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Research Article

Antifungal properties of Eucalyptus essential oils against devastating fungi in Australian wheat and canola cultivation

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Abstract

This study evaluated the antifungal activity of eucalyptus essential oils against three major phytopathogenic fungi: *Fusarium pseudograminearum*, *Pyrenophora tritici-repentis*, and *Sclerotinia sclerotiorum*, which are the causal agents of crown rot and yellow leaf spot in wheat, and stem rot in canola, respectively. Forty eucalyptus essential oils were initially screened at two concentrations: 10 µL per Petri dish (146.93 ppm v/v) and 50 µL per Petri dish (734.65 ppm v/v). The preliminary screening showed that oils applied at 50 µL per dish significantly inhibited the mycelial growth of all three pathogens compared to the untreated control ($p < 0.05$). Based on these results, the 20 most effective oils were selected for detailed dose–response assays across a broader concentration range, including 2.5, 5, 10, 20, 30 and 40 µL. Increasing concentrations consistently resulted in greater mycelial growth inhibition. The most active oils, ET289 and EA165, achieved ED_{90} values of 20 µL per Petri dish against *F. pseudograminearum*. Oils EP294 and EF17 suppressed *P. tritici-repentis* by 90–100% at just 5 µL per dish. For *S. sclerotiorum*, oils EF17 and EC162 produced 89–100% inhibition at 20 µL per dish. Overall, the results demonstrate that the selected eucalyptus essential oils possess strong, concentration-dependent antifungal properties and highlight their potential as environmentally sustainable alternatives to synthetic fungicides for crop protection.

1. Introduction

Wheat (*Triticum aestivum* L.) accounts for the largest grain production in Australia which is considered a world leader in the production of clean, dry, white, food-safe wheat [1]. Australian canola produces vegetable oil and is also known as an environmentally sustainable feedstock for biofuels [2]. However, disease pressure is a significant constraint in wheat and canola cultivation, causing substantial yield loss. Fusarium crown rot, caused by the soil-borne pathogen *F. pseudograminearum* results in significant yield loss (up to 100%), and potentially downgrades grains due to mycotoxins produced by the pathogen [3].

Yellow leaf spot (YLS), caused by the fungus *P. tritici-repentis,* has become a widespread stubble-borne foliar disease in Australian wheat cultivation [4], causing up to 50% yield loss. Although fungicides are commonly used to control this disease in wheat, it is difficult to completely eliminate [5]. On the other hand the control efficacy of fungicides is poor because the pathogen is a necrotrophic fungi which feeds on dead leaves [6, 7].

Sclerotinia stem rot caused by the pathogen *S. sclerotiorum*, is one of the most devastating soil-borne pathogens in Australian canola cultivation. It has been recognised as a sporadic disease in southern NSW regions [8]. In 1999, estimated losses due to *Sclerotinia* in NSW alone exceeded AU$170 million. In 2012 and 2013, increased inoculum pressure was observed in the high-rainfall zones of NSW, while an outbreak of stem rot disease was reported in most of the canola growing regions of Western Australia [9]. In several western and central parts of NSW, unusually high levels of Sclerotinia disease have developed in crops where the disease had previously rarely been reported. Surveys of commercial canola crops across central and southern NSW detected Sclerotinia in 66% of the assessed crops, indicating a widespread distribution of the pathogen in 2024. Infection incidence varied considerably, ranging from 0% to 76% of plants affected within individual crops [10].

Cereal crops such as wheat, barley, oats, triticale and rye are affected by the disease crown rot, with durum wheat being the most susceptible. The control of crown rot in wheat depends highly on chemical fungicides and the use of single-site fungicides to control plant pathogens is very popular. Triazole fungicides are known to be highly effective in controlling *F. pseudograminearum* however, the frequent use of fungicides has led to fungicidal resistance [11].

Therefore, alternative control measures are needed due to public concerns about the use of synthetic chemicals, resistance to fungicides among fungal pathogens, and the high development cost of new active chemicals [12]. The use of natural compounds of plant origin for plant disease control has attracted increasing attention because of their low environmental impact.

The eucalypts (genus *Eucalyptus* and its close relatives in the family Myrtaceae) are a group of Australian native plants, represented by approximately 700 species [13]. Eucalyptus oil has a wide range of biological, activities, including fungicidal, herbicidal, acaricidal, antimicrobial, and nematicidal properties [12, 14–16] .

The fungicidal properties of eucalyptus essential oils have been evaluated in several agricultural and horticultural crops. Eucalyptus essential oils are highly effective in inhibiting the growth of *Bipolaris sorokiniana*, the causal agent of spot blotch in wheat [17]. Essential oils from three species of Eucalyptus suppress the pathogen *Fusarium solani*, causing dry rot in potatoes and *Sclerotium rolfsii*, causing leaf spots in indoor plants [18]. Eucalyptus leaves often contain the highest concentration of essential oils [19] and the dominant compounds are 1,8-cineole, limonene, p-cymene, γ-terpinene, α-pinene, α-terpineol, camphene, linalool, ocimene, β-pinene, citronellol and citronellal [20, 21].

Several studies have highlighted the antifungal efficacy of essential oils derived from various *Eucalyptus* species against plant-pathogenic fungi. Essential oil extracted from *Eucalyptus citriodora* exhibited broad-spectrum antifungal activity and effectively suppressed the development of grey mould (*Botrytis cinerea*) in apples [22]. Similarly, Su et al. evaluated the antifungal properties of essential oils from four *Eucalyptus* species (*E. urophylla*, *E. grandis*, *E. camaldulensis*, and *E. citriodora*), identifying *E. citriodora* oil as the most effective against all tested mildew species [23].

