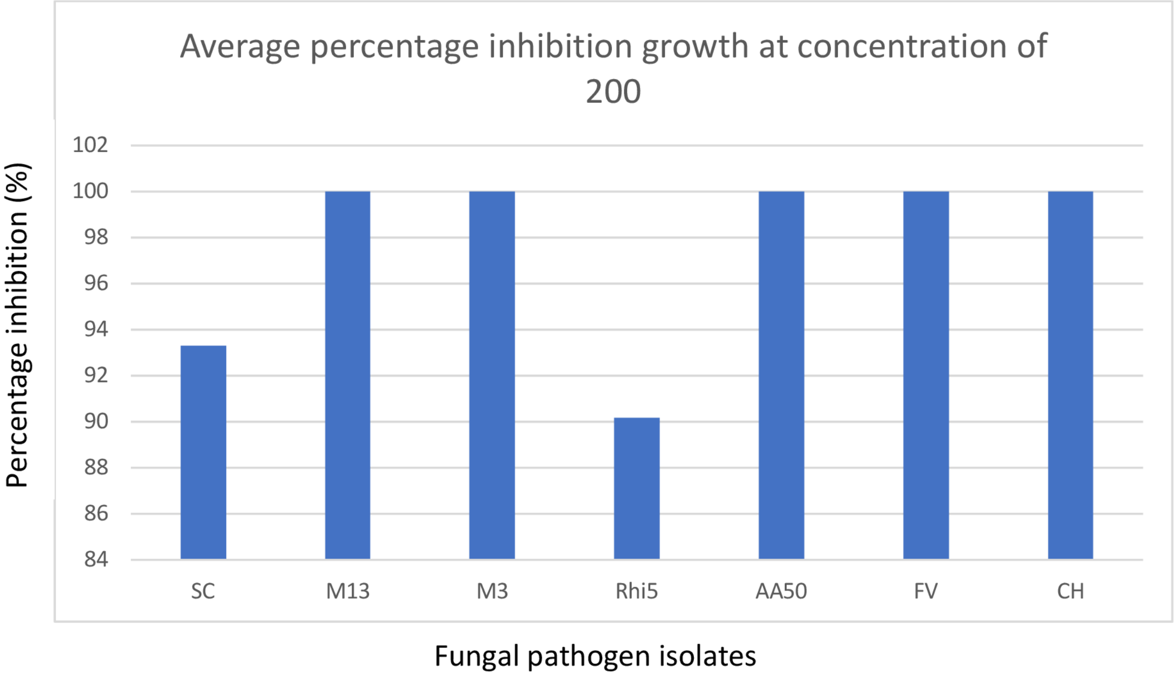

Figure 27: MIC of *Origanum vulgare* on Fungal Growth

The results show that the Minimum Inhibitory Concentration (MIC) of *Origanum vulgare* on fungal growth. In the case of OV, the MIC was determined to be between 200 and 1 with a range of 90.17-100 % for each pathogen isolate tested. Lower concentrations were not tested

For the 200 concentration, SC had 93 % inhibition, Rhi5 had 90 % inhibition while M12, M3, AA50, FV and CH had 100 % inhibition of fungal growth.

The 400, 600 and 800 concentrations showed 100 % inhibition of fungal growth for all the fungal. This indicates that Origanum vulgare oil has an inhibitory effect on the growth of fungi. At higher concentrations of Origanum vulgare oil (400, 600 and 800), shows 100 %, indicating complete inhibition of fungal growth at those concentrations.

