RESEARCH ARTICLE

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In-vitro efficacy of different essential oils against *Sclerotium rolfsii* (Sacc.)

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Abstract
This experimental study evaluated the effectiveness of different essential oils against the in vitro growth of *Sclerotium rolfsii*. The experiment employed a completely randomized design (CRD) with three concentrations (500, 1000, and 1500 ppm) of each essential oil, including thyme oil (*Thymus vulgaris* L.), cinnamon oil (*Cinnamomum zeylanicum* Blume), juniper oil (*Juniperus horizontalis* L.), neem oil (*Azadirachta indica* A. Juss.), lemon grass oil (*Cymbopogon citratus* (DC.) Stapf), peppermint oil (*Mentha piperita* L.), and an unamended control medium. This setup aimed to evaluate their efficacy against the mycelial growth of *S.* rolfsii. The data were analyzed using R software in R-Studio, and means were compared using Duncan's Multiple Range Test (DMRT) at a 5% level of significance. Mycelium growth data were recorded at 24 hours, 48 hours, and 72 hours of incubation. All tested essential oils significantly inhibited the mycelial growth of the pathogen compared to the control ($p<0.05$). After 72 hours, thyme oil at all concentrations and lemongrass oil at 1500 ppm both achieved 100% growth inhibition. In contrast, neem oil at 500 and 1000 ppm showed the lowest inhibitory effects, with rates of 27.56% and 34.62%, respectively. Lemongrass oil at 500 ppm (75.39%) showed statistical similarity to cinnamon oil at 1000 ppm (79.12%). Peppermint oil at 1500 ppm resulted in 82.73% inhibition, and cinnamon oil at 1000 ppm (75.73%) showed comparable results to peppermint oil at 1000 ppm. Thus, the study highlights the superior performance of thyme oil among the tested essential oils. These effective essential oils can potentially be used at lower concentrations to minimize potential hazards. However, further research and field trials are essential to validate these findings for practical applications.
Keywords: Incubation, Inoculation, Mycelium, Poisoned Food Technique, Potato Dextrose Agar

INTRODUCTION

Sclerotium rolfsii (Sacc.) is a soil-borne necrotrophic pathogen commonly found in tropical, subtropical, and warm temperate regions worldwide (Mullen, 2001; Roberts et al., 2014). This genus is associated with economically significant diseases such as root rot, stem rot, wilt, foot rot, and collar rot, affecting over 500 plant species, including almost all agricultural and horticultural crops (Fernando et al., 2004; Clarkson et al., 2007; Del Río et al., 2007; Sten et al., 2017). The economic losses attributed to diseases caused by this pathogen are substantial due to its wide host range, prolific development, and the formation of persistent sclerotia (Kokub et al., 2007). In the United States alone, *Sclerotium rolfsii* has infected more than 270 host genera (Farr and Rossman, 2006). It has been reported that

growing legumes, cucurbits, and other vegetable crops in rotation with beans can increase the incidence and severity of *S. rolfsii* (IITA, 1996). According to Mayee and Datar (1988), typical yield losses due to this pathogen and associated diseases have been reported to exceed 25%, with the potential to reach up to 80% under severe conditions. Globally, it is estimated that losses attributed to *S. rolfsii* range from 10 to 20 million dollars, resulting in yield depletions ranging from 1% to 60% in fields (Liamngee et al., 2015).

S. rolfsii is primarily characterized as a polyphagous, non-selective, moisture-dependent, and widely distributed facultative parasitic basidiomycete fungus. It can cause damage to plant tissues before colonization through the production of oxalic acid, poly-galacturonase, and cell wall-degrading enzymes (cellulase) as integral components of its pathogenicity (Punja, 1985; Chen et al., 2020). This pathogen, *S. rolfsii*, persists in the soil as resistant structures called sclerotia, which can remain viable for extended periods even under adverse climatic conditions in the absence of a susceptible host. These sclerotia act as the primary source of disease inoculation (Aycock, 1966; Kokalis-Burelle et al., 1997; Wu et al., 2008; Kokub et al., 2007). The pathogen possesses the ability to infect crops at various growth stages, presenting a formidable challenge in terms of management.