Mehani et al. demonstrated that essential oil from *E. camaldulensis* exhibited strong antifungal activity, with a minimum inhibitory concentration (MIC) of 60 μL, effectively inhibiting the growth of *Fusarium graminearum* and *F. culmorum* [24]. In a more recent study, Hajji-Hedfi et al. assessed the antifungal and phytochemical properties of *Eucalyptus globulus*, *Pistacia lentiscus*, and *Juniperus phoenicea* essential oils against *Colletotrichum gloeosporioides* and *Alternaria alternata*, which pathogens associated with postharvest diseases in apples. Under *in vitro* conditions, *E. globulus* oil at 2 and 4 μL/mL inhibited mycelial growth by more than 67% and spore germination by more than 99%. When applied to inoculated apples, the oil also reduced lesion diameters to less than 6.80 mm and lowered the disease severity index to below 15% [25].

Han et al. investigated the antimicrobial mechanism and preservative potential of an emulsion composed of *Eucalyptus* essential oil (EEO) and tamarind gum

(TG) against *Trichoderma harzianum* strain TPS2, isolated from infected *Agaricus bisporus*. The EEO–TG emulsion inhibited fungal growth in a concentration-dependent manner, with the 1:40 essential oil to gum ratio exhibiting the most pronounced preservation effect. Additionally, Pedrotti et al. Essential oils from *E. staigeriana* and *E. globulus* were effective against *B. cinerea* and *Colletotrichum acutatum*, two of the most significant fungal pathogens causing grape rot [26].

Although eucalyptus essential oils have been identified as potential antifungal agents, there are limited studies investigating the use of essential oils against plant pathogenic fungi in major broadacre agricultural crops, such as wheat and canola. Previous studies have often only evaluated a few eucalyptus species. This study investigated the potential antifungal activities of 40 Eucalyptus essential oils on selected pathogens of Australian wheat and canola crops.

2. Materials and methods

2.1. Fungal species

The three tested pathogens are *Fusarium pseudograminearum* (strain WAI1231), *Pyrenophora tritici-repentis* (strain WAI1682) and *Sclerotinia sclerotiorum* (strain 14ALM2060) (Fig. 1).

Figure 1. Three fungi used in the screening experiment. A- *Fusarium pseudograminearum* (WAI1231), B- *Pyrenophora tritici-repentis* (WAI1682) and C- *Sclerotinia sclerotiorum* (14ALM2060).

Fusarium pseudograminearum is the causal organism responsible for crown rot disease in wheat and barley, whereas yellow leaf spot disease in wheat is caused by the pathogen *Pyrenophora tritici-repentis*. *Sclerotinia sclerotiorum* is the causal agent of stem rot in canola. Cultures of *F. pseudograminearum* and *S. sclerotiorum* were maintained on Potato Dextrose Agar (PDA), while the culture of *P. tritici-repentis* was maintained on PDA-V8 medium, which incorporates V8 juice (commercially available) into PDA [27].

2.2. Extraction of essential oils

Essential oils were extracted from 40 Eucalyptus species (Table 1). The previously developed extraction protocol was followed [28]. Briefly, fresh leaves of each eucalyptus species (300 g) were subjected to steam distillation for 2.5 h. A Pyrex oil distillation apparatus with a flat-bottom flask (2 L) containing 1200 mL of distilled water was used to generate steam. The volatile essential oils from the leaves were condensed through cooling tubes and the essential oil afloat on top of the condensed water was collected through a separation funnel. The collected essential oil was stored in a sealed vial at 5 °C before use.

All the 40 oils were assessed in the initial screening against the three pathogens at two concentrations, and the selected oils used for the dose response studies were labelled A, B, and C for the respective fungi *Fusarium pseudograminearum* (WAI 1231), *Pyrenophora tritici-repentis* (WAI 1682) and *Sclerotinia sclerotiorum* (14ALM2060).

2.3. Preliminary in-vitro antifungal assay with 40 Eucalyptus essential oils

For the antifungal activity tests, 20 mL of Potato Dextrose Agar (PDA) was poured into a 90 mm diameter petri dish in a laminar flow. PDA-V8 media was also poured in the same way for the assay against *P. tritici-repentis*. The plates were allowed to set and stored until use.

Initially, the forty different eucalyptus oils at two concentrations,10 µL/petri dish (or 146.93 ppm v/v) and 50 µL/Petri dish (734.65 ppm v/v), were screened against the three pathogens, *F. pseudograminearum*, *P. tritici-repentis* and *S. sclerotiorum*. In a laminar flow cabinet, a 5-mm mycelial disk was cut from the growing edge of 3-day-old cultures of *F. pseudograminearum, S. sclerotiorum* or *P. tritici-repentis*, and placed in the middle of individually labelled petri dishes. A quadrant of an 85-mm filter paper (No. 4 filter paper, Whatman International Ltd., Maidstone, U.K.) was placed on the underside of the Petri dish lid and secured with a small piece of sterile sticky tape. The required amount of each essential oil was impregnated onto the filter paper disc, avoiding direct contact between the essential oil and the mycelial disk. The essential oil diffused within the petri dish to

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Table 1. Eucalyptus oils used in the initial screening and the subsequent dose response assays.