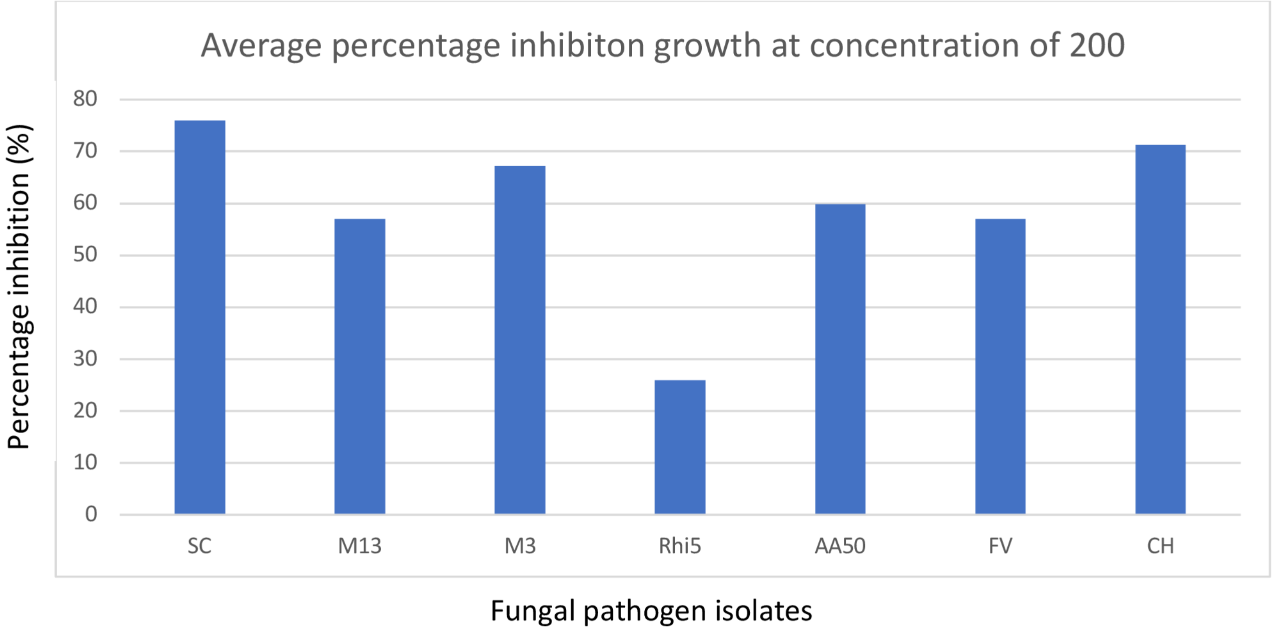

Figure 28: MIC of *Thymus vulgaris* on Fungal Growth at average 200

In the case of TV, the MIC was also determined to be in the range of 200-1 with an average pathogen growth inhibition of 25.9-75.9 % which is a significantly lower efficacy for growth inhibition compared to OV.

SC recorded a 76 % inhibition, M13 had 57 %, M3 had 67 %, Rhi5 had 26 %, AA50 had 60 %, FV had 57 % and CH also had 71 % of inhibition.

The results also show a significant difference in the inhibition of TV at a concentration of 400 with individual pathogens behaving differently as seen in the attached graph. SC had 45 % inhibition, AA50 had 56 % inhibition, FV had 66 % inhibition. While M13, M3, Rhi5 and CH had 100 % inhibition of fungal growth.

When it comes to concentrations of 600 and 800, all the fungi had 100 % inhibition in growth. This shows that Thymus vulgaris was able to achieve 100 % growth inhibition on SC, AA50, FV at concentrations of 600 and higher. The results suggest that Thymus vulgaris oil has inhibitory effects on the growth of fungal growth, and higher concentrations (from 600 to 800 concentrations) result in 100 % inhibition.