Common management measures for controlling *S. rolfsii* include the removal and destruction of diseased plants, which serve as sources of inoculum; the treatment of plants and seeds with fungicides like metalaxyl; and the use of resistant plants in crop rotations (Paparu et al., 2020). Moreover, in addition to these practices, effective strategies for managing the pathogen include various soil management techniques. Soil solarization, as researched by Chellemi (2002) and Flores-Moctezuma et al. (2006), utilizes solar heat to control the pathogen. Researchers have explored the use of both inorganic and organic soil fertility amendments, as recommended by Bulluck and Ristaino (2002) and Bonanomi et al. (2007). Another successful approach involves the cultivation of host plants with resistance, as highlighted by Woodward et al. (2008). Furthermore, natural plant products, which do not exhibit phytotoxicity and have systemic action, have garnered significant attention from scientists worldwide (Fawcett and Spencer, 2003; Gilbert, 1977; Dubey et al., 2008). These products, derived from higher plants and microorganisms, are generally characterized by their broad-spectrum efficacy, cost-effectiveness, biodegradability, and ecological soundness, making them ideal for use as agrochemicals (Cutler and Cutler, 1999).

Plant extracts and essential oils have evinced antifungal properties against a wide range of plant pathogenic fungi (Davidson, 1989; Kurita et al., 1981; Rice, 1995). Essential oils are volatile and naturally fragrant compounds, primarily composed of functional groups such as terpenoids. They are produced as secondary metabolites by aromatic plants and offer multi-purpose functional usage potential and enhanced safety (Wilson et al., 1997; Bakkali et al., 2008). Numerous studies have highlighted the antifungal potential of essential oils against various fungal pathogens, including *S. rolfsii*. El-Wakil et al. (2011) evaluated seven essential oils against *S. rolfsii* growth and found that thyme and basil oils, when applied at a 2% concentration, completely suppressed the fungal infection. Thyme oil, in particular, had a detrimental effect on the mycelium and sclerotia structures of seed-borne *S. rolfsii*. Similarly, El-Mohamedy et al. (2013) observed significant antifungal properties in lemongrass, thyme, citral, and nerol essential oils against the tomato root rot pathogen *S. rolfsii* in vitro. These essential oils, at a concentration of 1.5%, completely inhibited fungal growth. Moreover, as the concentration increased, their inhibitory effects became more potent. Thyme oil and lemongrass oil, in particular, completely halted mycelial growth and spore germination at a concentration of 100 µl/L each. Another study by Ragab et al. (2012) outlined that thyme, lemongrass, peppermint, clove, and mint oils showed superior mycelial inhibition percentages compared to lemon, cinnamon, and mustard oils. The inhibitory effect on fungal mycelial growth was positively correlated with increasing essential oil concentrations. Osman Mohamed Ali et al. (2017) reported significant antifungal activity of Neem Nano Emulsion Oil 10 (NNE10) and Citronella Nano Emulsion Oil 10 (CNE10) against *S. rolfsii*, with ED50 values of 14.71 mg L^{-1} and 20.88 mg L^{-1}, respectively. Moreover, Abdel-Kader et al. (2011) observed that peppermint oil caused 48.8% inhibition at a 1% concentration (v/v), while thyme oil achieved 100% inhibition at the same concentration (v/v). In addition, Kumar et al. (2007) observed the effective inhibition of the radial mycelial growth of *S. rolfsii* by Mentha oil at a concentration of 0.10 mg mL^{-1}. Gairhe et al. (2021) observed the highest inhibition of mycelial growth at 1000 ppm using cinnamon oil (98.15%), followed by mustard oil (40.00%) and coconut oil (32.04%). Chandra Sekhar et al. (2020) evaluated palmarosa, karanja, menthol, thyme, and lemongrass oils at 2%, revealing inhibition rates of 72.76%, 38.21%, 34.15%, 42.7%, and 34.15%, respectively. Shervin et al. (2019) reported thyme oil (400 ppm) and aloe vera (400 ppm) as highly effective, with inhibition rates of 99.98% and 99.82%, followed by garlic oil (89.15%) and cumin oil (59.75%) at the same concentration. Salome and Zacharia (2021) found neem oil and neem seed cake (5%) to have the least inhibitory effects on *S. rolfsii* growth, with inhibition percentages of 37.71% and 47.80%, respectively. Nurmansyah et al. (2022) found Citronella grass to be the most effective (95.75%) among five essential oils tested at 1500 mg/L against S. rolfsii, followed by lemongrass oil (92.70%) and cinnamon oil (92.15%).