Essential oil	Eucalyptus species	Pathogen tested
EP4	*Eucalyptus poliata*	*A*
EE12	*Eucalyptus exserta*	*AC*
EC13	*Eucalyptus canescens* subsp. *canescens*	*ND*
EF17	*Eucalyptus froggattii*	*ABC*
CC19	*Corymbia citriodora*	*AC*
EW20	*Eucalyptus wandoo* subsp. *wandoo*	*ND*
EC34	*Eucalyptus camphora* subsp. *humeana*	*ND*
ER40	*Eucalyptus resinifera* subsp. *hemilampra*	*AC*
EC54	*Eucalyptus carnei*	*BC*
EE59	*Eucalyptus erithronema* subsp. *erythonemay*	*B*
ED79	*Eucalyptus diversicola*	*ND*
EC136	*Eucalyptus caesia* subsp. *caesia*	*ND*
EC150	*Eucalyptus cabiana*	*AB*
EC151	*Eucalyptus crucis* subsp. *lanceolata*	*ABC*
EG156	*Eucalyptus globulus*	*BC*
EP160	*Eucalyptus pumila*	*B*
EC162	*Eucalyptus cooperiana*	*ABC*
EA165	*Eucalyptus amplifolia* subsp. *amplifolia*	*AC*
EC166	*Eucalyptus calycogona* subsp. *calycogona*	*A*
ET173	*Eucalyptus terebra*	*B*
EF179	*Eucalyptus fastigata*	*ND*
EM201	*Eucalyptus michaeliana*	*ND*
EV204	*eucalyptus vittata*	*B*
EH205	*Eucalyptus halophila*	*ND*
ER214	*Eucalyptus rhomboidea*	*B*
EA217	*Eucalyptus aspersa*	*AC*
ES226	*Eucalyptus selachiana*	*ABC*
EL245	*Eucalyptus leptocalyx* subsp. *leptocalyx*	*C*
ED250	*Eucalyptus dundassi*	*ND*
EA257	*Eucalyptus albida*	*ABC*
ED260	*Eucalyptus diversifolia* subsp. *hesperia*	*ND*
EG269	*Eucalyptus grandis* subsp. *grandis*	*ABC*
ED286	*Eucalyptus dissimulata*	*ABC*
ET289	*Eucalyptus thozetiana*	*A*
EP294	*Eucalyptus platycorys*	*BC*
EF296	*Eucalyptus fraseri* subsp. *melanobasis*	*ABC*
EL298	*Eucalyptus latens*	*A*
ES305	*Eucalyptus salmonophloia*	*BC*
ES310	*Eucalyptus salubris*	*ABC*
ES313	*Eucalyptus spathulate*	*ABC*

All the 40 oils were assessed in the initial screening against the three pathogens at two concentrations and the selected oils used for the dose response studies were labelled with A, B, C for the respective fugus, *Fusarium pseudograminearum* (WAI 1231), *Pyrenophora tritici-repentis* (WAI 1682) and *Sclerotinia sclerotiorum* (14ALM2060).

inhibit the mycelial growth. Untreated controls were prepared with fungal cultures and filter paper disks treated with 10 µL/petri dish and 50 µL/petri dish of distilled water. The volume of the petri dish was 68.06 cm^3. The plates were sealed with parafilm (American National Can, Greenwich, CT 06836) immediately after adding each essential oil or water and incubated at 25°C for 7 days (Model:Bioevopeak/ICB-CC175-3

Climate Chamber). The diameter of fungal mycelial growth was measured and compared with that of the untreated control 7 days after incubation.

2.4. Screening of 20 Eucalyptus Essential oils having highest antifungal activity against three pathogens

After the initial screening, 20 oils showing the highest antifungal properties against each of the three pathogens were selected (Table 1) and further screened *in-vitro*, using a series of concentrations viz. 2.5, 5,10,20, 30 and 40 μL to determine dose-dependent inhibition of mycelial growth of the respective fungi. The protocol used in the initial screening was followed in this assay. As soon as the required aliquot was added, the plate was sealed with parafilm and incubated at 25°C for seven days. Filter paper disks treated with respective amount of sterile distilled water were used as an untreated control in this experiment. The diameter of the fungal mycelium was measured at 7 days after incubation. All experimental procedures were undertaken in a clean environment using a laminar flow cabinet (AIRPURE-Westinghouse Pty Ltd).

2.5. Statistical analysis

The experiments were arranged in a completely randomised factorial design, with each treatment having four replicates. Data were analysed using R 2024 software, and analysis of covariance (ANCOVA) was performed to test the treatment effect, and Tukey's test was used to compare the means at a significance level of 0.05. A log-logistic model was employed to analyse the effective dose (ED_{90}) values for *Sclerotinia sclerotiorum* (equation 1). The observed inhibition was fitted to the proposed dose-response model [29] (equation 2).

$$y = c + (d - c) / (1 + exp (b * (log(x) - log (ED_{90})))) \qquad (1)$$

$$y = [c + ([(d-c) + f x]/ \{1 + exp [b*logx(x/ED_{90})]\}) \qquad (2)$$

In equation 1, y denotes the inhibition at essential oil concentration rate x, d is the maximum response and b is the slope. In Equation 1 and 2, d denotes the mean response of the untreated control, c the mean response at infinite rates, f the degree of hormetic increase, b the slope of the decreasing curve part, ED_{90} the dose causing 90% inhibition, and e parameter has no straightforward biological meaning [29]. The significance of hormesis was further verified using an analysis of variance (ANOVA). The candidate models were assessed based on the Akaike's Information Criteria (AIC) and mean square root (MSE) values. In particular, the nested models were compared with the MSE and non-nested models and were assessed based on the difference in AIC values (if the differences were > 2 then model with the lowest AIC was selected). ANCOVA was used to analyse *F. pseudograminearum*. R packages, including drc [30] and ggplot2 [31] were used for explanatory data analysis.