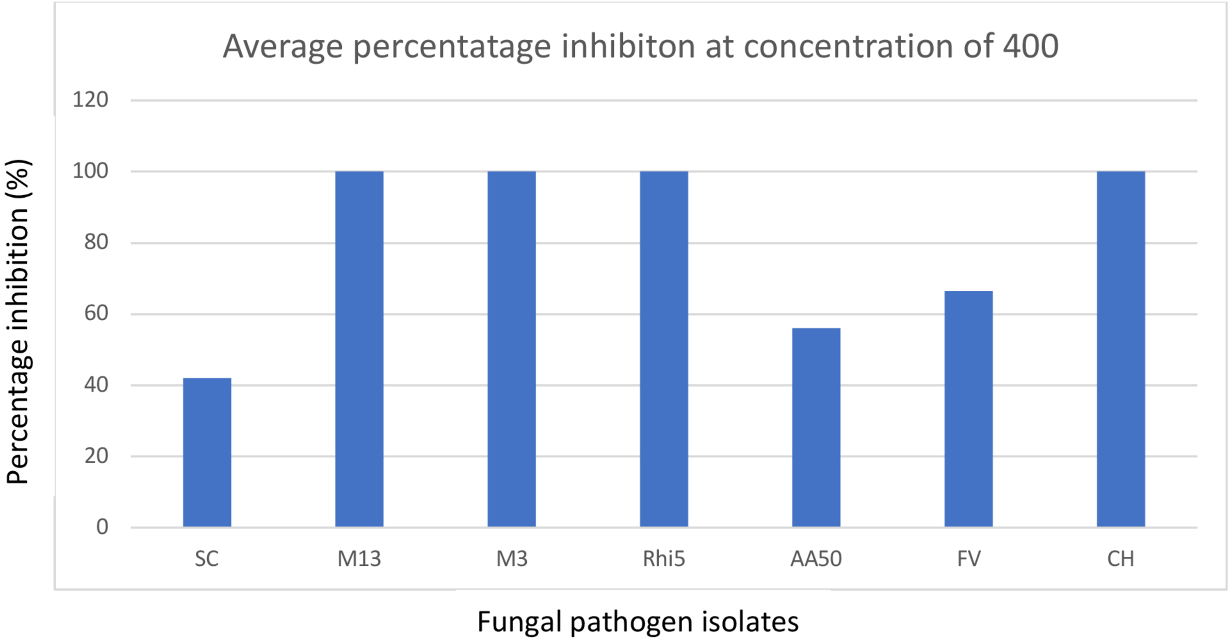

Figure 29: MIC of *Thymus vulgaris* on Fungal Growth at average 400

6. Discussions

The findings indicate that *Origanum vulgare* and *Thymus vulgaris* essential oils demonstrate significant inhibitory effects on the growth of *Alternaria alternata* compared to *Cinnamomum camphora* essential oil. *Origanum vulgare* and *Thymus vulgaris* essential oils both exhibited a growth value of 0, indicating complete inhibition of fungal growth. This implies that these essential oils are potent in suppressing the growth of *Alternaria alternata*. The growth value of 0 suggests that there was no visible growth of *Alternaria alternata* when exposed to these essential oils, highlighting their strong antifungal properties.

The findings suggest that *Origanum vulgare* and *Thymus vulgaris* essential oils are potentially utilized as natural and effective alternatives for controlling *Alternaria alternata* infestations in various applications such as sugarcane protection against fungal diseases. The findings of the study align with previous research that has elucidated the antifungal properties of *Origanum vulgare* and *Thymus vulgaris* essential oils. For instance, Failla & Romano (2020) conducted a comprehensive study assessing the antifungal activity of *Origanum vulgare* essential oil against a spectrum of fungal strains. Their findings corroborate our results, demonstrating the potent inhibitory effects of *Origanum vulgare* essential oil on *Alternaria alternata* growth.

Similarly, Rashad et al. (2022) investigated the antifungal activity of *Thymus vulgaris* essential oil and reported its strong inhibitory effects against *Alternaria alternata*, which supports our findings regarding the efficacy of *Thymus vulgaris* essential oil as an antifungal agent. Moreover, their study provides additional evidence regarding the broad-spectrum antifungal activity of *Thymus vulgaris* essential oil, further highlighting its potential for controlling fungal pathogens. In a comparative antifungal activity study, Sreenivasa et al. (2011) evaluated the efficacy of various essential oils and found that superior inhibitory effects of *Origanum vulgare* and *Thymus vulgaris* essential oils reaffirming the potency of *Origanum vulgare* and *Thymus vulgaris* essential oils as effective antifungal agents.

The findings on the *Schizophyllum commune* indicate that *Origanum vulgare*, *Thymus serpyllum* and *Thymus vulgaris*, have statistically significant inhibitory effects on the growth of *Schizophyllum commune*. However, *Cinnamomum camphora* and *Satureja montana* did not exhibit statistically significant inhibitory effects on the growth of *Schizophyllum commune*. This finding underscores the potential utility of these essential oils as effective agents for controlling the growth and proliferation of *Schizophyllum commune*, a common fungal pathogen

A study conducted by Nguefack et al. (2008) evaluated the antifungal activity of *Thymus serpyllum* and *Thymus vulgaris* essential oils against fungal pathogens. Their results demonstrated significant inhibitory effects of both *Thymus serpyllum* and *Thymus vulgaris* essential oils, supporting the findings regarding their efficacy in inhibiting the growth of *Schizophyllum commune*.

The study reveals that *Origanum vulgare* and *Thymus vulgaris* exhibit statistically significant efficacy in inhibiting the growth of *Fusarium verticillioides* while *Cinnamomum camphora, Thymus serpyllum and Satureja montana* do not exhibit statistically significant efficacy in inhibiting the growth of *Fusarium verticillioides*. This finding underscores the potential of *Origanum vulgare* and *Thymus vulgaris* as effective agents for controlling the growth and proliferation of *Fusarium verticillioides*, a common fungal pathogen associated with various crops and agricultural products such as sugarcane.