In recent years, Nepal has witnessed an increasing prevalence and severity of diseases caused by *S. rolfsii*, including southern blight in vegetables, seedling blight in rice, and collar rot in lentils. Prominent instances of *S. rolfsii* infections

include rice in Sunsari, Jhapa, Morang, and Udaypur districts in 2016/17 (RARS, 2017), onion in Dhading district in 2018/19 (PPD, 2018), lentil, rajma, chickpea, and mustard in Lumbini and Sudur Paschim provinces since 2015 (NGLRP, 2015), and chili in Chitwan since 2015. The excessive use of chemical fungicides by farmers to manage these diseases has raised concerns about food contamination and potential health risks, including toxicity, neurological effects, and reproductive health issues. In many regions, biological control methods utilizing antagonistic microbes have proven effective against various plant diseases (Sivan, 1987). Simultaneously, ongoing research seeks alternative and effective plant pathogen control compounds, aiming to reduce reliance on antimicrobial chemical fungicides either partially or completely and exploring their integration with biological compounds (Ons et al., 2020). Consequently, there is an urgent need to explore sustainable alternatives to control *S. rolfsii*. Essential oils emerge as promising candidates due to their inhibitory effects on pathogen proliferation. This study addresses this need by investigating the antifungal activity and efficacy of various essential oils against the in vitro growth of *S. rolfsii*. The findings of this study offer natural and effective alternatives like essential oils to address the rising challenges posed by *S. rolfsii*-induced diseases and the limitations of chemical fungicides in agricultural practices in Nepal and beyond.

MATERIALS AND METHODS

Experimental Site

The experimental study was conducted at the Central Laboratory of IAAS, Lamjung Campus, Sundarbazar, from August to September 2022, within a fully controlled laboratory environment.

Isolate Collection

The *S. rolfsii* isolate, collected from the Nepal Agricultural Research Council (NARC), Khumaltar, was cultured in the laboratory and incubated for one week at 27°C in a biological oxygen demand (BOD) incubator under complete darkness.

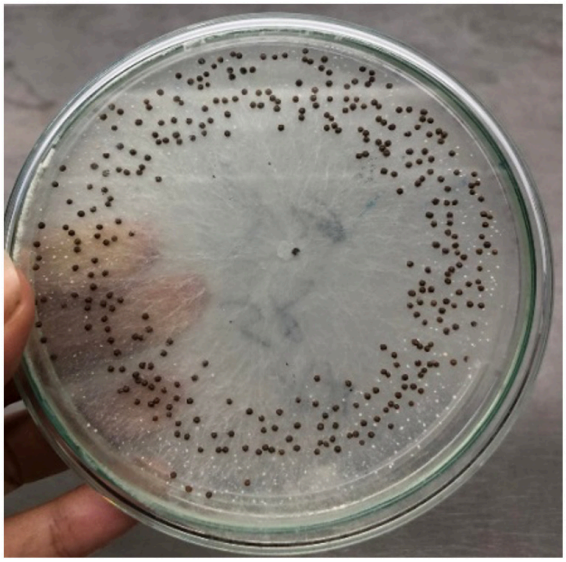

Figure 1. Mycelium and sclerotia of *S. rolfsii*

Design of the Experiments and Treatment Details

The experiment was conducted using a completely randomized design (CRD) with seven distinct treatments, as indicated in Table 1. Each treatment, except for the control, was subjected to three different concentrations (500 ppm, 1000 ppm, and 1500 ppm), and three replications were conducted for each concentration. The treatments included thyme oil (*Thymus vulgaris*) as T1, peppermint oil (*Mentha piperita*) as T2, juniper oil (*Juniperus horizontalis*) as T3, cinnamon oil (*Cinnamomum zeylanicum*) as T4, neem oil (*Azadirachta indica*) as T5, lemon grass oil (*Cymbopogon citratus*) as T6, and a control group as T7.