3. Results

3.1. Preliminary screening with 40 Eucalyptus essential oils

Fig. 2 depicts the mycelial growth inhibition by the 40 different eucalyptus oils on the two concentrations on three selected fungi. The results demonstrated that the higher concentration (50 μL/petri dish) exhibited greater suppression of fungal growth compared to the lower concentration of 10 μL/petri dish (Fig. 2).

Fourteen eucalyptus oils (EH205, ED260, EA257, CC19, EC162, EC136, EG269, EP286, EE12, EF296, EM201, EC151, ES313 and EP4) at 10 μL suppressed the mycelial growth of *F. pseudograminearum* to less than 50 mm, while others had little effect at the same concentration of 10 μL.

When applied at the concentration of 50 μL, twenty-six oils out of the 40 oils (65%) screened completely suppressed the mycelial growth of *F. pseudograminearum*. *F. pseudograminearum* mycelial growth was below 25 mm in the presence of oils EM201, ED250, EC136, EP160, EC54, EE59 and EC166 at the concentration of 50 μ /petri dish (734.65 ppm v/v), However, a few essential oils (ER40, EC150 and ES226) at 50 μL were less effective, with the mycelium growth of *F. pseudograminearum* > 50 mm (Fig. 2).

Among the 40 oils screened against *P. tritici-repentis* at the dose of 10 μL, the oil CC19 completely inhibited fungal growth. Mycelial growth of the pathogen *P. tritici-repentis* in the presence of oils EC150, EC166 and EV204 were 65 mm, 55 mm and 55 mm, respectively, with the inhibition being 35-45% compared to the control treatment, which had a fungal growth of 90 mm. The remaining 36 oils inhibited the mycelial growth of the pathogen around 50-90% at the same concentration (Fig.2).

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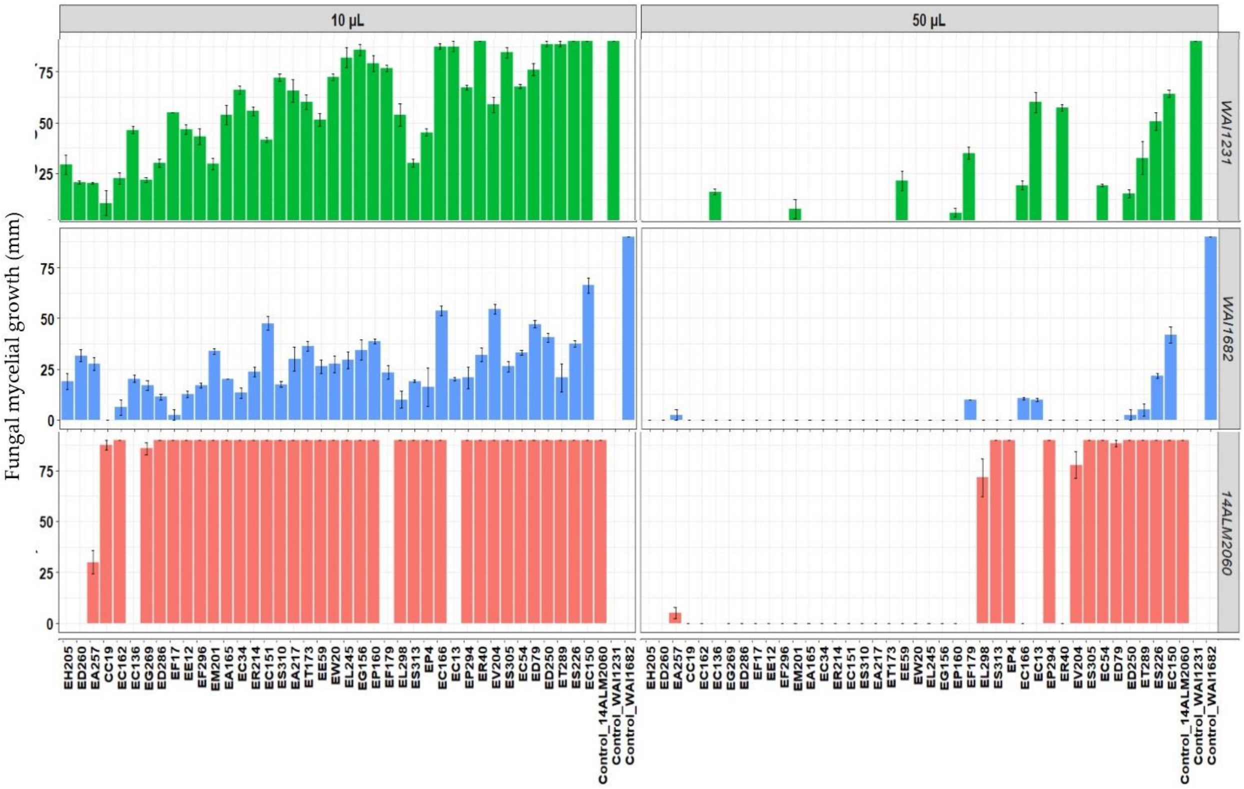

Figure 2. Initial screening of 40 eucalyptus essential oils against *Fusarium pseudograminearum* (WAI1231) and *Pyrenophora tritici-repentis* (WAI1682) and *Sclerotinia sclerotiorum* (14ALM2060).