In a study by Isman (2020), the antifungal activity of various essential oils such as *Thymus vulgaris* essential oil was examined against Fusarium strains. The results indicated significant inhibitory effects of *Thymus vulgaris* essential oil, aligning with our findings regarding their efficacy against *Fusarium verticillioides*. A study by Raveau et al. (2020) evaluated the antifungal activity of *Origanum vulgare* essential oil against various fungal pathogens, including Fusarium species. Their findings revealed potent inhibitory effects of *Origanum vulgare* essential oil, which supports the study's findings regarding its efficacy against *Fusarium verticillioides*.

These studies provide robust evidence supporting the antifungal properties of *Origanum vulgare* and *Thymus vulgaris* essential oils against *Fusarium verticillioides*. The statistically significant efficacy observed in our study further validates the potential utility of these essential oils as natural alternatives for controlling fungal growth and mitigating fungal-related issues in agricultural settings such as sugarcane production.

The study reveals that *Satureja montana*, *Origanum vulgare*, *Thymus serpyllum*, and *Thymus vulgaris* exhibit statistically significant efficacy in inhibiting the growth of *Chalara sp*. In contrast, *Cinnamomum camphora* does not show statistically significant efficacy in inhibiting the growth of *Chalara sp*. This finding underscores the potential of *Satureja montana, Origanum vulgare, Thymus serpyllum*, and *Thymus vulgaris* as effective agents for controlling the growth of *Chalara sp*., a fungal pathogen known to affect various plant species. Research by Bhavaniramya et al. (2019) investigated the antifungal activity of *Satureja montana* essential oil against various fungal pathogens, including Chalara species. Their results demonstrated significant inhibitory effects of *Satureja montana* essential oil, supporting our findings regarding its efficacy against *Chalara sp.*

Regards to *Rhizoctonia sp*., *Satureja montana*, *Origanum vulgare*, *Thymus serpyllum*, and *Thymus vulgaris* are statistically significant in their efficacy in inhibiting the growth of *Rhizoctonia sp*. as compared to *Cinnamomum camphora*. A study by Prakash et al. (2015) evaluated the antifungal activity of *Origanum vulgare* essential oil against a range of fungal strains, including *Rhizoctonia sp*. Their findings revealed potent inhibitory effects of *Origanum vulgare* essential oil, which aligns with the findings regarding its efficacy against *Rhizoctonia sp.*

The findings on *Setophoma terrestris* show that *Satureja montana*, *Origanum vulgare*, *Thymus serpyllum*, and *Thymus vulgaris* are statistically significant in their efficacy in inhibiting the growth of *Setophoma terrestris* compared to *Cinnamomum camphora*. In a study by Aksit et al. (2022), the antifungal activity of *Thymus serpyllum* and *Thymus vulgaris* essential oils was examined against various fungal pathogens, including *Setophoma terrestris*. The results indicated significant inhibitory effects of both *Thymus serpyllum* and *Thymus vulgaris* essential oils, further supporting the findings regarding their efficacy against *Setophoma terrestris*.

When it comes to *Fusarium sp*. the findings reveal that *Satureja montana, Origanum vulgare, Thymus serpyllum,* and *Thymus vulgaris* are statistically significant in their efficacy in inhibiting the growth of Fusarium sp. compared to *Cinnamomum camphora.* In a study by Park et al. (2023), the antifungal activity of *Satureja montana* essential oil was investigated against various fungal strains. The results indicated strong inhibitory effects of *Satureja montana* essential oil, which corroborates the findings regarding its effectiveness against Fusarium sp.

With respect to the Minimum Inhibitory Concentrations (MIC), the findings show that *Thymus vulgaris* was able to achieve 100 % growth inhibition of all the fungal growth at concentrations of 0.06 % and 0.08 %. The results suggest that *Thymus vulgaris* oil has inhibitory effects on the growth of fungal and higher concentrations (from 0.06 % to 0.08 % concentrations) result in greater inhibition and 0 fungal growth. The findings indicating the inhibitory effects of *Thymus vulgaris* oil on fungal growth are consistent with an existing study by Teigiserova et al. (2021) on the antimicrobial properties of thyme oil and its constituents. *Thymus vulgaris*, commonly known as thyme, contains bioactive compounds such as thymol, carvacrol, and p-cymene, which have been extensively studied for their antimicrobial activities.