Table 1. Treatment details used in the experimental study

Treatments Symbol	Essential Oils	Composition	Manufacturer (Company)
T1	Thyme oil (*Thymus vulgaris* L.)	Thymol (44.7%), P-cymene (18.6%), g-terpinene (16.5%), terpinone (16.5%), B-caryophyllene (3.07%), carvacrol (3.04%)	Everest Aroma Pvt. Ltd.
T2	Peppermint oil (*Mentha piperita* L.)	Menthol (41%), Menthone (23.4%), menthyl acetate (4%)	Kanti Herbal Industries
T3	Juniper oil (*Juniperus horizontalis* L.)	α-pinene (50.5%), myrcene (7.5%), sabinene (5.2%), limonene (5.0%) and β-pinene (5.0%)	Everest Aroma Pvt. Ltd.
T4	Cinnamon oil (*Cinnamomum zeylanicum* Blume)	Linalool (52.5 %), a- pinene (8.7 %), p-cymene (5.4 %), B-pinene (4.5 %),	Kanti Herbal Industries
T5	Neem oil (*Azadirachta indica* A. Juss.)	Azadirachtin (65%)	Kanti Herbal Industries
T6	Lemon grass oil (*Cymbopogon citratus* (DC.) Stapf)	Geranial (30.6%), neral (29.7%) and myrcene (16.1%)	Sara Foods Nepal
T7	Control		

These essential oils were chosen due to their diverse chemical compositions and potential anti-microbial properties. Thyme oil (T1) is rich in hydrocarbons and phenolic compounds such as borneol, carvacrol, and thymol, which can disrupt the pathogen's cell membrane integrity (Porte and Godoy, 2008; Anìovar et al., 2014). Peppermint oil (T2) is characterized by its high menthol content, menthofuran, menthyl acetate, menthone, and 1,8-cineole, which contribute to its antimicrobial properties (Behnam et al., 2006; Saharkhiz et al., 2012; Marwa et al., 2017). Juniper oil (T3) is composed of monoterpene hydrocarbons like α-pinene, δ-3-carene, limonene, and myrcene, which enhance antioxidant-related enzyme activities while reducing pathogenicity-related enzymes (Höferl et al., 2014; Zheljazkov et al., 2021). Cinnamon oil (T4) contains E-cinnamaldehyde, linalool, β-caryophyllene, eucalyptol, and eugenol, which can inhibit the growth of pathogenic bacteria by disrupting cell envelopes (Alizadeh Behbahani et al., 2020). Neem oil (T5) comprises active ingredients like azadirachtin, nimbin, salannin, meliantriol, nimbolinin, and sodium nimbinate, offering biopesticidal properties (Elteraifi and Hassanali, 2011; Alzohairy, 2016; Chaudhary et al., 2017). Lemongrass oil (T6) primarily contains citral (>45%), known for its antibacterial and antifungal activities (Moore-Neibel et al., 2012).

This experimental design allowed for a comprehensive exploration of the effects of these oils across different concentrations, offering valuable insights into the potential impact of varying dosage levels on the inhibition of pathogen growth.

General Laboratory Procedure

Sterilization

The inoculation needle, forceps, and cork borer were sterilized by heating them until they were red-hot, and this process was repeated 2-3 times. The Petri plates were washed with liquid detergent under running tap water, rinsed with distilled water, air-dried, wrapped in aluminum foil, and then placed in a hot air oven at 105°C for up to 24 hours.

Disinfection of the Inoculation Chamber

Laminar airflow was used to conduct all experiments, including isolation, sub-culturing, and other studies, under aseptic conditions. The laminar airflow chamber was sterilized for fifteen minutes using UV light, followed by wiping the inner walls and base with 70% ethanol.

Preparation of Culture Media

Potato dextrose agar (PDA) containing 2% agar, readily available in the market, was used as the growth medium for *S. rolfsii*. According to the label instructions, the required amount of PDA powder was mixed with distilled water to

prepare the medium, with a mixing ratio of 7.8 grams of powder per 1000 ml of distilled water. The prepared medium was then sterilized in an autoclave at 15 psi and 121°C for 20 minutes and allowed to cool. When the temperature of the medium reached approximately 40°C, it was poured into sterile petri plates inside a laminar flow cabinet and left to solidify. Subsequently, the medium was infused with essential oils and used to inoculate the pathogen.