The 40 essential oils at the concentration of 50 μL also significantly suppressed the mycelial growth of *P. tritici repentis*. At a concentration of 50 μL, 32 out of the 40 oils (80%) completely inhibited the mycelial growth of *P. tritici-repentis* (Fig. 2). The remaining 8 oils EA257, ED250, EF179, EC166, EC13, ET289, ES226 and EC150 inhibited mycelial growth by 65 - 75%.

Among the screened 40 eucalyptus oils, the mycelial growth of *S. sclerotiorum* was completely suppressed by 5 essential oils (EH205, ED260, EF179, EC136 and EC13) at the concentration of 10 μL. In the presence of oil EA257, mycelial growth inhibition was 70%. None of the other oils inhibited the fungal growth of S. *sclerotiorum* (Fig. 2).

At 50 μL, there are twenty-seven oils out of the 40 oils (68%) showed complete suppression of the mycelial growth of *S. sclerotiorum*. The oil EA257 inhibited the mycelial growth of the pathogen to 10 mm, with the inhibition rate of 90%. The remaining 13 oils exhibited lower suppression on *S. sclerotiorum* (Fig. 2).

In general, different oils acted differently on the 3 selected pathogens. For example, oils ED205, ED260, EF179 and EC13 completely suppressed the growth of *S. sclerotiorum* at the concentration of 10 μL, whereas the same oils did not suppress the growth of *P. tritici-repentis* and *F. pseudograminearum* at the same concentration. Another example is oil EP294, which completely inhibited the growth of the pathogens *P. tritici-repentis* and *F. pseudograminearum,* while the same oil showed no inhibition of mycelial growth of the pathogen *S. sclerotiorum* (Fig. 2). In another example, oils EP4 and ES313 completely suppressed mycelial growth of *F. pseudograminearum* and *P. tritici-repentis* at a concentration of 50 μL. However, at the same concentration, these oils did not suppress the mycelium growth of *S. sclerotiorum*. Furthermore, the oil, EC13 at a concentration of 50 μL inhibited the mycelial growth of *S. sclerotiorum* (100%) and *P. tritici-repentis* (90%), while the same oil only exhibited 30% inhibition of *F. pseudograminearum* (Fig. 3).

The *P. tritici-repentis* (WAI1682) is generally the most sensitive when exposed to essential oils, followed by *F. pseudograminearum* (WAI1231) and *S. sclerotiorum* (14ALM2060). On average across the 40 oils, the inhibition of mycelial growths were 70.4 % (± 15.74), 34.66 % (± 24.23) and 2.14 % (± 10.16) at the concentration

Figure 3. *In-vitro* screening of *Eucalyptus fraseri* subsp. *melanobasis* (EF296) against *Fusarium pseudograminearum* (photos taken at 7 days after the treatment).

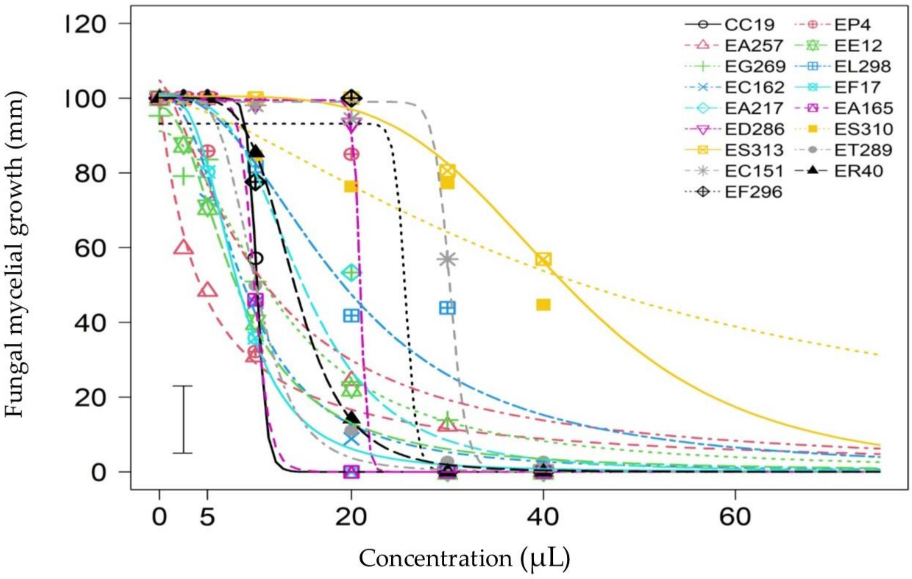

Figure 4. Dose response of 17 eucalyptus oils on the mycelial growth of *F. pseudograminearum* under *in vitro* conditions. The vertical bar in the graph represents the least significant difference (LSD) at the 0.05 significance level.

of 10 μL/petri dish and were 96.50 % (±10.34), 89.28 % (±18.29) and 67.7 % (±41.35) at the concentration of 50 μL/petri dish for *P. tritici-repentis*, *F. pseudograminearum* and *S. sclerotiorum*, respectively.

3.2. Dose response on F. pseudograminearum

A 20 eucalyptus essential oils that performed best in suppressing the mycelial growth of *F. pseudograminearum* in the initial screening were further evaluated using a concentration series of 0, 2.5, 5, 10, 20, 30 and 40 μL per Petri dish. The typical dose response of *F. pseudograminearum* when exposed to the essential oil EF296 is shown in Fig. 4.

The results indicated that mycelial growth was reduced as the concentration of the selected eucalyptus oil increased. A similar pattern was observed for all the selected oils screened.