The findings indicate that with increases in the concentration of *Origanum vulgare* oil, there is a decrease in the growth percentages of fungal growth. This indicates that *Origanum vulgare* oil has an inhibitory effect on the growth of fungal growth. At higher concentrations of *Origanum vulgare* oil (0.04 %, 0.06 % and 0.08 %), the MIC percentage was 100 %, indicating complete inhibition of fungal growth at these concentrations. The findings regarding the inhibitory effects of *Origanum vulgare* oil on fungal growth align with Raveau et al. (2020) study in the field of plant pathology and natural products research. Raveau et al. (2020) also found that *Origanum vulgare*, commonly known as oregano, possesses various bioactive compounds such as carvacrol, thymol, and p-cymene, which have been extensively studied for their antimicrobial properties.

7. Conclusion

The evaluation of new means of sugarcane protection against fungal diseases under in vitro conditions has yielded compelling insights into the efficacy of essential oils derived from *Origanum vulgare, Thymus vulgaris, Thymus serpyllum*, and *Satureja montana*. These findings underscore the potential of natural compounds as viable alternatives for controlling fungal pathogens, particularly *Alternaria alternata, Schizophyllum commune, Fusarium verticillioides, Chalara sp., Rhizoctonia sp., Setophoma terrestris*, and *Fusarium sp*.

The study reveals that *Origanum vulgare* and *Thymus vulgaris* essential oils exhibit exceptional inhibitory effects against all fungi examined, with complete suppression of fungal growth at higher concentrations. This highlights their promising role in mitigating infestations and underscores their potential as natural fungicides for safeguarding sugarcane crops against fungal diseases. The robust antifungal properties observed align with previous research, affirming the potency of *Origanum vulgare* and *Thymus vulgaris* essential oils in inhibiting various fungal strains.

Moreover, the significant inhibitory effects of *Origanum vulgare*, *Thymus vulgaris, Thymus serpyllum*, and *Satureja montana* essential oils against *Chalara sp., Rhizoctonia sp., Setophoma terrestris*, and *Fusarium sp.* highlight their broad-spectrum efficacy. These findings suggest the potential utility of these essential oils in diverse agricultural applications, including sugarcane protection, due to their ability to suppress the growth and proliferation of common fungal pathogens.

The comparative analysis against *Cinnamomum camphora* underscores the superior efficacy of *Origanum vulgare, Thymus vulgaris, Thymus serpyllum*, and *Satureja montana essential* oils in inhibiting fungal growth across multiple strains. This suggests the feasibility of incorporating these natural compounds into integrated pest management strategies aimed at enhancing crop resilience and reducing reliance on synthetic fungicides.

The study provides compelling evidence supporting the efficacy of *Origanum vulgare, Thymus vulgaris, Thymus serpyllum,* and *Satureja montana* essential oils as promising candidates for the development of sustainable and environmentally friendly approaches to fungal disease management in sugarcane cultivation and other agricultural sectors.

7.1 Implication for Policy

The implications of the findings on policy can be significant, especially in the realms of agriculture, environmental protection, and public health.

Encouraging Sustainable Agriculture Practices

Policymakers may consider incentivizing or promoting the adoption of sustainable agricultural practices, including the use of natural compounds such as *Origanum vulgare, Thymus vulgaris, Thymus serpyllum*, and *Satureja montana* essential oils. This could be through subsidies, grants, or educational programs aimed at transitioning farmers away from synthetic pesticides toward more environmentally friendly alternatives.

Regulatory Frameworks for Natural Products

There might be a need for policymakers to reassess existing regulatory frameworks concerning the registration, approval, and usage guidelines for natural products like essential oils in agriculture. This could involve streamlining approval processes, ensuring safety and efficacy standards, and providing clearer guidelines for farmers and manufacturers.

Reduction of Chemical Inputs

The findings suggest an opportunity for policies aimed at reducing the reliance on chemical inputs in agriculture. Governments may consider phasing out or restricting the use of synthetic fungicides that pose environmental and health risks in favor of safer alternatives such as essential oils.

Research and Development Funding

Policymakers could allocate funding for research and development initiatives focused on further exploring the potential of natural compounds for crop protection. This could involve supporting interdisciplinary research collaborations, technology transfer programs, and public-private partnerships aimed at advancing innovation in agricultural practices.

Integrated Pest Management Strategies

Policies could promote the adoption of integrated pest management (IPM) strategies that incorporate natural products alongside other control methods. This holistic approach emphasizes preventive measures, biological controls, and cultural practices to manage pest and disease pressures while minimizing environmental impacts.

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