Dilution of Essential Oils

All activities were conducted inside a laminar airflow chamber under sterile conditions. Six different essential oils were assessed against *S. rolfsii* on Potato Dextrose Agar (PDA) medium using the poisoned food technique at concentrations of 500, 1000, and 1500 ppm. To prepare the stock solution, 1 ml of each essential oil was mixed with 19 ml of acetone, resulting in concentrations of 50,000 ppm for each essential oil. The required concentrations of 500 ppm, 1000 ppm, and 1500 ppm for the stock solutions were meticulously prepared using the following formula:

$$C_1V_1=C_2V_2$$

where,

C_1 = concentration of essential oil (ppm)

V_1 = desired volume of essential oils (ml)

C_2 = desired concentration of essential oils (ppm)

V_2 = measured volume of PDA (ml)

Thus-prepared essential oil dilutions were thoroughly mixed by vortexing using a lab vortex mixer before their application to ensure uniform distribution. Streptomycin (0.25 g/l) was added to a sterilized medium to inhibit bacterial growth. For the Potato Dextrose Agar (PDA) medium, a specific quantity of the stock solution was added to achieve final concentrations of 500 ppm, 1000 ppm, and 1500 ppm.

Inoculation and Incubation

On sterile petri plates, 20 ml of culture medium (PDA) mixed with the poisoned medium was poured and allowed to solidify. Mycelium with a 6 mm diameter was cut from the mother culture using a cork borer and placed in the center of a Petri plate with the poisoned medium. All six treatments, including controls, were replicated three times following the same procedure. Finally, parafilm wax was used to seal the Petri plates, which were then incubated at 27°C. The Petri plates were stored inverted in the incubator.

Growth Inhibition Test

The petri plates were positioned within a laminar flow cabinet, and data were collected using a Vernier caliper. The initial data collection was taken 24 hours after inoculation, followed by subsequent measurements at 24-hour intervals for up to 72 hours post-inoculation. The percentage inhibition of mycelial growth was calculated using the formula described by Vincent (1947).

$$\text{PGI} = \frac{C-T}{C} X\ 100$$

where,

PGI = Percent Growth Inhibition

C is the average diameter of the colony in the control treatment.

T is the average diameter of the colony in essential oil treatment.

Statistical Analysis

All data were entered into Microsoft Excel and analyzed using R-Stat (version 4.2.1). The data were subjected to analysis of variance, and treatment means were separated using the least significant difference (LSD) and Duncan's multiple range test (DMRT) at a significance level of 5%.

RESULTS AND DISCUSSION

The in vitro evaluation of various essential oils (thyme, peppermint, cinnamon, lemongrass, neem, and juniper) in inhibiting the growth of *S. rolfsii* was assessed using the Poisoned Food Technique, and the results are presented in Table 2.

Table 2. Efficacy of different essential oils on the growth of S. *rolfsii* in vitro

S.N	Essential Oils	Concentration (ppm)	Percentage Growth Inhibition (%)		
			24 hours	48 hours	72 hours
1	Thyme Oil	500	100^{a}	100^{a}	100^{a}
		1000	100^{a}	100^{a}	100^{a}
		1500	100^{a}	100^{a}	100^{a}
2	Lemon Grass Oil	500	73.49^{c}	73.93^{c}	75.39^{c}
		1000	83.90^{b}	86.31^{b}	86.47^{b}
		1500	100^{a}	100^{a}	100^{a}
3	Cinnamon Oil	500	52.95^{d}	53.76^{d}	59.78^{d}
		1000	71.63^{c}	75.73^{c}	79.12^{c}
		1500	96.55^{a}	98.06^{a}	99.38^{a}
4	Peppermint Oil	500	44.40^{e}	50.23^{d}	51.76^{e}
		1000	75.80^{c}	76.15^{c}	78.36^{c}
		1500	82.69^{b}	82.73^{c}	85.29^{b}
5	Juniper Oil	500	33.58^{g}	34.03^{fg}	36.27^{gh}
		1000	36.62^{fg}	37.19^{ef}	37.84^{fgh}
		1500	38.34^{f}	39.86^{e}	41.79^{f}
6	Neem Oil	500	25.68^{h}	26.77^{h}	27.56^{i}
		1000	29.11^{h}	31.27^{gh}	34.62^{h}
		1500	38.28^{f}	38.41^{ef}	39.26^{fg}
	Grand Mean		65.73	66.91	68.49
	SE_m ()		1.47	1.67	1.43
	CV (%)		3.87	4.33	3.63
	LSD (5%)		4.21	4.79	4.12

LSD: Least Significant Difference, SE_m: Standard error of the mean deviation, CV: Coefficient of Variance, Treatment means separated by DMRT and columns represented with different letter (s) are significant based on DMRT P = 0.05.