Seventeen of the twenty essential oils had a significant impact on *F. pseudograminearum* (Fig. 4). The fungicidal activity of these essential oils was dose-dependent. Although, 20 oils were screened, only 17 oils performed well within the selected range of concentrations, and their data were fitted into the graph (Fig. 4). The three oils, EC166, ES226, and EC150 are not included in the graph as they did not exhibit any suppression at the tested concentrations (2.5, 5, 10, 20, 30 and 40 μL per Petri dish), although these oils showed significant suppression (40-80%) of *F. pseudograminearum* mycelial growth at a concentration of 50 μL in the initial bioassays (Fig. 2).

The 17 selected oils differed significantly in suppressing the mycelial growth of *F. pseudograminearum in-vitro*. Oils ET289 and EA165 suppressed the mycelial growth of *F. pseudograminearum* by 90% (ED_{90}) at a concentration of

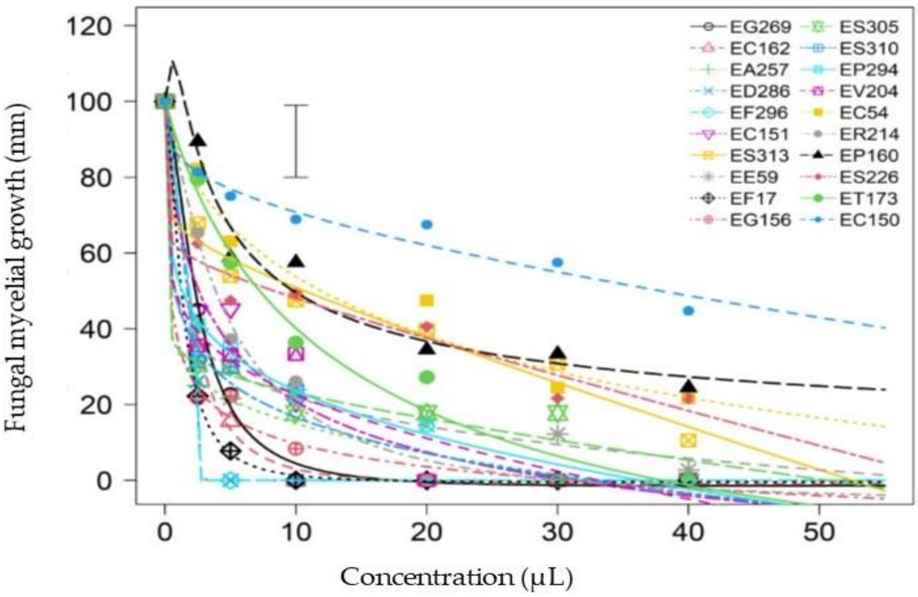

Figure 5. Dose-dependent response of 20 eucalyptus oils on the mycelial growth of *P. tritici-repentis* under *in vitro* conditions. The vertical bar in the graph represents the least significant difference (LSD) at the 0.05 significance level.

Control (0 μL) **2.5. μL** **5 μL** **10 μL** **20 μL** **30 μL** **40μL**

Figure 6. Dose-response effect of *Eucalyptus fraseri* subsp. *melanobasis* (EF296) against *S. sclerotiorum* (photos taken at 7 days after the treatment).

20 μL, whereas the oils EC151 and ER40 required 30 μL and 40 μL, respectively to achieve 90% inhibition (Fig. 4). The oil EA257, suppressed the mycelial growth of the pathogen by ≥ 50% at a concentration of 5 μL while a few other oils (i.e.: EE12 and EG269) inhibited ≥ 50% of the pathogen mycelial growth at a concentration of 10 μL (Fig. 4).

3.3. Dose response on P. tritici-repentis

The oils ED286, EP294 and EF296 completely suppressed the mycelial growth of the pathogen at a concentration of 5 μL and the oil EF17 suppressed the mycelial growth by 90% at the same concentration. The ED_{90} values for oils ES226 and EC162 were 10 and 20 μL, respectively (Fig. 5).

Oils EC162, EA257, ED286, EF296, EE59, EF17, EG156, ES305, ES310, EP294 and EV204 significantly inhibited the mycelial growth of *P. tritici-repentis* ranging from 60-80% at a concentration of 2.5 μL. However, at a concentration of 10 μL the oils EG269, EC162 and EF17 completely suppressed the mycelial growth of the respective pathogens (Fig. 5).

3.4. Dose response on Sclerotinia sclerotiorum

As shown in Fig. 6, the control treatments resulted in the production of sclerotia, which are survival structures formed by the pathogen under adverse conditions through the aggregation, thickening, and hardening of the undifferentiated hyphae. Increasing concentrations of the essential oil from *Eucalyptus fraseri* subsp. *melanobasis* resulted in a greater reduction or complete inhibition of sclerotia production by the fungus. A similar pattern was observed when screening for other oils.

Of the 20 oils screened against *S. sclerotiorum*, oil EA257 inhibited mycelial growth at all tested concentrations (Table 2). The oil EA257 inhibited *S. sclerotiorum* mycelium growth by 10% at a

Table 2. Inhibition of 20 Eucalyptus essential oils on mycelium growth of *Sclerotinia sclerotiorum* (14ALM2060).

Essential oil	Eucalyptus species	Inhibition (% control)					
		2.5 µL	5 µL	10 µL	20 µL	30 µL	40 µL
EE12	*Eucalyptus exserta*	0	0	0	0	100	100
EF17	*Eucalyptus froggattii*	0	0	0	88.9±0.90	100	100
CC19	*Corymbia citriodora*	0	0	0	0	57.27±1.06	100
ER40	*Eucalyptus resinifera* subsp. *hemilampra*	0	0	0	0	100	100
EC54	*Eucalyptus carnei*	0	0	0	0	0	0
EC151	*Eucalyptus crucis* subsp. *lanceolata*	0	0	0	0	52.55±1.23	100
EG156	*Eucalyptus globulus*	0	0	0	0	50.05±3.89	79.19±1.05
EC162	*Eucalyptus cooperiana*	0	0	0	100	100	100
EA165	*Eucalyptus amplifolia* subsp. *amplifolia*	0	0	0	0	54.27±1.65	88.35±0.55
EA217	*Eucalyptus aspersa*	0	0	0	0	100	100
ES226	*Eucalyptus selachiana*	0	0	0	0	0	0
EL245	*Eucalyptus leptocalyx* subsp. *leptocalyx*	0	0	0	0	0	100
EA257	*Eucalyptus albida*	11.2±0.9	34.24±2.45	59.21±1.52	80.02±2.39	81.13±1.92	89.46±0.55
EG269	*Eucalyptus grandis* subsp. *grandis*	0	0	7.6±2.19	57.27±1.06	77.8±0.90	88.9±0.9
ED286	*Eucalyptus dissimulata*	0	0	0	0	100	100
EP294	*Eucalyptus platycorys*	0	0	0	0	100	100
EF296	*Eucalyptus fraseri* subsp. *Melanobasis*	0	0	0	0	100	100
ES305	*Eucalyptus salmonophloia*	0	0	0	0	0	100
ES310	*Eucalyptus salubris*	0	0	0	0	22.3±0.9	43.67±1.65
ES313	*Eucalyptus spathulata*	0	0	0	0	0	0

concentration of 2.5 µL, with the inhibition increasing as the oil concentration increased. Oils EE12, EF17, ER40, EC162, EA217, ED286, EP294 and EF296 completely inhibited mycelium growth of *S. sclerotiorum* at a concentration of 30 µL, while The oils EG156, EC151, EA165, and CC19 exhibited moderate inhibition (50-60%) at the concentration of 30 µL. Oils EC54, ES226 and ES313 showed no inhibition of *S. sclerotiorum* mycelial growth at any of the concentrations tested in this screening. However, these three oils showed considerable inhibition of 20%, 50% and 100% inhibition, respectively, on the pathogen *S. sclerotiorum* at a concentration of 50 µL (Fig. 2).

4. Discussion

To date, there appears to be very little published literature on the potential fungicidal effects of eucalyptus essential oils against *Fusarium pseudograminearum*, *Pyrenophora tritici-repentis*, and *Sclerotinia sclerotiorum*. Initial screening of 40 eucalyptus essential oils showed a potential inhibitory effect on the mycelial growth of the three selected fungi. The inhibition increased with increasing oil concentration. The same pattern was observed for the three pathogens screened, with the high concentration of 50 µL being more inhibitory than the low concentration of 10 µL.

Out of the 40 oils initially screened, the top 20 most effective oils on each of the three fungi were further evaluated at a series of concentrations. Mycelial growth inhibition was found to be dose dependent. Ayed et al. investigated the antifungal activity of essential oils from eight Tunisian *Eucalyptus* species against four *Fusarium* species, *F. oxysporum matthioli*, *F. oxysporum solani*, *F. culmorum*, and *F. redolens*. They concluded that all tested *Eucalyptus* essential oils suppressed mycelial growth of the four *Fusarium* species in a dose-dependent manner [32]. Six different essential oils, including *Eucalyptus* essential oil, were tested *in vitro* against four phytopathogenic fungi, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Alternaria brassicicola*, and *Cylindrosporium concentricum*. The application of *Eucalyptus* essential oil at increasing doses showed high efficacy in reducing the mycelial growth of all tested pathogenic fungi [33]. These findings further support our results, which demonstrated dose-dependent mycelial growth inhibition by *Eucalyptus* essential oils in the three tested pathogens, including *F. pseudograminearum*.

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Researchers have found that *E. globulus* has potential antifungal properties against *A. alternata* and *C. gloeosporioides* on apple fruits under *in vivo* conditions and observed reduced lesion diameter [25]. Our findings also confirmed that the essential oil of *E. globulus* (EG156) completely suppressed mycelial growth of all three fungi tested at a concentration of 50 μL under *in vitro* conditions. The essential oil of *Eucalyptus citriodora* significantly reduced the biomass production of the fungi *Aspergillus niger*, *Fusarium oxysporum*, *Fusarium udum*, and *Magnaporthe grisea*. Furthermore, E. citriodora oil delayed sporulation of *A. niger* [34].

Occasionally, the same essential oil exhibited similar effects on all three fungal pathogens. For example, the oils ES310, EC151, and EA217 at a concentration of 50 μL completely suppressed the mycelial growth of the three fungi. Likewise, oil EF17 completely suppressed the mycelial growth of all three pathogens at a concentration of 30 μL/per Petri dish. Additionally, the oil EC162 at a concentration of 20 μL/per petri dish completely suppressed the mycelial growth of *S. sclerotiorum* and *P. tritici-repentis*, while showing 92% inhibition against *F. pseudograminearum*.