The results indicated significant differences in the ability of essential oils to inhibit the growth of *S. rolfsii* under in vitro conditions. Enhanced efficacy was observed as the concentration of the essential oils increased. Thyme oil and lemongrass oil resulted in the most substantial inhibition of growth at various concentrations. Regardless of the concentration used, the range of growth inhibition ranged from 27.56% to 100% after 72 hours of inoculation. Different essential oils displayed varying levels of fungicidal properties against the tested fungus, and a significant difference ($P \leq 0.001$) was observed among different extracts in their inhibitory effects.

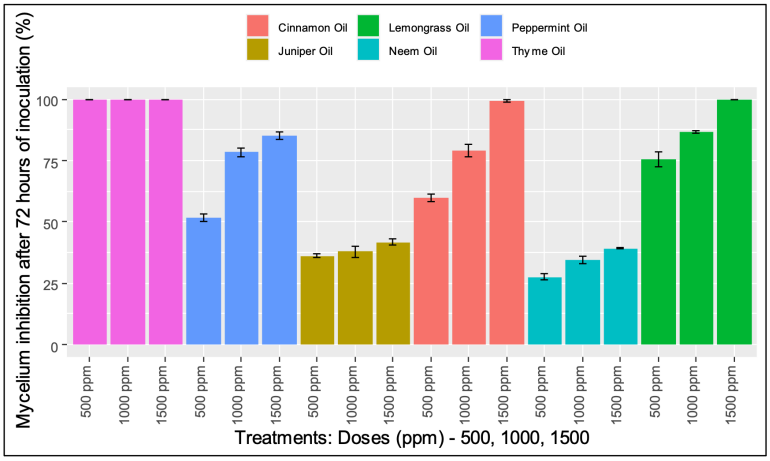

Figure 2. Mycelium inhibition (%) after 72 hours of inoculation across different treatments

As the concentration of the plant extracts increased, a proportional rise in the suppression of *S. rolfsii* mycelial growth was observed. After 24 hours of inoculation, thyme oil achieved 100% inhibition at all concentration levels, followed by 1500 ppm of lemongrass oil. The lowest inhibition was observed with 500 ppm of neem oil (25.68%), which was statistically similar to its 1000 ppm concentration (29.11%). After 36 hours of inoculation, thyme oil at all concentrations, 1500 ppm of lemongrass oil, and cinnamon oil showed the highest percentage of inhibition. Lemongrass oil at 500 ppm (73.93%) showed similar efficacy to cinnamon oil at 1000 ppm (71.63%). At a concentration of 1500 ppm, peppermint oil led to the highest inhibition rate of 82.73%. Cinnamon oil at 1000 ppm (75.73%) showed statistically similar results to peppermint oil at 1000 ppm. Neem oil at 500 ppm was the least effective among the tested essential oils, with the lowest growth inhibition rate at 25.68%.