On the other hand, the same oil does not always have consistent effects across the three pathogens. For example, the oil EC162 significantly inhibited the mycelial growth of *F. pseudograminearum* and *P. tritici-repentis* by 75% and 90%, respectively, at a concentration of 10 μL, whereas it only inhibited *S. sclerotiorum* by 10 % at the same concentration. However, the same oil completely suppressed all three pathogens at a concentration of 50 μL. Generally, *P. tritici-repentis* was the most sensitive to the essential oils tested, while *S. sclerotiorum* was the least sensitive.

Different oils are highly likely to differ in their metabolite profiles. Khedhri et al. conducted a metabolic profiling of four Tunisian *Eucalyptus* essential oils and evaluated their insecticidal and antifungal activities [35]. The study highlighted a dose-dependent fungicidal effect, where an increased concentration of eucalyptus oil led to higher fungicidal activity against the selected pathogens. Furthermore, the observed effects varied due to differences in the chemical composition of the essential oils. Similarly, four eucalyptus essential oils *E. griffithsii*, *E. longicornis*, *E. lesouefii*, and *E. obliqua* were screened against three strains of the pathogen *Fusarium*, namely *F. lycopersici*, *F. redolens*, and *F. culmorum*, under *in-vitro* conditions [35]. They suggested that the efficacy of the essential oils can be attributed to their elevated sesquiterpene content, with β-eudesmol potentially being one of the primary compounds responsible for their inhibitory properties [35].

Our research further demonstrated that the three pathogens reacted differently when screened with various eucalyptus essential oils. The findings highlight variations in pathogen susceptibility, with some oils exhibiting stronger inhibitory effects than others. This suggests that the efficacy of the oils tested in our study may be due to differences in their chemical compositions.

Eucalyptus essential oils are known for their antifungal properties, which can be attributed to a combination of direct and indirect effects on the microorganisms. These essential oils exert their antifungal action through two primary mechanisms such as direct vapour absorption by the microorganism and an indirect effect through the medium that absorbs the vapour [36]. In this experiment, the essential oils impregnated in the filter paper were directly absorbed by the fungal cells, leading to the disruption of their cellular functions and structures. This direct interaction can inhibit the mycelial growth and reproduction (sexual or asexual) of fungi, effectively reducing their presence. It was reported that the mechanism of the eucalyptus essential oil-tamarind emulsion could be due to the change in the membrane permeability of the pathogens, which increased the leakage of nucleic acids, proteins, and other substances. Hence, the pathogen lost its protection and was inactivated [37].

The three fungal pathogens used in this screening experiment were cultured on Potato Dextrose Agar (PDA) or PDA-V8 media. These media could absorb the vapours produced by eucalyptus essential oils, which could partly contribute to the inhibition of fungal mycelial growth.

Zhou et al. studied the mechanism of action of eucalyptus essential oils in the inhibition of mycelial growth by the pathogenic fungus *Magnaporthe grisea* and investigated the morphological variations of *M. grisea* in the presence of eucalyptus oils *E. grandis* and *E. urophylla*. Electron scanning microscopy studies revealed, structural changes in the hyphae including irregular shapes, cavities in the outer surface of the fungal hyphae, swelling at the tops of the spores, and adhesion of the mycelium. Due to these alterations, the pathogen's ability to infect and its biological activity are significantly reduced, leading to a substantial inhibition of mycelial growth by eucalyptus oils [19]. They also reported that the expression level of the glucose oxidation gene was largely reduced after the introduction of eucalyptus oil, thereby affecting glycolysis, which is a critical process for providing the energy required for spore production. However, whether a similar mechanism occurs with the screened fungi in our experiments, requires further investigation.

The production of sclerotia, which are persistent survival structures, is common in *S. sclerotiorum*. However, our study revealed a reduced number of sclerotia, or even no sclerotia production in the presence of eucalyptus oils. This finding further confirms the structural changes in fungi in the presence of eucalyptus oil. However, further studies are needed to investigate the effects of eucalyptus oil on sclerotia production and the mechanism underlying the inhibition of sclerotial production in *S. sclerotiorum*. Some oils had no effect on fungal mycelial growth but suppressed sclerotia production. For example, in the presence of oil ES310, mycelial growth was observed at all concentrations used in the screening. However, this oil completely inhibited sclerotia production at the oil concentration of > 5 µL/petri dish. This behaviour could be useful in reducing inoculum production in canola crops over time.

5. Conclusions

The most effective eucalyptus essential oils demonstrated substantial inhibitory activity against all three pathogens investigated in this study, namely *Fusarium pseudograminearum*, *Pyrenophora tritici-repentis*, and *Sclerotinia sclerotiorum*. The inhibition depends on the essential oil used and the pathogen tested. Oils ET289 and EA165 achieved ED_{90} values of 20 µL per Petri dish against *F. pseudograminearum*, while EP294 and EF17 suppressed *P. tritici-repentis* by 90–100% at only 5 µL per dish. Similarly, EF17 and EC162 inhibited *S. sclerotiorum* by 89–100% at 20 µL per dish. This study identified potent essential oils for each of the three pathogens, which could be further developed as an environmentally friendly disease management practices by creating a biopesticides. However, for the further development of eucalyptus essential oil as an alternative to synthetic fungicides, additional studies are required to evaluate suitable methods of application to plants, as well as its effects on associated soil microbes.

Disclaimer (artificial intelligence)

Author(s) hereby state that no generative AI tools such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators were utilized in the preparation or editing of this manuscript.

Authors' contributions

Conceptualization, H.W.; conducting the experiments, writing and revision, S.R.; data analysis and review, providing fungal isolates, A.M., K.L.; review, A. M., K.L.K.L., A.A.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest.

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