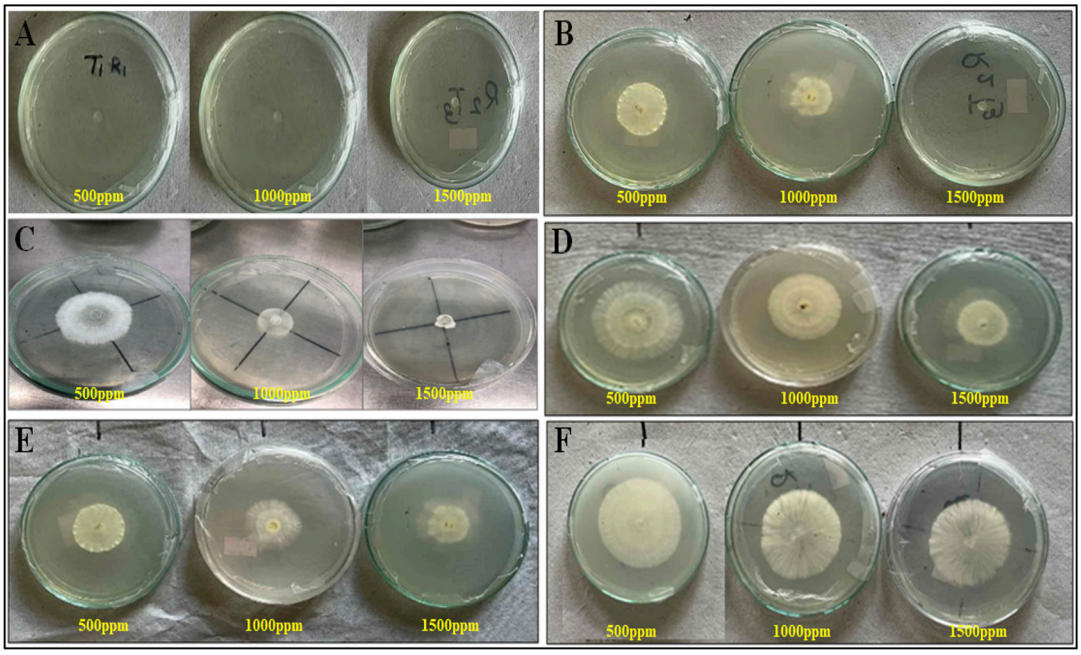

Figure 3. Mycelial growth of *S. rolfsii* in PDA amended media with various essential oils used at different concentrations; A) Thyme oil, B) Lemongrass oil, C) Cinnamon oil, D) Juniper oil, E) Peppermint oil, F) Neem oil

After 72 hours of inoculation, thyme oil at all concentrations achieved 100% inhibition, which was statistically similar to lemongrass oil and cinnamon oil at 1500 ppm (99.38%). Significant results were observed for lemongrass oil at 500 ppm and 1000 ppm (75.39% and 86.47%, respectively) and cinnamon oil at 500 ppm and 1000 ppm (59.78% and 79.12%, respectively). Lemongrass oil at 1000 ppm (86.47%) showed statistically similar efficacy to peppermint oil at 1500 ppm (85.29%). Juniper oil at 1500 ppm (41.79%) was statistically similar to neem oil at 1500 ppm (39.26%). Neem oil at 500 ppm and 1000 ppm resulted in the lowest inhibition rates (24.56% and 34.62%, respectively).

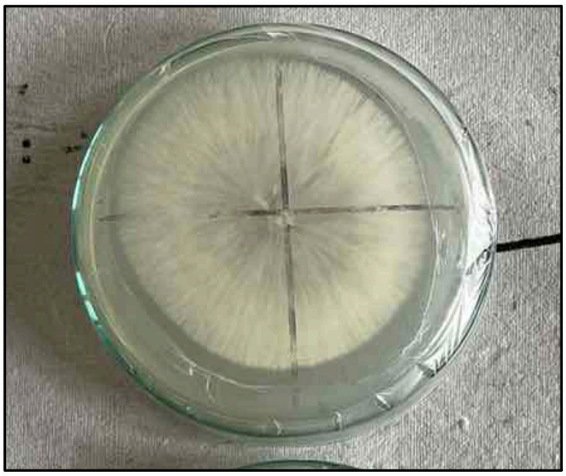

Figure 4. Mycelial growth of *S. rolfsii* in PDA-unamended media as a control

Natural compounds have a profound impact on the internal processes of the fungus. They lead to significant alterations in essential cellular structures, such as the formation of cytoplasmic granules, disruption of cell contents, disturbances in the plasma membrane, and the inhibition of fungal enzymes. These effects, in turn, hinder germination and germ tube elongation, potentially leading to a reduction or complete cessation of mycelial growth (Kim et al., 1995; Schwan-Estrada et al., 2000; Kotzekidou et al., 2008). The current findings are consistent with previous studies by El-Mohamedy et al. (2013) and Abdel-Kader et al. (2011), which also identified thyme oil as the most potent inhibitor of mycelial growth in various *S. rolfsii* strains. This inhibitory effect is attributed to thymol and carvacrol, which constitute a significant portion (20%–54%) of thyme oil's total content (Porte and Godoy, 2008). Thymol's hydrophobic properties allow it to interact with the pathogen's outer cytoplasmic membrane, leading to pathogen death. Thyme oil had exceptional effectiveness, achieving complete mycelial inhibition of *S. rolfsii* at concentrations ranging from 1% to 4% (Abdel-Kader et al., 2011). Additionally, the vapors of lemongrass and thyme oils, each at a concentration of 100 µl/L, resulted in the complete inhibition of linear growth and spore germination. Similar outcomes were observed for the vapors of thyme, oregano, and lemongrass, along with their major components, which manifested complete growth inhibition against Botrytis cinerea and Alternaria arborescens, as documented by Plotto et al. (2003).

Lemongrass demonstrated significant effectiveness in inhibiting the growth of *S. rolfsii* across all concentrations tested. This complete inhibition of pathogen growth can be attributed to the presence of antimicrobial constituents, including citral-a (33.1%), citral-b (30.0%), geranyl acetate (12.0%), and linalool (2.6%) in lemongrass oil, as reported by Nurmansyah et al. (2022). These constituents contribute to its dual antibacterial and antifungal properties, which include reducing spore germination, inhibiting germ tube development, and effectively suppressing fungal sporulation.

Our results at 1500 ppm of cinnamon oil have similar implications and closely align with the findings of Sukatta (2008), who reported 100% antifungal activity of cinnamon oil against various postharvest pathogens, including *Aspergillus niger*, *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae*, and *Phomopsis viticola*. Kowalska et al. (2020) also reported an 81.4% inhibition in the mycelium growth of *Botrytis cinerea* using cinnamon water filtrates at a 1% concentration. These consistent findings highlight the potential of cinnamon's active ingredient as an effective antifungal agent in various studies and applications.

Similarly, peppermint oil also showed pronounced mycelium growth inhibition in *Sclerotium rolfsii*, consistent with the findings reported by Souza et al. (2014) and Chandra Sekhar et al. (2020). Furthermore, in an in vitro antifungal assessment conducted by Falasca et al. (2016), they observed that the essential oil extracted from both green and ripe juniper berries effectively inhibited the growth of *S. rolfsii*, which is in accordance with our findings. This antifungal activity appeared to be closely linked to the concentration of sesquiterpenes. Additionally, it might be influenced by potential synergistic or antagonistic interactions among different terpenoid components, as suggested by several researchers (Jing et al., 2014). Several potential mechanisms have been proposed to explain this antifungal effect. One possibility is that terpenes such as α-pinene, p-cymene, and β-pinene could increase the levels of lipid peroxides, leading to cellular death (Filipowicz et al., 2003; Lucini et al., 2006). Alternatively, they may act on the mycelium's hyphae, causing the release of cytoplasmic components and ultimately resulting in the death of the mycelium (Sharma and Tripathi, 2008).

Besides, Salome and Zacharia (2021) observed that neem oil and neem seed cake at a 5% concentration showed the least inhibition of *S. rolfsii* growth, with rates of 37.71% and 47.80%, respectively, when compared to other essential oils, which is in accordance with our findings. This antifungal activity is primarily attributed to bioactive compounds like nimbin, nimbidin, and gedunin, which are found in neem oils and neem leaf extracts. These compounds disrupt fungal cell membranes, hinder fungal enzyme activity, and interfere with fungal metabolic processes (Suleiman, 2011; Raghavendra and Balsaraf, 2014).

CONCLUSION

In conclusion, it is advisable to use thyme oil at lower concentrations to effectively inhibit mycelium growth in vitro while reducing the risks associated with chemical fungicides. Thyme oil, along with other essential oils, presents a promising eco-friendly alternative to chemical fungicides, given their environmental hazards and potential impact on human health. Future research should focus on investigating the mechanisms of action of essential oils to enhance efficacy and reduce the likelihood of resistance development. Comprehensive field trials, covering diverse crop types and application methods, are essential for validating their real-world efficacy. Furthermore, further evaluation is necessary to understand how regional variations in essential oil compounds may affect their effectiveness against fungal pathogens such as *S. rolfsii* and others.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The author order aligns with their respective contributions, with K.R. Pandey contributing more to major aspects of the experiment and paper publishing, followed by A. Pant and N. Gajurel, both making significant contributions to experiments, data analysis, material provision, and paper writing.

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